Safety Assessment and Detection Method of Genetically Modified Chinese Kale (*Brassica oleracea* cv. *alboglabra*)

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Sporamins are tuberous storage proteins and account for 80% of soluble protein in sweet potato tubers with trypsin-inhibitory activity. The expression of sporamin protein in transgenic Chinese kale (line BoA 3-1) conferred insecticidal activity toward corn earworm [*Helicoverpa armigera* (Hübner)] in a previous report. In this study, we present a preliminary safety assessment of transgenic Chinese kale BoA 3-1. Bioinformatic and simulated gastric fluid (SGF) analyses were performed to evaluate the allergenicity of sporamin protein. The substantial equivalence between transgenic Chinese kale and its wild-type host has been demonstrated by the comparison of important constituents. A reliable real-time polymerase chain reaction (PCR) detection method was also developed to control sample quality. Despite the results of most evaluations in this study being negative, the safety of sporamin in transgenic Chinese kale BoA 3-1 was uncluded because of the allergenic risk revealed by bioinformatic analysis.

KEYWORDS: Sporamin; safety assessment; real-time PCR; allergenicity

INTRODUCTION

The rapid progress of biotechnology and molecular biology has made genetically modified (GM) crops a practical 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 of GM foods is still in debate. To ensure the safety of GM crops, every novel GM crop event 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. The Department of Health (DOH) of Taiwan has also included these general guidelines into their safety assessment (*2*).

Sporamins are tuberous storage proteins and account for 80% of soluble protein in sweet potato tubers (*3*). Sporamin genes belong to a gene family with more than 10 members. On the basis of nucleotide homology, they can be grouped into two gene subfamilies, *sporamins* A and B (*4*). A *sporamin* A subfamily gene SpTI-1 was isolated from sweet potato. The

SpTI-1 product functions as a serine protease inhibitor (Kunitz type) with trypsin-inhibitory activity (*5, 6*). The protein inhibitor is an important element of natural plant defense strategies (*7*). Protein inhibitors are usually nonphytotoxic but either toxic or reducing the digestibility of the nutrient for pests. The defensive role of *sporamin* in protecting plants from herbivorous damage was confirmed in transgenic tobacco, which after transformation with the *SpTI*-1 gene displayed resistance to tobacco cutworm (*Spodoptera litura*) (*8*). The *SpTI*-1 expressed in Taiwan cauliflower (*Brassica oleracea* var. *botrytis* L.) cultivars, which resulted in transgenic plants showing resistance to *Spodoptera* spp. (*9*). Other studies have shown that sporamin inhibits the development of cyst nematodes in transgenic sugar beet hairy root (*10*).

Chinese kale (*B. oleracea* cv. *alboglabra*) and many other *Brassiceae* are popular vegetables in Taiwan. Chen et al. constructed a transgenic Chinese kale expressing the *sporamin* gene with a P*spoa* promoter and *spo*MAR region (**Figure 1**). This transgenic Chinese kale (line BoA 3-1) expressed a constant level of sporamin and showed insecticidal activity from the feeding test of corn earworm [*Helicoverpa armigera* (Hübner)] (*11*). The broad insecticidal activity of transgenic plants with sporamin expression has implied commercial potential. In this study, the allergenicity of transgenic-Chinese-kale-expressed sporamin was investigated by a bioinformatic database search and simulated gastric fluid (SGF) digestibility test. The sub-

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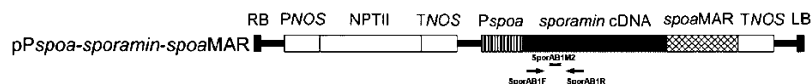
Figure 1. Schematic representation of the T-DNA construct of transgenic Chinese kale BoA 3-1. NPTII, neomycin phosphoesterase II; PNOS, nopaline synthase promoter; P*spoa*, *sporamin* promoter; *spoa*MAR, sporamin matrix attached region-like segment; TNOS, nopaline synthase terminator; RB and LB, right and left T-DNA border.

Table 1. Primers and Probes Used in This Study

primer ID	sequence 5′–3′	target gene	amplicon size (bp)	reference
qHMG R	GGTCGTCCTCCTAAGGCGAAAG			
qHMG F	CTTCTTCGGCGGTCGTCCAC			
qHMG-P	FAM-CGGAGCCACTCGGTG-CCGCAACTT-TMARA	*hmg*I/Y	99	*12*
Spor-AB1F	GCCTCCTCTGAAACTCCAGTAC			
Spor-AB1R	GGCGGAGACCATGTAGTAGTTC			
Spor-AB1M2	FAM-CTCGTCGCCGTTGATG-TAMRA	*sporamin*	78	this study

stantial equivalent between transgenic and wild-type Chinese kale was also evaluated by major nutrient analysis.

The detection method of GM crops is essential to provide sufficient product information for consumers and regulation. To trace and control the material, we used safety tests and also developed both qualitative and quantitative real-time polymerase chain reaction (PCR) detection methods for the sporamin gene in the transgenic Chinese kale.

MATERIALS AND METHODS

Plant Material. The transgenic Chinese kale line BoA 3-1 and its wild-type host Boa 2301 seeds were kindly provided by professor Kai-Wen Yeh of the National Taiwan University. Plants were grown under white florescent light (28 μmol m^{-2} s^{-1}) with 16 h of light/8 h of dark photoperiod at 25 °C for 50 days.

Isolation and Quantification of Genomic DNA. The isolation of kale DNA was carried out by the GeneMark plant genomic DNA purification kit (GeneMark Technolgy Co., Ltd., Tainan, Taiwan). Fresh leaves of kale were ground in liquid nitrogen, and 100 mg of leaf powder was used for DNA extraction. All DNA samples were quantified using the DNAQF DNA quantitation kit (Sigma-Aldrich, St. Louis, MO) based on the fluorescent-dye-binding method.

Primer Design and DNA Sequencing. Qualitative and quantitative PCR primers and probes were designed according to the sporamin gene sequence (accession number IBU12436) by software Vector NTI, version 9.0. High-mobility-group protein I/Y (*hmg*I/Y) was used as an endogenous reference gene for real-time PCR analysis (*12*). The sequences of primers and probes used in this study are presented in **Table 1**.

Qualitative and Quantitative Real-Time PCR Analysis. Qualitative PCR was carried on a Thermo PCR Sprint thermocycler (Thermo Scientific, Waltham, MA). PCR amplifications were performed in a final volume of 50 μL. Each reaction mixture had 1× PCR buffer, 50 μM dNTPs, 200 nM of each primer, 100 ng of DNA sample, and 0.5 unit of Super-Therm DNA polymerase (Bertec Enterprise, Taipei, Taiwan). The following program was used for qualitative PCR: 5 min at 95 °C, 35 cycles of 30 s at 95 °C, and 30 s at 72 °C. The PCR product was analyzed by 1.5% agarose electrophoresis. Real-time PCR was performed on an ABI 7700 sequence detection system (Applied Biosystems, Foster, CA). The real-time PCR reaction was carried out in a 20 μL final assay volume and contained 1× TaqMan Universal Master Mix, sample DNA, 100 nM of each primer, and 200 nM of probe. For real-time PCR, the following program was used: 2 min at 50 °C, 10 min at 95 °C, 40 cycles of 15 s at 95 °C, and 1 min at 60 °C. Triplicate PCR was performed for each sample.

Bioinformatic Analysis of Sporamin. The sporamin amino acid sequence (accession number AAA86670) was used for the database search. The AllergenOnline version 8.0 database (1313 peer-reviewed sequences; http://www.allergenonline.com/), Structural Database of

Allergenic Proteins database (SDAP; 737 allergen sequences; http://fermi.utmb.edu/SDAP/), and the Allergen Database for Food Safety database (ADFS; 2108 registered allergens; http://allergen.nihs.go.jp/ADFS/index.jsp) were used for bioinformatic analysis. FASTA overall search and FASTA 80-mer amino acid segment search were performed using AllergenOnline database. FAO/WHO allergenicity prediction by 80-mer amino acid FASTA alignment and 8 contiguous amino acid search were performed using ADFS and SDAP databases. For the full FASTA overall search, matches of low E score values ($<1 \times 10^{-7}$) and/or greater than 50% identity were used as an indication of potential cross-activity (*13*). The FASTA 80-mer amino acid search was performed on the basis of the criterion of 35% identity as a recommendation of Codex (*14*). The criterion of IgE-linear epitopes was 8 contiguous amino acids or longer (*15*–*17*).

Nutrient Analysis. Compositional analyses were conducted to measure the moisture content, total fat, total protein, total carbohydrate, ash content, dietary fibers, and vitamins A, C, B$_6$, and B$_{12}$. A total of 10 of plants were used for compositional analysis for each group (WT and GM), and triplicate tests were performed for every plant. The moisture content was determined as a constant weight loss under 110 °C (*18*). The total fat was extracted using the Soxhlet apparatus with diethyl ether and determined by the weight of extract (*19*). The total protein was determined by Kjeldahl total nitrogen analysis (*20*). The ash content was determined by the weight of residue after 550 °C heating in an oven for 16 h (*21*). The dietary fiber content was determined by the weight difference of ash and protein content in the sample residue of α-amylase, protease, and amyloglucosidase digestion (*22*). The total carbohydrate content was calculated by the difference from the total fat, protein, moisture content, and ash (*23*). Vitamin A was determined by a modified Carr–Price method, in which Vitamin A–CHCl$_3$ solution reacts with SbCl$_3$ solution and shows a blue color, and then the absorbance at 620 nm is measured with a spectrophotometer (*24*). Vitamins B$_6$ and B$_{12}$ were determined by a microbioassay using *Lactobacillus delbrueckii* subsp. *lactis* ATCC 7830 (BCRC 11051) based on the AOAC 961.15 (*25*) and AOAC 952.20 (*26*) methods, respectively. Vitamin C was determined by the 2,6-dichloroindophenol titrimetric method (*27*).

Stimulated Gastric Fluid (SGF) Assay and Western Analysis. GM Chinese kale leaf was wounded by a surgical blade at 30 min before harvest to induce sporamin expression. To investigate the stability of sporamin in transgenic Chinese kale in routine food processes, both fresh and cooked (5 min in boiling water) Chinese kale were evaluated with the SGF assay. The sample tissue was ground into fine powder with liquid nitrogen, and then a protein extraction buffer [50 mM Tris-HCl, 0.05% phenylmethylsulfonyl fluoride (PMSF), 1% 2-mercaptoethanol, 0.2% Triton X-100, 10% sucrose, 0.3 M NaCl, and 10 mM L-ascorbic acid at pH 8.0] was added with gentle shaking (3:1 buffer/sample). The crude extract was centrifuged twice at $10,000 \times g$ at 4 °C for 15 min to completely remove plant tissue debris, and the total amount of protein was quantified by the Bradford method (*28*).

The SGF [0.084 N HCl, 35 mM NaCl at pH 1.2, and 4000 units of pepsin (6887; Sigma-Aldrich, St. Louis, MO) within a total volume of 1.52 mL] was prepared as previously described (*29*). For each SGF test, 500 μg of crude protein extract was mixed with 200 μL of SGF and incubated at 37 °C for 0, 2, 4, and 8 min. To terminate the reaction, 75 μL of 0.2 M Na$_2$CO$_3$ (pH 11.0) was added to the SGF reaction mixture (*30*). Samples (16 μL) from each time point were subjected to 12.5% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) separation according to the method of Laemmli (*31*). 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. For Western blot detection of sporamin, the gel was equilibrated in transfer buffer and then

Table 2. Repeatability Analysis of Serial Copy Numbers of pSpor and pHMG by the TaqMan Quantitative System

true copy number	Ct 1	Ct 2	Ct 3	mean Ct	mean copy number	SD
			pSpor			
5000000	15.08	15.09	15.11	15.09	4995355.61	0.02
500000	18.62	18.54	18.55	18.58	508682.04	0.04
50000	22.29	22.08	22.04	22.14	49259.53	0.13
5000	25.83	25.89	25.61	25.78	4526.35	0.15
500	29.35	29.19	29.09	29.21	476.29	0.13
50	32.44	32.60	32.16	32.40	58.79	0.22
			pHMG			
5000000	15.33	15.29	15.43	15.35	4923630.70	0.07
500000	18.67	18.82	18.74	18.74	515034.90	0.08
50000	22.34	22.13	22.13	22.20	51652.18	0.12
5000	25.75	25.72	25.56	25.68	5111.66	0.10
500	28.95	29.41	29.04	29.13	512.64	0.24
50	32.32	32.75	32.86	32.64	49.62	0.29

Table 3. Accuracy and Precision Statistics for TaqMan Quantitative System[a]

		*sporamin/hmg*I/Y ratio						
			mean		accuracy		precision	
true value (%)	coefficient value	1	2	3	mean value (%)	bias (%)	SD_R	RSD_R (%)
5.0	0.91	4.34	4.29	4.37	4.76	−4.76	0.04	0.85
3.0		2.67	2.78	2.33	2.85	−5.01	0.23	8.23
1.0		0.89	0.73	0.72	0.86	−14.29	0.10	11.13
0.5		0.32	0.42	0.25	0.36	−27.47	0.09	23.56
0.0		0.00	0.00	0.00	0.00	NA	NA	NA

[a] NA, not available; RSD_R, reproducibility.

proteins were transferred to a polyvinylidene difluoride (PVDF) membrane, which were blocked with 1% skimmed milk and washed twice. Rabbit polyclonal antibodies were raised against sporamin purified from recombinant *Escherichia coli*. The membrane was developed with 5-bromo-4-chloro-3-indolyl phosphate-toluidine salt (BCIP) and *p*-nitroaziltetrazolium chloride (NBT) substrates, until color appeared.

RESULTS

Real-Time PCR Quantification System of Transgenic Chinese Kale. A real-time PCR quantification system specific for the sporamin gene inserted in the BoA 3-1 transgenic Chinese kale was developed on the ABI PRISM 7700 sequence detection system instrument. The probe (Spor-AB1M2) and primers (Spor-AB1F and Spor-AB1R) were designed specifically to the sporamin gene sequence (accession number IBU12436). The high-mobility group gene (*hmg*I/Y) of *Brassica napus* was adopted as a real-time PCR reference gene (*32*). Standard curves of real-time PCR using a serial dilution of plasmids were constructed for both reference (plasmid pHMG) and sporamin (plasmid pSpor) genes. Linearity was assessed for both the genomic DNA samples and the plasmid standard curve. The range of the plasmid DNA samples was defined between 50 and 5 000 000 copies. The correlation coefficient (r^2) of the regression line was >0.993, whereas the range of genomic DNA samples was defined between 0.5 and 5%. The regression correlation between plasmid and genomic DNA standard curves was >0.990. Repeatability, accuracy, and precision of this quantification system were also tested (**Tables 2** and **3**). The PCR efficiency of sporamin and *hmg*I/Y gened was 92.47 and 95.51%, respectively. The coefficient value between sporamin and hmg I/Y genes was 0.91, and the bias ranged from −4.76 to −27.47%, with 5.0−0.5% transgenic DNA content. According to the approach suggested by Codex, the limit of detection (LOD) should correspond to the lowest level of analyte, for which the relative standard deviation for reproducibility (RSD_R) is 33% or less, and the limit of quantification (LOQ) should correspond to the lowest level of analyte, for which the RSD_R is 25% or less (*33*). The RSD_R of the lowest concentration level (0.5%) in this study was below the 25% criteria (23.56%). In conclusion, according to the Codex Alimentarius guidelines, both the LOD and LOQ of this method were <0.5%, which is a feasible level for GM crop detection. All of materials used in this study were qualified with this detection system to ensure quality.

Bioinformatic Analysis of Sporamin. Sweet potato has a history of safe use in humans because it is widely consumed, especially in Africa. Despite being widely harvested for food, published allergic reactions to sweet potato are rare. There is only one report about allergic reactions following consumption of sweet potato from three patients. The allergenic bands founded in the IgE serum test were 54−64, 19−24, and 74 kD (*34*). There are no known accounts of the storage protein sporamin being an allergen.

The sporamin cDNA sequence (accession number IBU12436), which contains 219 amino acids, was subjected to sequence search. There was no positive result of the sporamin protein in the 8-mer search against SDAP and ADFS databases. In the 80-mer amino acid FASTA search of sporamin protein, five positive records were identified from the AllergenOnline version 8.0 database, nine positive records were identified from the ADFS database, and two positive records were identified from the SDAP database. In the full FASTA search of sporamin protein, eight positive records were identified in the AllergenOnline database, two positive records were identified from the ADFS database, and six positive records were identified in the SDAP database, with an *E* score < 1 × 10⁻⁷. In summery, nine allergen records with significant homology to sporamin were identified from three allergen databases (**Table 4**). All of the allergenic proteins with positive results were protease (trypsin) inhibitors, similar to the function of sporamin protein.

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Table 4. Positive Records of the Similarity Search of the Allergen Protein Sequence[a]

allergen name	accession number	database	maximum 80-mer similarity (%)	E score (full FASTA)	description
Sola t 4	P30941	AO	37.00	2.5×10^{-11}	serine protease inhibitor
		AD	NA	7.7×10^{-9}	
		SD	—	2.9×10^{-11}	
	CAA45723	AO	37.00	1.1×10^{-11}	aspartic protease inhibitor
		AD	NA	7.9×10^{-9}	
		SD	37.50	1.3×10^{-11}	
Gly m TI	P01070	AO	35.30	1.7×10^{-10}	Kunitz-type trypsin inhibitor
		AD	NA	1.4×10^{-7}	
		SD	38.75	1.9×10^{-10}	
	Q39898	AO	35.30	1.7×10^{-10}	trypsin inhibitor A
		AD	NA	1.4×10^{-7}	
	Q39869	AO	—	1.4×10^{-9}	Kunitz-type trypsin inhibitor precursor
		AD	NA	1.6×10^{-7}	
		SD	—	1.5×10^{-9}	
	Q39899	AO	36.50	3.3×10^{-10}	trypsin inhibitor subtype B
		AD	NA	1.6×10^{-7}	
	P25272	AO	—	1.6×10^{-10}	Kunitz-type 1 trypsin inhibitor precursor
		AD	NA	2.0×10^{-6}	
		SD	—	1.9×10^{-10}	
Sola t 3	P20347	AO	—	7.2×10^{-9}	serine protease inhibitor II
		AD	NA	6.2×10^{-5}	
		SD	—	7.6×10^{-9}	
Tri a F073/F170	P16347	AD	NA	1.1×10^{-8}	α-amylase and trypsin inhibitor

[a] Database: AO, AllergenOnline; AD, ADFS; and SD, SDAP. Similarity: NA, not available; —, negative result.

These nine positive records belong to four allergen types: *Solanum tuberosum* (potato) protease inhibitors Sola t 4 and Sola t 2, *Glycine max* (soy bean) trypsin inhibitor family Gly m TI, and *Triticum aestivum* (wheat) α-amylase/trypsin inhibitor. Although no linear epitope was found in the 8-mer search, the significant homology of both protein sequence and function between sporamin and known allergens suggested the allergenic risk of sporamin. According to the safety assessment guideline recommended by Codex, further IgE serum evaluation of sporamin protein would have to be conducted before the safety of sporamin protein can be determined. The IgE serum test of potential allergenic protein requires human serum from an anaphylaxis patient. The reactivity of IgE in patient serum to protein allergen was detected using the immunoblot method. However, sweet potato is a minor source of food allergen, and a patient anaphylaxis to sweet potato was very rare (*34*), especially in Taiwan. For this reason, the IgE serum test was impossible without sufficient serum from an anaphylaxis patient at this time.

Nutrient Analysis of Chinese Kale. The substantial equivalence concept of GM crops safety assessment has relied on a comparative approach that focuses on similarities and differences between GM crops and its conventional counterparts. In this study, the nutrient compositional equivalence between GM and wild-type Chinese kale was revealed by major nutrient composition analysis. The moisture content, total fat, total protein, total carbohydrate, ash content, dietary fibers, and vitamins A, C, B_6, and B_{12} were analyzed for 10 of each (transgenic and wild-type host) Chinese kale plants. The most different component between GM and wild-type Chinese kale was the fat content (0.47 and 0.71%, respectively). Although there was some componental variation, no significant difference ($p < 0.05$) of major nutrient composition between GM and wild-type Chinese kale was found (**Table 5**). The results of nutrient analysis have demonstrated that GM Chinese kale BoA 3-1 is equivalent to its conventional counterparts with respect to these important constituents.

SGF Assay of Transgenic Chinese Kale with Sporamin Expression. The digestibility of sporamin expressed in the transgenic Chinese kale was tested by SGF and immunoblot

**Table 5.** Nutrient Analysis of Transgenic and Wild-Type Chinese Kale

nutrient	mean		SD		p value
	WT	GM	WT	GM	
moisture (%)	88.72	89.43	0.91	1.04	0.12
ash (g/100 g)	1.35	1.32	0.10	0.12	0.56
fat (g/100 g)	0.71	0.47	0.26	0.19	0.07
protein (g/100 g)	1.63	1.54	0.23	0.13	0.28
carbohydrate (g/100 g)	7.85	7.28	1.02	1.02	0.23
dietary fiber (g/100 g)	3.42	3.19	0.79	0.89	0.55
vitamin A (RE[a]/100 g)	691.00	682.00	7.38	13.17	0.08
vitamin C (mg/100 g)	34.52	29.72	5.77	7.46	0.13
vitamin B_6 (mg/100 g)	0.02	0.02	0.01	0.01	0.31
vitamin B_{12} (μg/100 g)	ND	ND	ND	ND	

[a] RE, retinol equivalents = 10 international units (IU) = 0.3 μg of retinol or 0.6 μg of β-carotene; ND, not detectable. $n = 10$ for all assay.

analyses. Because the consumption of Chinese kale without cooking is very rare in Taiwan, a SGF assay of sporamin after a routine cooking process (boiled in water for 5 min) of transgenic Chinese kale was also performed. Transgenic Chinese kale with or without cooking was subjected to SGF analysis. The results of the SGF assay of raw transgenic Chinese kale demonstrated that sporamin degraded in SGF rapidly (**Figure 2**). The signal of sporamin that disappeared within 2 min on both SDS−PAGE and Western blot has shown that sporamin is susceptible to pepsin digestion, which indicates that it is less likely to be an allergen (*35*). The disappearance of sporamin also showed that sporamin is easy to release (into boiling water) or degrade in a routine cooking process. For a food routinely consumed after cooking, the heat-unstable property of sporamin expressed in transgenic Chinese kale has greatly diminished the exposure of sporamin to the human body.

DISCUSSION

With the development of GM 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 (*35−37*). The key feature of such a decision tree is that it takes into consideration
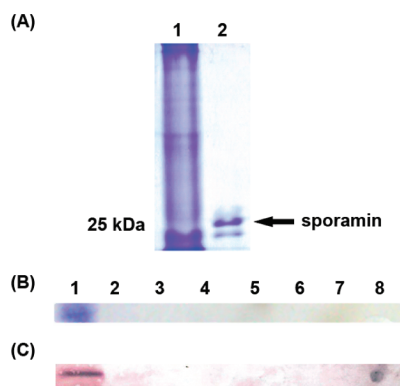
Figure 2. SGF assay of transgenic Chinese kale with sporamin expression. (A) SDS−PAGE analysis of transgenic Chinese kale. Lane 1, crude total protein extract; lane 2, purified recombinant sporamin. (B) SDS−PAGE stained with Coomassie Blue. Lanes 1−4, raw transgenic Chinese kale with SGF digest for 0, 2, 4, and 8 min; lanes 5−8, cooked transgenic Chinese kale with SGF digest for 0, 2, 4, and 8 min. (C) Western blot analysis.

multiple features of the protein; thus, enabling a judgment to be made on the probability of a protein being allergenic. In this paper, the sporamin expressed in Chinese kale was assessed using the most recent FAO/WHO decision tree (*35, 37, 38*). Here, we have focused on the source of the protein, sequence similarity with known allergens, and resistance to pepsin hydrolysis.

In this study, we present a preliminary safety assessment of transgenic Chinese kale with sporamin expression. A reliable real-time PCR detection method was developed to control sample quality. The substantial equivalence between transgenic Chinese kale and its wild-type host has been demonstrated by the comparison of important constituents. The result of the SGF assay showed that sporamin protein is pepsin-susceptible, which indicates less possibility to be an allergen. The heat-unstable and wound-induced expression properties have minimized the exposure of sporamin to the human body. Although there were no other reports describing the safety of sporamin, a bioinformatics search of various allergen databases indicated that there was a significant homology of sporamin to known allergens, in both protein sequence and function. Despite most of the results in this study implying the safety of sporamin, allergenicity of sporamin protein and transgenic Chinese kale BoA 3-1 could not be concluded without a further serum IgE test according the decision tree of allergenicity evaluation (*35, 37, 38*). An IgE serum test of potential allergenic protein requires human serum from an anaphylaxis patient. The reactivity of IgE in patient serum to protein allergen was detected using an immunoblot method. However, sweet potato is a minor source of food allergen, and patient anaphylaxis to sweet potato was very rare (*34*), especially in Taiwan. For this reason, an IgE serum test is impossible at this time until sufficient serum from an anaphylaxis patient is collected.

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