DOI:10.1021/jf903487f

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Allergenicity Assessment of Genetically Modified Cucumber Mosaic Virus (CMV) Resistant Tomato (*Solanum lycopersicon*)

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Cucumber mosaic virus (CMV) has been identified as the causal agent of several disease epidemics in most countries of the world. Insect-mediated virus diseases, such as those caused by CMV, caused remarkable loss of tomato (*Solanum lycopersicon*) production in Taiwan. With expression of the CMV coat protein gene (*Cmvcp*) in a local popular tomato cultivar L4783, transgenic tomato line R8 has showed consistent CMV resistance through T_0 to T_8. In this report, the allergenicity of the CMV coat protein (CMV cp) expressed in transgenic tomato R8 was assessed by investigation of the expression of the transgene source of protein, sequence similarity with known allergens, and resistance to pepsin hydrolysis. There is no known account for either the CMV or its coat protein being an allergen. The result of a bioinformatic search also showed no significant homology between CMV cp and any known allergen. The pepsin-susceptible property of recombinant CMV cp was revealed by a simulated gastric fluid (SGF) assay. Following the most recent FAO/WHO decision tree, all results have indicated that CMV cp was a protein with low possibility to be an allergen and the transgenic tomato R8 should be considered as safe as its host.

KEYWORDS: CMV resistant; safety assessment; allergenicity; genetically modified.

INTRODUCTION

The rapid progress of biotechnology and molecular biology has made genetically modified (GM) crops become a part of agricultural production. From 1996 to 2006, a total of 539 approvals of GM crops have been granted worldwide (*1*). Despite the success of GM crops, safety issues about GM foods are still debated. In order to ensure the safety of GM crops, every novel GM crop has to be thoroughly evaluated before commercialization. Many worldwide organizations such as the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) of the United Nations have suggested general guidelines for GM crop safety assessment. Department of Health (DOH) of Taiwan has also incorporated these general guidelines into GMO safety assessments (*2*).

Cucumber mosaic virus (CMV) is the type member of the genus *Cucumovirus*, family *Bromoviridae* (*3*, *4*). Since its first discovery in Michigan and New York (*5*), CMV has been found in most countries of the world and has been identified as the causal agent of several disease epidemics. Its host range exceeds 800 plant species, making CMV one of the most important viruses for its economic impact. Because of its importance and the absence of resistance genes in the germplasm of most crops, CMV has been one of the primary targets for development of transgene-mediated resistance (*6*). A transgenic plant with coat protein-mediated resistance (CP-MR) to CMV was reported in 1987, the year following the first description of CP-MR (*7*). As for other viruses, CP-MR has been used widely to create CMV resistance, with positive results reported with six plant species (tobacco, cucumber, tomato, melon, squash, and pepper) in more than 20 publications (*6*).

Tomato (*Solanum lycopersicon*) is a popular vegetable in the world. Insect-mediated virus diseases, such as those caused by CMV, not only caused remarkable loss of production but also raised the price of tomatos in Taiwan. Dr. Liu of the Asian Vegetable Development and Research Center (AVDRC) has developed a CMV resistant transgenic tomato. With expression of the CMV coat protein gene (*Cmvcp*) in a local tomato cultivar L4783, transgenic tomato line R8 has shown consistent CMV resistance through T_0 to T_8. The CMV resistant property of transgenic tomato has reduced 10% to 15% the yield loss caused by plant disease in the test field. Preliminary tests showed that there was no significant difference between transgenic tomato line R8 and its wild type host in agronomic properties (*8*). In this study, transgene expression level, source of protein, sequence similarity with known allergens, and pepsin susceptibility of the CMV coat protein were investigated to assess the allergenicity of transgenic CMV resistant tomatos.

MATERIALS AND METHODS

Plant Material. The transgenic tomato line R8 and its wild-type host L4783 were kindly provided by Dr. Liu of AVRDC. Plants were grown in a transgenic greenhouse and a test field of AVRDC for 120 days.

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 Published on Web 01/26/2010

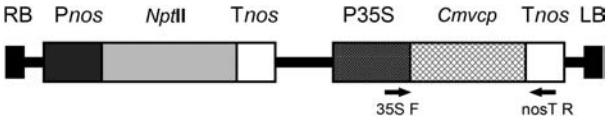

Figure 1. Schematic representation of a T-DNA construct of transgenic tomato R8. RB and LB: right and left T-DNA border. P*nos*: nopaline synthase promoter. *Npt*II: neomycin phosphoesterase II. T*nos*: nopaline synthase terminator. P35S: cauliflower mosaic virus 35S promoter. *Cmvcp*: cucumber mosaic virus coat protein gene.

Table 1. Primers Used in This Study

primer ID	sequence 5′-3′
35S F	CCACAGATGGTTAGAGAGGCTTAC
nosT R	GGCCGTTGCTGTCGTAATGAT
CMVCP RTQF	TTGCCGCCATCTCTGCTATGTT
CMVCP RTQR	GCATCGCCGAGAGATCGTACAA
nptII RTQF	GAAGTGCCGGGGCAGGATCT
nptII RTQR	AGCCGCCGCATTGCATCAG
GAPDH RTQF	AGGGTGGTGCCAAGAAGGTTG
GAPDH RTQR	GGAGACAATGTCCAGCTCTGGC

Isolation of *Cmvcp* Gene in Transgenic Tomato. The isolation of tomato DNA was carried out with a GeneMark Plant Genomic DNA Purification kit (GeneMark Technolgy Co., Ltd., Tainan, Taiwan, ROC). A fresh leaf of tomato was ground in liquid nitrogen, and 100 mg of leaf powder was used for DNA extraction. All DNA samples were quantified using a DNAQF DNA Quantitation Kit (Sigma-Aldrich, St. Louis, MO) based on a fluorescent dye-binding method. The DNA sequence of *Cmvcp* was amplified using primer set 35S F/nosT R (**Figure 1** and **Table 1**). The PCR condition was at 95 °C for 5 min with 35 cycles of 30 s at 95 and 72 °C for 30 s. PCR product was cloned and sequenced using a T&A cloning kit (Yeastern Biotech Co., Ltd., Taipei, Taiwan). The *Cmvcp* sequence expressed in transgenic tomato line R8 was identical to a record of CMV Taiwan isolate (accession number: DQ004597).

Bioinformatic Analysis of CMV cp. A CMV coat protein amino acid sequence derived from the *Cmvcp* DNA sequence (accession number: AAY21160) was used for the database search. The AllergenOnline version 8.0 database (1313 peer reviewed sequences; http://www.allergenonline.com/), the Structural Database of Allergenic Proteins (SDAP; 737 allergen sequences; http://fermi.utmb.edu/SDAP/), and the Allergen Database for Food Safety (ADFS; 2108 registered allergens; http://allergen.nihs.go.jp/ADFS/index.jsp) were used for bioinformatic analysis. A FASTA overall search and a FASTA 80 mer amino acid segment search were performed using the AllergenOnline database. A FAO/WHO allergenicity prediction by 80 mer amino acid FASTA alignment and an 8 contiguous amino acid search were performed using the ADFS and SDAP databases. For the full FASTA overall search, matches of low *E* score values ($< 1 \times 10^{-7}$ for AllergenOnline and < 0.01 for SDAP) and/or greater than 50% identity indicate potential cross-activity (*9*). A FASTA 80 mer amino acid search was performed based on the criterion of 35% identity as a recommendation of Codex (*10*). The criterion of IgE-linear epitopes was 8 contiguous amino acids or longer (*11*−*13*).

Production of Recombinant CMV cp. Although the transgenic tomato R8 expressed consistent CMV resistance, there was no detectable CMV cp on Western blot analysis (data not shown). For a protein whose expression level was below the detection limit of Western analysis, direct purification of CMV cp from transgenic tomato was practically impossible. For this reason, recombinant CMV cp (rCMV cp) was produced using the *Escherichia coli* expression system to provide sufficient protein for the SGF digestibility test.

The *Cmvcp* gene was constructed into a pET32 expression vector (Novagen, Madison, WI) and transformed into *E. coli* BL21(DE3) as the host for expression of rCMV cp. The incubation and induction procedure for protein expression was as follows: 10 mL of Luria−Bertani (LB) broth (20 g of tryptone, 10 g of yeast extract, and 20 g of NaCl per liter) containing 50 μg/mL of kanamycin was inoculated with a bacterial colony and incubated overnight at 37 °C with 125 rpm shaking. An overnight seed culture of *E. coli* was subsequently transferred into 100 mL of LB broth containing 50 μg/mL of kanamycin and incubated at 37 °C with 125 rpm shaking. As the culture density reached an OD_{600} of 0.7−0.8, isopropyl β-D-1-thiogalactopyranoside (IPTG) was added to the final concentration 1 mM and subsequently incubated for 6 h to induce rCMV cp expression. After incubation, the *E. coli* cells were harvested by centrifugation at 6,000*g* for 15 min and frozen at −20 °C.

Purification and Identification of rCMV cp. The pellet of recombinant *E. coli* was resuspended in lysis buffer (50 mM sodium phosphate, 0.5% Triton X-100, 0.5% Na deoxycholate, 0.1 M NaCl, and 1 mM 1,4-dithioerythritol (DTT) at pH 7.5) and adjusted to the final $OD_{600} = 200$. Bacterial cells were subsequently disrupted on ice with a sonicator (XL-2020, MISONIX, Farmingdale, NY) for a total of 15 min (15 s pulse and 10 s pause time). The insoluble fraction of the recombinant *E. coli* cell lysate was collected as inclusion bodies (IBs) by centrifugation at 12,000*g* for 30 min and washed with lysis buffer twice. Inclusion bodies of rCMV cp were further redissovled in 8 M urea solution and diluted slowly to a final urea concentration of 4 M. The pellet was discarded after centrifugation at 12,000*g* for 30 min, and rCMV cp was precipitated from supernatant by adding an equal volume of 50% polyethylene glycol (PEG) 4000. A pellet of rCMV cp was redissovled in 50 mM sodium phosphate with 0.1% Triton X-100 and concentrated using an Amicon Ultra-15 (MWCO 30 kDa; Millipore, Billerica, MA) device according to the manufacturer's instructions. Protein samples were quantified by the Bradford method (*14*) and subjected to 10% SDS-PAGE separation according to the method of Laemmli (*15*). After electrophoresis, gel was stained with 0.1% Coomassie brilliant blue R-250 for 15 min and destained in 25% methanol with 7.5% glacial acetic acid. The purity of rCMV cp was determined by image analysis using software ImageJ version 1.37 (*16*). The band of rCMV cp on SDS-PAGE was excised and LC-MS MS sequenced by Mission Biotech Co. Ltd. (Taipei, Taiwan). The probability based MOWSE score was used to identify the protein sequence (*17*). For Western blot detection of rCMV cp, the gel was equilibrated in transfer buffer, and then proteins were transferred to a PVDF membrane, which were blocked with 1% skimmed milk and washed twice. Rabbit polyclonal antibodies were raised against rCMV cp purified from recombinant *E. coli*. Membrane was developed with a Chemilucent ECL Detection System (Millipore) and detected by a BioSpectrum Imaging System (UVP, Upland, CA). For N-terminal sequencing, rCMV cp was transferred to a PVDF membrane and stained with Amindo Black 10B. The band of rCMV cp on the membrane was excised for N-terminal sequencing analysis (Mission Biotech Co. Ltd.).

Production of Monoclonal Antibody against rCMV cp. The concentration of purified rCMV cp was diluted to 500 μg/mL with adjuvant (10% aluminum potassium sulfate solution). BALB/c mice were immunized by intraperitoneal injection at two week intervals. After three time immunizations, the mice were given a final booster with antigen complex five days before the fusing program. Splenocytes (1×10^8) were fused with $(1-2) \times 10^7$ X63 myeloma in 50% PEG solution. Hybridoma cells were selected using HAT medium (20% FCS RPMI1640, 10 mM hypoxanthanine, 40 mM aminopterin, and 1.6 mM thymidine). After 7−14 days, when the clones were visible, ELISA was performed to test all wells. The positive wells were then subcloned using the limit dilution method and then the positive clones were frozen and stored.

Determination of CMV cp Expression Level using ELISA. Mouse anti-rCMV cp mAb (100 μL, 1:100 dilution) was coated on a 96-well microtiter plate at 4 °C overnight. The plates were then washed with PBS-T buffer (PBS with 0.5% Tween 20) three times and incubated with blocking buffer (PBS-T with 10% BSA) at 37 °C for 1 h. The plates were washed three times with PBS-T, and the sample (supernatant of homogenized tomato fruits) was added at the corresponding dilution and incubated at 37 °C for 1 h. After washing three times, the plates were incubated with 100 μL of biotinylated anti-rCMV cp mAb (1:100) at 37 °C for 1 h. The plates were then washed three times with PBS-T buffer and incubated with 100 μL of avidin-horseradish peroxidase (1:500) at 37 °C for 1 h. After washing seven times, the reaction was developed with H_2O_2 and 0.05% 2,2′-azinobis-3-enthylbenzthioazoline-6-sulfonic acid (ABTS) for 30 min. The absorbance was measured at 405 nm (OD_{405}).

Transgene mRNA Expression Analysis. The transgene expression level was evaluated by quantitative reverse transcriptase PCR (qRT-PCR). The glyceraldehyde 3-phosphate dehydrogenase (*Gapdh*) gene was used as a housekeeping gene to normalize the expression level of transgene. The tomato *Gapdh* gene sequence (accession number: U97257.1) was used to

Table 2. Most Relevant Records of the Similarity Search of the Allergen Protein Sequence

allergen name	organism	accession no.	database[a]	identity, %	*E* score[b] (full FASTA)	description
Dol m 5	*Dolichovespula maculata* (Hornet)	P10736	AO	20.61	1.9	venom allergen 5.01
			SD	12.39	>0.01	
Cla h 4	*Davidiella tassiana*	P40918	AO	23.35	2.4	heat shock 70 kDa protein
			SD	19.72	>0.01	
Der p 8	*Dermatophagoides pteronyssinus* (home dust mite)	P46419	AO	40.47	3.5	glutathione transferase μ class
			SD	10.55	>0.01	
Der p 15	*Dermatophagoides pteronyssinus* (home dust mite)	AAX37326	AO	40.47	3.5	glutathione transferase homologues
Ory s TAI	*Oryza sativa* (rice)	BAA07710	AO	30.00	6.0	α-amylase/trypsin inhibitor
			SD	10.55	>0.01	
Cyp c 1.01	*Cyprinus carpio* (common carp)	CAC83658	SD	8.72	>0.01	parvalbumin
Ani s 4	*Anisakis simplex* (herring worm)	P16347	AO	34.21	3.8	allergen

[a] Database: "AO" = AllergenOnline; "SD" = SDAP. [b] *E* score $<1 \times 10^{-7}$ indicates significant homology for the AllergenOnline database.

design the primer set GAPDH QF/QR. The expression level of the CMV coat protein was determined using the primer set CMVCP RTQF/RTQR, which designed according the *Cmvcp* sequence isolated from transgenic tomato line R8. The expression level of the *Npt*II gene was determined using the primer set nptII RTQF/RTQR, which designed according to the *Npt*II sequence isolated from transgenic tomato line R8. Total RNA was extracted from 0.25 g of both transgenic and wild-type tomato leaf using Plant RNA Purification reagent (Invitrogen) under RNase-free conditions. Reverse transcription was carried out using a SuperScript III Platinum Two-Step qRT-PCR Kit (Invitrogen), and 2 μg of tomato total RNA was used as template. Reverse transcription was carried out using oligo dT_{20} primer under the conditions suggested by the manufacturer. Real-time PCR was carried out using an ABI Prism 7700 sequence detection system (Life Technologies Corporation, Carlsbad, CA). The real-time PCR reaction was carried out in a 20 μL final assay volume that contained 1X Platinum Quantitative Supermix-UDG (Invitrogen), 0.5 μL of cDNA, and 100 nM of each primer. For real-time PCR, the following program was used: 2 min at 50 °C, 10 min at 95 °C, and 40 cycles of 15 s at 95 °C and 1 min at 60 °C. Triplicate PCR was performed for each sample.

Stimulated Gastric Fluid (SGF) Assay. To validate the method we used in this study, bovine serum albumin (BSA) and soybean trypsin inhibitor (STI) were subjected to SGF as pepsin susceptible and pepsin resistant control. The SGF (0.084 N HCl, 35 mM NaCl, pH 1.2, and 2400 U pepsin (#6887; Sigma-Aldrich) within total volume 1.52 mL) was prepared as previously described (*18*). The concentration of all protein samples (purity > 85%) was adjusted to 5 mg/mL. Eighty microliters of protein sample was mixed with 1.52 mL of SGF prewarmed at 37 °C to make the final ratio of pepsin to sample protein 6000 U/mg. The SGF reaction mixture was incubated at 37 °C for 0, 0.5, 2, 5, 10, 30, and 60 min. At each time point, 200 μL of SGF reaction mixture was mixed with 75 μL of 0.2 M Na_2CO_3 (pH 11.0) and 75 μL of 5× SDS sample buffer immediately to terminate the reaction. Samples (16 μL) from each time point were subjected to 12.5% SDS-PAGE separation. After electrophoresis, gel was stained with 0.1% Coomassie brilliant blue R-250 for 15 min and destained in 25% methanol with 7.5% glacial acetic acid.

RESULTS

Bioinformatic Analysis of rCMV cp. Although CMV has been identified as the causal agent of several disease epidemics in most countries of the world, there are no known accounts of CMV or its coat protein being an allergen. Cheng and Peng (*19*) have carried out a field survey of tomato virus diseases including those caused by Tomato Mosaic Virus (ToMV), Tomato Yellow Leaf Curl Virus (TYLCV), Cucumber Mosaic Virus (CMV), Potato Virus Y (PVY), and Tomato Spotted Wilt Virus (TSWV), in the tomato production area of southwest Taiwan. Most tomato samples have one or more virus diseases. In this survey, 71.85% of tomato plants were infected by the Cucumber Mosaic Virus. Since tomato may fruit under the infection of CMV in the reproductive phase, the high infect ratio of CMV has implied that the probability of consumption of tomatoes carrying CMV by humans was relatively high. For this reason, the protein CMV cp could be considered as safe in history.

The CMV cp amino acid sequence (accession number: AAY21160), which contains 218 of amino acids, was subjected to a sequence search. There was no positive result of the CMV cp protein in an 8-mer search against the SDAP and ADFS databases. In the 80-mer amino acid FASTA search of sporamin protein, no positive record was identified from the AllergenOnline v8.0 database, the ADFS database, and the SDAP database. In the full FASTA search of the sporamin protein, there was also no record identified in the AllergenOnline database and the SDAP database. The three most relevant allergen records in the full FASTA search were Dol m 5, Cla h 4, and Der p 8 with *E* scores of 1.9, 2.4, and 3.5, respectively. In summary, none of allergens recorded with significant homology to CMV cp were identified in all of the search results from allergen databases. The low similarity between CMV cp and known allergens has indicated the criteria for suspected cross-activity were not reached (table **2**). This demonstrates that there is not expected to be any significant risk of cross-reactivity for those who are allergic to known allergens.

Purification and Identification of rCMV cp. Due to the fact that the expression of transgenic CMV cp in tomato was a trace amount, recombinant CMV cp produced in the *E. coli* expression system was used as an alternate in the SGF test. The rCMV cp was expressed in *E. coli* BL21(DE3) mainly as inclusion bodies (IBs). The strong aggregative trend of rCMV cp makes it easy to precipitate and could not be purified using conventional chromatography methods such as hydrophobic interaction, gel filtration, or ion-exchange (data not shown). Purification of rCMV cp was achieved by slow dilution of IBs solution and subsequent precipitation with PEG 4000. After redissolution and concentration, the final purity of rCMV cp was 87.76%. Purified protein was confirmed by Western blot analysis using rabbit polyclonal anti-rCMV cp antibodies (**Figure 2**).

To ensure the equivalence of rCMV cp to native protein, LC/MS/MS and N-terminal sequence analysis of purified rCMV cp was carried out. Purified rCMV cp was LC/MS/MS identified as CMV coat protein (accession number: AAY21160) with a significant score of 625 (individual ion scores >42 indicate identity or extensive homology ($P < 0.05$)) with 65% coverage. The result of the N-terminal sequencing also matched the record of the CMV coat protein (accession number: AAY21160).

Expression of CMV cp in Transgenic Tomato R8. An ELISA method using mouse anti-rCMV cp mAb was established to determine the expression level of CMV cp in tomato. The linear range of this ELISA method was from 0.03 to 10 μg/mL ($r^2 = 0.994$). Fruits of wild-type tomato L4783 were used to determine the background level of this method. The expression level of CMV cp in transgenic tomato fruit was 0.159 ± 0.078 μg/mL, which equals 0.016% of total protein. Although the expression level of CMV cp protein was trace, the result of mRNA expression analysis has

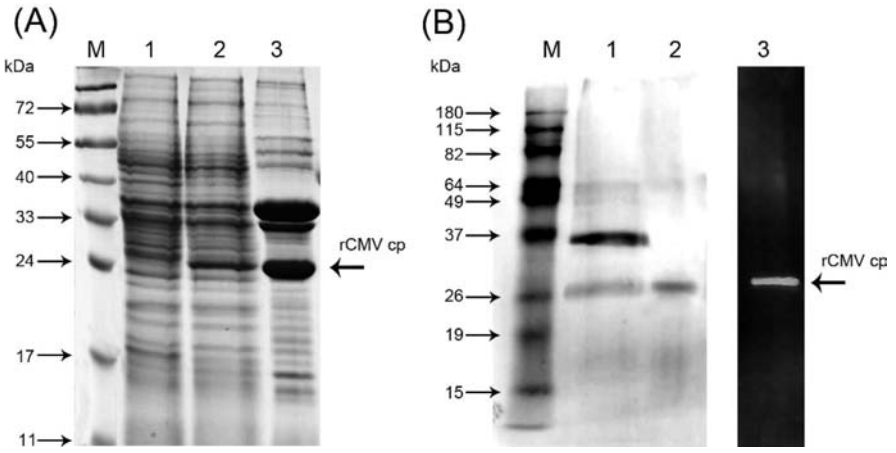

Figure 2. Purification of rCMV cp. (**A**) SDS-PAGE analysis of rCMV cp expressed in *E. coli.* Lane 1: no IPTG induction. Lane 2: 1 mM IPTG induction. Lane 3: inclusion bodies. (**B**) Purified rCMV cp. Lane 1: supernatant of IBs solution after dilution. Lane 2: purified rCMV cp. Lane 3: Western blot analysis of rCMV cp.

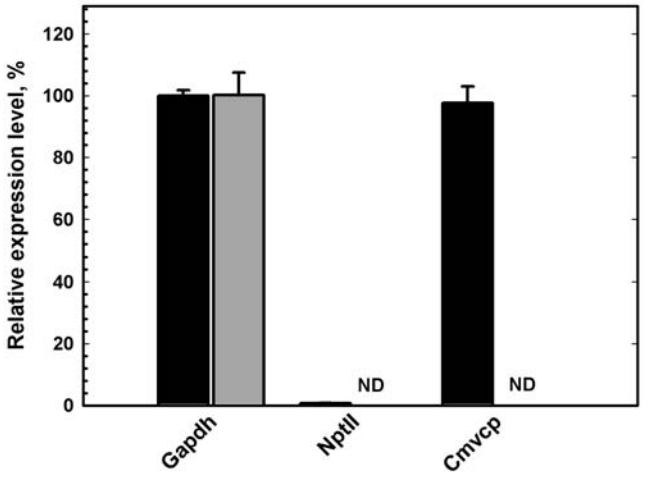

Figure 3. Transgene expression level in transgenic tomato R8. Black bar: transgenic tomato R8. Gray bar: wild-type tomato L4783 for negative control. Gapdh: glyceraldehyde 3-phosphate dehydrogenase. NptII: neomycin phosphoesterase II. Cmvcp: Cucumber Mosaic Virus coat protein gene. ND: not detected.

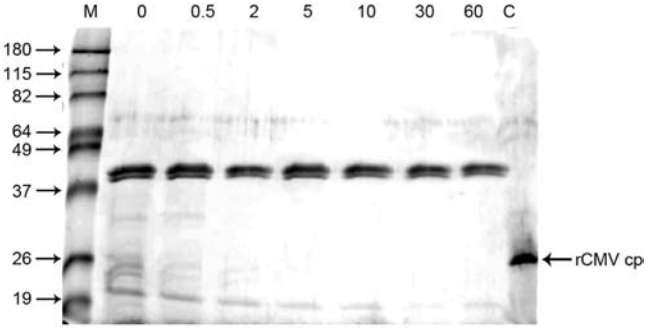

Figure 4. Simulated gastric fluid assay of rCMV cp. Lane 1−7: rCMV cp with SGF digestion for 0, 0.5, 2, 5, 10, 30, and 60 min. Lane C: rCMV cp control (without SGF).

shown that the expression level of *Cmvcp* mRNA was 97.6% of the housekeeping gene *Gapdh* (**Figure 3**), which indicates a relatively high expression level of *Cmvcp* mRNA in transgenic tomato R8.

Simulated Gastric Fluid Assay of rCMV cp. The ability of food allergens to reach the intestine is a prerequisite to allergenicity. This ability necessarily implies survival to gastric digestion in the stomach. The digestibility of rCMV cp was tested by the SGF assay. To validate the SGF method we used in this study, one of each well-known pepsin-susceptible (BSA) and resistant (STI) protein was first subjected to the SGF test. As expected, BSA degraded rapidly in SGF (< 30 s), and STI showed significant resistance to digestion (> 60 min) (data not shown). In the case of rCMV cp, the result of the SGF assay was shown in **Figure 4**. The band of rCMV cp that disappeared within 30 s on SDS-PAGE has demonstrated that rCMV cp degraded in SGF rapidly. This result has shown that rCMV cp is susceptible to pepsin digestion, which indicates less possibility to be an allergen (*20*, *21*).

DISCUSSION

With the development of genetically modified crops, there has been a growing interest in the approaches available to assess the potential allergenicity of novel gene products. To provide assurance that a novel protein is not a potential allergen, approaches have been proposed that are based on a decision tree (*22*−*24*). The key feature of such a decision tree is that it takes into consideration multiple features of the protein, thus enabling a judgment to be made on the probability of a protein being allergenic. Here, we have focused on the source of the protein, sequence similarity with known allergens, and resistance to pepsin hydrolysis.

Cucumber mosaic virus (CMV) has been found in most countries of the world and has been identified as the causal agent of several disease epidemics. Insect-mediated virus diseases, such as those caused by CMV, caused remarkable loss of tomato production in Taiwan. With expression of the CMV coat protein gene (*Cmvcp*) in a local tomato cultivar L4783, transgenic tomato line R8 has shown consistent CMV resistance. Although CMV has been identified as the causal agent of several disease epidemics, there are no known accounts either of the CMV or its coat protein being an allergen. The amino acid sequence of CMV cp (accession number: AAY21160) expressed in transgenic tomato R8 was subjected to a sequence search. No allergen record with significant homology to CMV cp was identified using Full FASTA, 80 mer FASTA, and 8-mer contiguous amino acid search methods from allergen databases including AllergenOnline, SDAP, and ADFS. This demonstrates that there is not any significant risk of cross-reactivity expected for those who are allergic to known allergens.

Although the expression level of *Cmvcp* mRNA was comparable to that of the housekeeping gene *Gapdh*, the expression level of CMV cp protein in tomato fruit was very low (0.016% of total

protein, undetectable by Western blot). Due to the low expression of CMV cp protein, the mechanism of CMV resistance will less possible to be coat protein mediated protection (CP-MR). Instead of protein mediated virus resistance, RNA related post-transcriptional gene silencing (PTGS) may contribute to the virus resistance. RNAi is the most possible mechanism among mechanisms of PTGS, since there was significant mRNA expression in the transgenic tomato R8 (*25*). The low abundance of CMV cp in transgenic tomato R8 may indicate lower safety risk as the consequence of diminished human exposure. Due to the trace expression of transgenic CMV cp in tomato, recombinant CMV cp was produced and purified using the *E. coli* expression system as an alternate for the SGF test. The equivalence of rCMV cp and native protein was proved by LC/MS/MS and N-terminal sequence analysis. The rapid degradation of rCMV cp in SGF has demonstrated that rCMV cp is susceptible to pepsin digestion, which also indicates less possibility to be an allergen.

In this report, the allergenicity of CMV cp expressed in transgenic tomato R8 has been assessed using the most recent FAO/WHO decision tree (*22*, *23*). The expression of transgene, source of protein, sequence similarity with known allergens, and resistance to pepsin hydrolysis were investigated. Following the decision tree, all results have indicated that CMV cp has a low possibility of being allergenic and that transgenic tomato R8 should be considered as safe as its host.

ACKNOWLEDGMENT

This work was supported by the Department of Health of Taiwan, ROC (project code: DOH95-FS031).

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Received for review October 3, 2009. Revised manuscript received December 5, 2009. Accepted December 14, 2009.