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Transgenic Tobacco Plants Expressing the Coat Protein of Cucumber Mosaic Virus Show Different Virus Resistance

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Transgenic tobacco (*Nicotiana tabacum* cv. Xanthi-nc) plants were regenerated after cocultivation of leaf explants with *Agrobacterium tumefaciens* strain LBA4404 harboring a plasmid that contained the coat protein (CP) gene of cucumber mosaic virus (CMV-As). PCR and Southern blot analyses revealed that the CMV CP gene was successfully introduced into the genomic DNA of the transgenic tobacco plants. Transgenic plants (CP^+) expressing CP were obtained and used for screening the virus resistance. They could be categorized into three types after inoculation with the virus: virus-resistant, delay of symptom development, and susceptible type. Most of the CP^+ transgenic tobacco plants failed to develop symptoms or showed systemic symptom development delayed for 5 to 42 days as compared to those of nontransgenic control plants after challenged with the same virus. However, some CP^+ transgenic plants were highly susceptible after inoculation with the virus. Our results suggest that the CP-mediated viral resistance is readily applicable to CMV disease in other crops.

Keywords: CMV, coat protein, tobacco, transgenic plant, virus resistance

INTRODUCTION

With the development of molecular biological tools for introduction of a foreign gene into plant cells and regeneration of transformed plants, various approaches have been taken to attempt to produce plants resistant to virus diseases (Beachy *et al.*, 1990; Namba *et al.*, 1991; Van der Vlugt *et al.*, 1992; Van der Vlugt and Goldbach, 1993; Zaitlin *et al.*, 1994; Prins and Goldbach, 1996). Sanford and Johnston (1985) first hypothesized the idea that the trasferring of a gene derived from the viral genome into a plant chromosome can confer resistance against the homologous virus. This new concept of non-conventional protection, so called as pathogen derived resistance (PDR), has been successfully exploited for developing virus resistance in many crop plants (Beachy *et al.*, 1990; Wilson, 1993).

Coat protein-mediated resistance (CP-MR), one type of PDR, has been widely used to develop protection in monocots and dicots against a number of different plant viruses (Powell Abel *et al.*, 1986; Cuozzo *et al.*, 1988; Kawchuk *et al.*, 1990; Namba *et al.*, 1991; MacKenzie and Ellis, 1992; Xue *et al.*, 1994; Leclerc and AbouHaidar, 1995; Prins *et al.*, 1995). Beginning with CP-MR, the range has been extended to the use of other viral genes as a source of PDR (Harrison *et al.*, 1987; Cuozzo *et al.*, 1988; Lawson *et al.*, 1990; Van der Vlugt *et al.*, 1992; Zaitlin *et al.*, 1994; Leclerc and AbouHaidar, 1995).

The cucumber mosaic virus (CMV), the type species of the genus *Cucumovirus* in the family *Bromoviridae*, possesses a segmented genome consisting of three single-stranded genomic RNAs and two subgenomic RNAs (Palukaitis *et al.*, 1992). Both the first and second RNAs encode components of the viral replicase which are responsible for CMV replication. The third RNA, coding for the 3a movement protein, can be synthesized into a fourth subgenomic RNA, which translates into the coat protein. The CMV has the widest host range of over 800 species in 85 families (Palukaitis *et al.*, 1992). In nature, the virus is spread by more than 60 aphid species, and this property is a potential cause of virus epidemics in economically important crops. To date, a number of CMV strains has been reported and characterized (Palukaitis *et al.*, 1992). They can be divided into two subgroups, I and II, by serological properties and nucleotide sequence homologies (Palukaitis *et al.*, 1992). In this study, the CMV-As strain was isolated from *Aster yomena* M. in Korea

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as a virus source (Park *et al.*, 1990a). Previous results showed that the CMV-As strain belongs to subgroup I (Park *et al.*, 1990a). Recently, the complete nucleotide sequence of the third RNA of the virus has been reported (Kim *et al.*, 1996). The tobacco plant, *Nicotiana tabacum* cv. Xanthi-nc, used in this study has resistance to TMV that is conferred by the *N* gene, but the plant is susceptible to CMV.

In this study, the coat protein gene derived from a cDNA of CMV-As RNA 3 was transformed into tobacco plants and their resistance to the homologous virus was evaluated.

MATERIALS AND METHODS

Virus Source and Plant Material

The CMV-As strain, a Korean isolate, was used for the source of virus in this study (Park *et al.*, 1990a). The virus was purified from the tobacco plants inoculated with the virus as described by Park *et al.* (1990a). Viral genomic RNA was extracted from purified virion particles by using SDS/proteinase K and phenol extraction followed by ethanol precipitation by the method of Park *et al.* (1990b). Tobacco (*N. tabacum* L. cv. Xanthi-nc) was used as plant material for transformation. Genomic DNA of tobacco plants was extracted from leaf tissues by the method of Cuozzo *et al.* (1988) for PCR and Southern blotting analyses (Sambrook *et al.*, 1989).

Engineering the CMV CP Gene and Tobacco Transformation

A cDNA of the CMV-As CP gene was inserted in the sense orientation between the CaMV 35S promoter and NOS-terminator site of the pBI121 to replace the β-glucuronidase gene, and then the resulting construct pCMASCP121-123 was transferred into tobacco plants by the *Agrobacterium*-mediated transformation method. Transformation and regeneration were carried out as described by Horsch *et al.* (1985). Leaf tissues from *in vitro* cultured tobacco tissues were used for transformation. The tissues were cut into small pieces (0.7 cm^2) and the sections were incubated overnight in an MS medium. The sections were soaked in a fresh overnight culture of *Agrobacterium tumefaciens* (ca. 10^8 bacterial cells/mL) for 20 min. Explants were then blotted dry on a sterilized filter paper and placed onto solidified media in disposable plastic petri dishes for cocultivation with the bacteria. Calli were induced from the leaf tissues treated with the bacteria on an MS selective medium containing 300 μg/mL kanamycin and 500 μg/mL carbenicillin. After two successive transfers for 10 weeks, the calli were transferred to an MS medium not supplemented with kanamycin to promote callus proliferation. Shoots were regenerated, and they were subsequently induced to establish whole plants. Each leaf piece from kanamycin-resistant putative transformants developed many calli on the selection media within 2 weeks, whereas nontransgenic control plants did not form calli and turned yellow rapidly. Regenerated plants were selected on kanamycin and then rooted. Young plantlets with roots were transferred to soil and grown in a greenhouse. Integration of the CP gene into a plant chromosome was checked by Southern blot (Sambrook *et al.*, 1989) with a ^{32}P-labeled CMV-As CP probe (Park *et al.*, 1990b).

PCR Analysis

Transgenic tobacco plants were initially screened for the presence of the CMV CP gene by PCR. Genomic DNA isolated from putatively transformed tobacco leaves was PCR-amplified using two primers specific to the CMV CP gene. PCMCP1 (5'-AACACGGATTCAAACTGG-3': EMBL AJ001131) and PCMCP2 (5'-GAGTCATGGACAAATCTG-3': EMBL AJ001132) produce a 671 bp DNA fragment representing a portion of the CP gene (Park *et al.*, 1995). pCMASCP121-123 plasmid DNA and DNA extracted from nontransformed tobacco plants were used as positive and negative controls, respectively. PCR amplification was carried out in 50 μL volumes with a denaturation step at 94°C for 1 min, an annealing step at 42°C for 1 min, and an extension step at 72°C for 2 min, for a total of 35 cycles. Each PCR-amplified sample was electrophoresed in 1.0% agarose gel in 20 mM Tris-acetate, 0.5 mM EDTA buffer.

Plant Inoculation and Quantification of CMV

Two leaves of each plant were dusted with Carborundum (300 mesh) and inoculated with purified virus diluted with a 50 mM phosphate buffer (pH 7.0). Four to five-leaf stage transgenic tobacco lines transformed with the CMV CP gene and nontransgenic control plants were challenged with CMV by mechanical inoculation. The CMV-As strain was used as an inoculum source. *Chenopodium amaranticolor*, a local lesion host of the virus, was used to check for latent infection in inoculated plants (Park *et al.*, 1990a). Plants were observed daily for symp-

tom development after inoculation of the virus. Quantification of the CMV was assessed by enzyme-linked immunosorbent assay (ELISA) with immunoglobulin G from polyclonal antiserum against CMV by the method of Park *et al.* (1990a). Total proteins were extracted from whole leaf tissues by homogenizing the samples in a 0.01 M phosphate buffer (pH 7.0) containing 1 mM EDTA and 0.1% PVP.

RESULTS

Confirmation of Transformation

Regenerated tobacco plants were screened for transgene incorporation in the plant genome using PCR. CMV CP-specific primers were used to amplify the CMV CP gene (Park *et al.*, 1995). Amplification of the transgene from genomic DNA containing the CMV CP gene with the primers resulted in a 671 bp product (Fig. 1, lanes 2-8). Nontransformed tobacco plant DNA yielded no PCR product (Fig. 1, lane 9). In addition, Southern blot analysis revealed the presence of the CP gene in the plant genome (data not shown).

Expression of CMV CP in Transgenic Tobacco Plants

Immunological analysis by ELISA indicated that the CMV CP gene was expressed in transgenic tobacco plants. Initial experiments showed that ELISA was effective in monitoring the relative expression levels of the transgene (Namba *et al.*, 1991; Ryu and Park, 1995). A total of 200 kanamycin resistant putative transgenic plants was obtained and analyzed for CP expression by ELISA; 152 plants (76%) expressed CP genes (CP^+). They produced detectable levels of the viral CP as measured by ELISA, and the amounts of the CP gene were differed slightly from one another (absorbance 0.06-0.21). Transgenic line X054 revealed the highest expression of the transgene (absorbance 0.21) among the tested transgenic tobacco which showed thereafter high resistance against homologous virus infection. All the selected CP^+ transgenic plants showed morphologically normal and were visually indistinguishable from wild type nontransformed control tobacco plants.

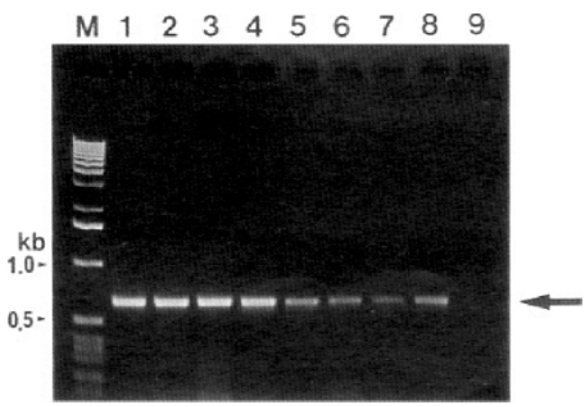

Fig. 1. Agarose gel electrophoresis (1.0%) for tobacco transformants analysis by PCR with CMV-specific primers. Lane M: size maker DNA, 1: positive control DNA (pCMASCP121-123), 2-8: DNA from putatively transformed tobacco plants; X006(2), X022(3), X073(4), X005(5), X076(6), X004(7), X111(8), 9: negative control (nontransformed tobacco). The arrow indicates the 671 bp PCR product.

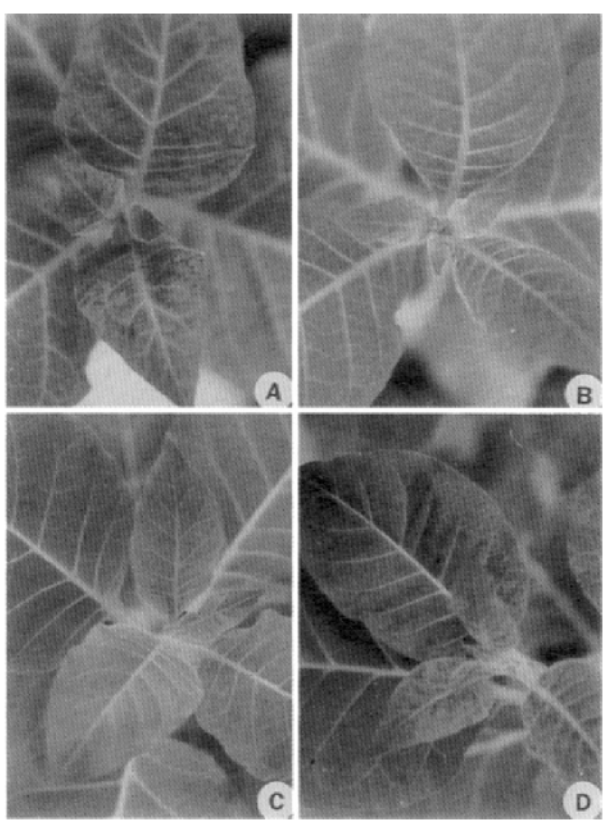

Fig. 2. Symptoms of transgenic tobacco plants expressing the coat protein gene of cucumber mosaic virus (CMV) and a nontransgenic control tobacco plant after inculation with CMV. A, nontransformed control; B, C, and D: CP expressing transgenic tobacco plants (B, delayed in symptom development; C, resistant; D, susceptible).

Protection of CP$^+$ Transgenic Tobacco Against CMV Infection

A total of twenty-five CP$^+$ transgenic plants was analyzed for protection effects against the CMV. They could be categorized into three types by resistance types and disease indexes after challenged with CMV: delayed symptom development type (Fig. 2B), virus-resistant type (Fig. 2C), and susceptible type (Fig. 2D). Some CP$^+$ transgenic plants that accumulated detectable levels of CMV CP showed significant levels of resistance either in delayed symptom development or resistant type against the homologous virus. However, others were revealed susceptible to the virus.

Table 1. Assessment of virus resistance of transgenic tobacco lines expressing the coat protein gene and nontransgeni control tobacco plants to cucumber mosaic virus (CMV) infection

Plant line[a]	Symptom[b]	Disease index[c] (Days of delayed symptom)
X006	mM	D(22)
X007	VC	D(42)
X022	mM	D(22)
X037	mM	D(21)
X039	M	D(5)
X073	mM	D(31)
X005	–	R
X030	–	R
X044	–	R
X047	–	R
X054	–	R
X076	–	R
X079	–	R
X101	–	R
X103	–	R
X120	–	R
X004	M	S(0)
X017	M	S(-3)
X032	M	S(0)
X038	M	S(-1)
X045	M, St	S(-5)
X049	M, St	S(-5)
X050	M	S(-4)
X066	M	S(-5)
X111	M, St	S(-5)
Control-1	M, St	S(0)
Control-2	M	S(0)
Control-3	M	S(0)
Control-4	M	S(0)

[a]Plant line: X006-X111, transgenic tobacco plants; control-1-4, nontransformed tobacco plants for negative controls.
[b]Symptom: mM, mild mosaic; VC, vein clearing; M, mosaic; St, stunting; -, no symptom.
[c]Disease index: D, delayed in symptom development; R, resistant; S, susceptible.

Severe systemic mosaic symptoms developed in all of the control plants within 10 days after inoculation, whereas some transgenic tobacco plants showed a significant level of resistance. Of twenty-five CP$^+$ transgenic plants, six plants showed significant delays in systemic symptom development after inoculation with the virus compared to the nontransgenic control plants (Table 1). These transgenic plants developed very mild symptoms consisting of slight vein clearing and mild mosaic, and the symptom development was delayed for 5 to 42 days compared to those of the controls. Inoculated leaf samples from the X037 line, which belongs to the delayed symptom type, contained significantly lower concentrations of the CMV than did the control plants at all time points. Ten CP$^+$ transgenic plants exhibited a high level of resistance, nearly immune, to CMV infection up to 100 μg/mL, and symptom development and virus accumulation were absent in these plants until flowering stage. When the concentration of CMV increased to 200 μg/mL, they retained the resistance to the infected virus. Nine CP$^+$ transgenic tobacco plants including the X111 line showed no protection against the virus at 10 μg/mL although they were CP$^+$ transgenic lines. They were severely stunted about 60% reduction in both shoot length and leaf size as compared to nontransformed control tobacco plants (Fig. 3). No comprehensive correlation occurred in such CP$^+$ transgenic plants

Fig. 3. Protection effects of transgenic tobacco plants expressing the coat protein of the cucumber mosaic virus (CMV) challenged with CMV as compared to a nontransgenic control plant. DT; delayed in symptom developed type; ST, susceptible type; RT, resistant type; CON, control.

between the expression level of CP and virus resistance. The F1 seeds of self-fertilized X005 and other transgenic lines were assayed for kanamycin resistance, CP expression, and protection effects. Their segregation ratio was about 3:1 (CP^+:CP^-) indicating insertion of the transgene in a single locus. The progeny of resistant transgenic tobacco plants expressing the gene were protected from CMV infection.

DISCUSSION

To date, coat protein-mediated resistance (CP-MR) has been reported for many virus genera in several plant species (Powell Abel *et al.*, 1986; Cuozzo *et al.*, 1988; Kawchuk *et al.*, 1990; Namba *et al.*, 1991; MacKenzie and Ellis, 1992; Xue *et al.*, 1994; Leclerc and AbouHaidar, 1995; Prins *et al.*, 1995). Most of the effort that has been directed towards interpreting the mechanism of CP-MR has utilized the virus-host combination of TMV and tobacco plants (Osbourn *et al.*, 1989). Plants transformed with the CP genes of several viruses showed susceptibility to infection with RNA of the corresponding virus (Powell Abel *et al.*, 1986; Osbourn *et al.*, 1989). This demon- strates that a major component of CP-MR against virus is affected at or before uncoating of the virus (Register and Beachy, 1988). It was postulated that a host factor that is involved in virus disassembly might be blocked or occupied by transgenic CP (Beachy *et al.*, 1990). Register and Beachy (1988) reported that expression of the TMV CP gene in transgenic plant cells prevented the TMV from the uncoating process and resulted in the virus resistance. The uncoating of the virus is an early event in virus infection in the plant, and most of CP-MR in various plants versus viruses can be explained by the mechanism of this blockage model (Register and Beachy, 1988; Register and Nelson, 1992). On the other hand, plants expressing the potato virus X (PVX) CP showed a significant resistance to infection with PVX RNA as well as a virion of the PVX (Lawson *et al.*, 1990), suggesting that other steps can also be affected. Recently, RNA-mediated protection has been explained as some virus resistance mechanism such as the latter case (Prins and Goldbach, 1996).

In our experiments, some CP^+ transgenic lines such as X005 did not develop virus symptoms and contained no detectable virus in the systemic leaves as well as the inoculated leaves after virus infection until the flowering stage. This result demonstrates and confirms that the strategy of expression of the CP can be used to confer protection against CMV. Transgenic tobacco plants that expressed and accumulated detectable levels of CP exhibited varying levels of resistance to CMV infection, and they could be classified into three types although levels of expressed CP varied only slightly: delayed symptom development type, highly virus resistant type, and susceptible type. Some transgenic lines remained symptomless and highly resistant to the virus, while other lines showed a delay in the symptom development and reduction in symptom severity. Other lines failed to display any level of virus resistance. Moreover, CMV could not be detected by ELISA nor recovered from both inoculated and uninoculated leaves of all the highly resistant transgenic tobacco plants. Namba *et al.* (1991) reported that tobacco plants expressing the CMV-WL CP gene showed a broad spectrum of protection against infections by the various CMV strains including subgroup I and II. We assume that our resistant lines may be also be protected against other strains of CMV.

In the case of TMV CP transgenic tobacco plants, the degree of protection was directly proportional to the levels of accumulated CP (Register and Beachy, 1988). However, no correlation in the levels of expressed CP with the degree of protection was observed in some viruses such as CMV, potato virus Y (PVY), and plum pox virus (PPV) (Cuozzo *et al.*, 1988; Regner *et al.*, 1992; Van der Vlugt *et al.*, 1992; Okuno *et al.*, 1993). No direct explanation has yet been obtained to correlate the levels of expressed CP with the degree of protection.

The *Agrobacterium*-mediated transformation method results in the incorporation of a variable number of the transgene into variable locations of host chromosomes (Beachy *et al.*, 1990). Thus, many independent lines of transgenic plants can be produced in a single experiment. Due to the position effects of the transgene in plant chromosomes, independent lines that contain the same transgene will have different phenotypes. Moreover, random insertion of the transgene into host DNA makes it difficult to analyze the mechanism of pathogen-derived resistance. These phenomena could explaine why no direct correlation was observed between the level of expression of the transgene and the degree of resistance in our CMV CP-mediated tobacco system. Accumulated CP can interfere with different steps of virus infection cycle, depending on the virus-host combination (Prins and Goldbach, 1996). Accumulated CP in resistant transgenic tobacco plants may aggregate with CP subunits of the infecting virus, and such aggregation

would lead to stabilization of virion and prevent virus replication. It is possible that the CMV CP is involved in the transport of infectious units, and that accumulation of CP in transgenic plants may interfere with this process. It is likely that there are multiple mechanisms involved in the protection phenotype that may reflect virus life cycle (entry, uncoating, replication, translation, movement, etc.) for each virus.

The transgenic plant possessing the CP gene and exhibiting the virus resistance can be useful as a genetic source with the agronomic characteristic of the virus resistance. Recently, an alternative approach to achieve a broad spectrum of resistance against multiple viruses has been demonstrated (Lawson *et al.*, 1990; Prins *et al.*, 1995) and this will also be a valuable option for improving various crops.

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LITERATURE CITED

Beachy, R.N., S. Loesch-Fries and N.E. Turner. 1990. Coat protein-mediated resistance against virus infection. *Ann. Rev. Phytopathol.* **28**: 451-474.

Cuozzo, M., K.M. O'Connel, W. Kaniewski, R.X. Fang, N.H. Chua and N.E. Turner. 1988. Viral protection in trnasgenic tobacco plants expressing the cucumber mosaic virus coat protein or its antisense RNA. *Bio/Technology* **6**: 549-557.

Harrison, B.D., M.A. Mayo and D.C. Baulcombe. 1987. Virus resistance in transgenic plants that express cucumber mosaic virus satellite RNA. *Nature* **328**: 799-802.

Horsch, R.B., J.E. Fry, N.L. Hoffmann, D. Eichholtz, S. G. Rogers and R.T. Fraley. 1985. A simple and general method for transferring genes into plants. *Science* **227**: 1229-1231.

Kim, S.H., W.M. Park, S.Y. Lee and Y.I. Park. 1996. Determination of nucleotide sequences of cDNA from cucumber mosaic virus-As RNA4. *Korean J. Plant Pathol.* **12**: 176-181.

Kawchuk, L., R. Martin and J. McPherson. 1990. Resistance in transgenic plants expressing the potato leafroll luteovirus coat protein gene. *Mol. Plant-Microbe Interact.* **3**: 301-307.

Lawson, C., W. Kaniewski, L. Haley, R. Rozeman, C. Newell, P. Sanders and N.E. Turner. 1990. Engineering resistance to mixed virus infection in a commercial potato cultivar: resistance to potato virus X and potato virus Y in transgenic Russet Burbank. *Bio/Technology* **8**: 127-134.

Leclerc, D. and M.G. AbouHaidar. 1995. Transgenic tobacco plants expressing a truncated form of the PAMV capsid protein (CP) gene show CP-mediated resistance to potato aucuba mosaic virus. *Mol. Plant-Microbe Interact.* **8**: 58-65.

MacKenzie, D.J. and P.J. Ellis. 1992. Resistance to tomato spotted wilt virus infection in transgenic tobacco expressing the viral nucleocapsid gene. *Mol. Plant-Microbe Interact.* **5**: 34-40.

Namba, S., K. Ling, C. Gonsalves, D. Gonsalves and J. L. Slightom. 1991. Expression of the gene encoding the coat protein of cucumber mosaic virus CMV strain WL appears to provide protection to tobacco plants against infection by several different CMV strains. *Gene* **107**: 181-188.

Okuno, T., M. Nakayama, S. Yoshida, I. Furusawa and T. Komiya. 1993. Comparative susceptibility of transgenic tobacco plants and protoplasts expressing the coat protein gene of cucumber mosaic virus to infection with virions and RNA. *Phytopathology* **83**: 542-547.

Osbourn, J.K., J.W. Watts, R.N. Beachy and T.M.A. Wilson. 1989. Evidence that nucleocapsid disassembly and a later step in virus replication are inhibited in transgenic tobacco protoplasts expressing TMV coat protein. *Virology* **172**: 370-373.

Palukaitis, P., M.J. Roosinck, R.G. Dietzgen and R.I.B. Francki. 1992. Cucumber mosaic virus. *Adv. Virus Res.* **41**: 281-348.

Park, W.M., K.H. Ryu and J.K. Choi. 1990a. Properties and purification of cucumber mosaic virus. *Korean J. Plant Pathol.* **6**: 393-401.

Park, W.M., K.H. Ryu, H.K. Koh and S.Y. Lee. 1990b. Diagnosis of cucumber mosaic virus using ^{32}P and biotinylated cDNA probe. *Korean J. Plant Pathol.* **6**: 417-422.

Park, W.M., K.H. Ryu, S.J. Kim and J.K. Choi. 1995. Rapid detection and identification of cucumber mosaic virus by reverse transcription and polymerase chain reaction (RT-PCR) and restriction analysis. *J. Plant Biol.* **38**: 267-274.

Powell Abel, P., R.S. Nelson, B. De, N. Hoffmann, S.G. Rogers, R.T. Fraley and R.N. Beachy. 1986. Delay of disease development in transgenic plants that express the tobacco mosaic virus coat protein gene. *Science* **232**: 738-743.

Prins, M. and R. Goldbach. 1996. RNA-mediated virus resistance in transgenic plants. *Arch. Virol.* **141**: 2259-2276.

Prins, M., P. de Haan, R. Luyten, M. van Veller, M.Q. J.M. van Ginsven and R. Goldbach. 1995. Broad resistance to tospoviruses in transgenic tobacco plants expressing three tospoviral nucleoprotein gene sequences. *Mol. Plant-Microbe Interact.* **8**: 85-91.

Register, J.C. and R.N. Beachy. 1988. Resistance to TMV in transgenic plants resulted from interference with an early event in infection. *Virology* **166**: 524-532.

Register, J.C. and R.S. Nelson. 1992. Early events in plant virus infection: relationships with genetically engineered protection and host gene resistance. *Sem. Virol.* **3**: 441-451.

Ryu, K.H. and W.M. Park. 1995. Construction of a plant expression vector for the coat protein gene of cucumber mosaic virus-As strain for plant transformation. *Korean J. Plant Pathol.* **11**: 66-72.

Sambrook, J., E.F. Fritsch and T. Maniatis. 1989. *Molecular cloning:a laboratory manual*, 2nd ed. Cold Spring Harbor Laboratory, New York.

Sanford, J.C. and S.A. Johnston. 1985. The concept of pathogen derived resistance. *J. Theor. Biol.* **113**: 395-405.

Van der Vlugt, R.A.A. and R.W. Goldbach. 1993. Tobacco plants transformed with the potato virus Y^N coat protein gene are protected against different PVY isolates and against aphid-mediated infection. *Transgenic Res.* **2**: 109-114.

Van der Vlugt, R.A.A., R.K. Ruiter and R. Goldbach. 1992. Evidence for sense RNA-mediated protection to PVY^N in tobacco plants transformed with the viral coat protein cistron. *Plant Mol. Biol.* **20**: 631-639.

Wilson, T.M.A. 1993. Strategies to protect crop plants against viruses: pathogen-derived resistance blossoms. *Proc. Natl. Acad. Sci. USA* **90**: 3134-3141.

Xue, B., C. Gonsalves, R. Provvidenti, J.L. Slightom, M. Fuchs and D. Gonsalves. 1994. Development of transgenic tomato expressing a high level of resistance to cucumber mosaic virus strains of subgroups I and II. *Plant Dis.* **78**: 1038-1041.

Zaitlin, M., J.M. Anderson, K.L. Perry, L. Zhang and P. Palukaitis. 1994. Specificity of replicase-mediated resistance to cucumber mosaic virus. *Virology* **201**: 200-205.

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