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Assessment of the Allergenic Potential of Foods Derived from Genetically Engineered Crop Plants*

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I. INTRODUCTION

Over 60 different plant species, including the most economically important crops, have been successfully genetically engineered; and the list is growing[1] (see Table 2 in "Genetic Modification of Proteins in Food" by Peter R. Day in this issue). Traits being introduced into these crops include insect protection, delayed ripening, virus resistance, modified starch, herbicide tolerance, modified oils, disease resistance, male sterility, and many others[2] (refer to article cited above). More than 20 different genetically engineered plant products are predicted to be in the marketplace within the next 4 or 5 years[3] (see Table 3 in the article cited above).

Prior to market introduction, each of these products is subjected to extensive food, feed, and environmental safety assessments. This article presents a consensus and a science-based approach to assess the allergenic potential of foods derived from genetically engineered crops as one component of that food safety evaluation. Most traits introduced into crops result from the expression of one or a few new proteins. In some cases the desired trait will be produced by introduction of a gene that turns off another gene (e.g., antisense or cosuppression). Therefore, there may be no new protein introduced into the crop, with the exception of a marker gene protein. Typically, these proteins are expressed at low levels and represent a minor percentage of the total plant protein. In contrast, a specific plant may express tens of thousands of discrete proteins, some of which are present in high concentrations (see "Genetic Modification of Proteins in Food"). Despite this enormous variety, allergy to food proteins occurs in less than 1 to 2% of the population.[4,5] Where food allergy is confirmed, patients are usually allergic to only a few specific proteins within one or two specific foods.

Eight foods or food groups (peanuts, soybeans, tree nuts, milk, eggs, fish, crustacea, wheat) account for over 90% of the documented food allergies worldwide (see Table 1 in "Allergenic Foods" by Steven L. Taylor and Samuel B. Lehrer in this issue). This list of foods is based on discussions at the recent expert consultation on food allergies sponsored by the Food and Agriculture Organization (FAO).[6] The majority of individuals with documented immunologic reactions to foods exhibit immunoglobulin E (IgE)-mediated immediate hypersensitivity reactions that can be sudden, severe, and life-threatening;[7,8] and that are thus the focus of general concern. Although other forms of food protein-induced hypersensitivity exist (see "Allergic Reactions to Foods" by John A. Anderson in this issue), the IgE-mediated form provides the most sensitive indicator for the transfer of a protein that induces an immunologic response. It will be the only immune response specifically addressed in this article. Gluten-sensitive enteropathy (celiac disease), a distinct clinical pathologic entity that is observed in specific individuals sensitive to gluten in certain foods, is not specifically addressed in this article. The assessment approach suggested in this article is not appropriate for celiac disease.

* Please note: The content of this article was developed through a series of joint meetings and discussions involving all authors.

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Assessment of the allergenic potential of foods derived from genetically engineered plants should focus on a multilevel analytical process. This approach takes into account the source from which the gene is obtained, amino acid sequence comparisons with known allergens, *in vitro* and *in vivo* immunologic analyses, and an assessment of key physicochemical characteristics.

A rational assessment of allergenic potential should be conducted in a careful step-wise process, using a decision tree strategy (Figure 1). The totality of these assessments provides reasonable assurance that foods derived from new plant varieties will not introduce allergenic concerns beyond those that exist relative to the current food supply. If allergens are introduced, the foods will be appropriately labeled so that they can be avoided by susceptible individuals. This decision tree approach will be illustrated, as appropriate, with examples of proteins introduced into plants by genetic modification.

II. THE SOURCE

The Food and Drug Administration (FDA), in their Policy on "Foods Derived from New Plant Varieties,"[9] recognized the need to address the potential transfer of food allergens. The FDA stated that if a gene was obtained from an allergenic source "FDA considers it prudent practice for the producer initially to assume that the transferred protein is the allergen. Appropriate *in vitro* or *in vivo* allergenicity testing may reveal whether food from the new variety elicits an allergenic response in the potentially sensitive population (i.e., people sensitive to the food in which the protein is ordinarily found)." The FDA further stated that "labeling of foods newly containing a known or suspect allergen may be needed to inform consumers of such potential." The label should disclose the source from which the gene was transferred.*

The source from which the gene is derived is the critical parameter in the assessment of potential allergenicity. It dictates the need to verify whether a gene encoding an allergenic protein was transferred and expressed in a food component, and mandates labeling should that be the case.

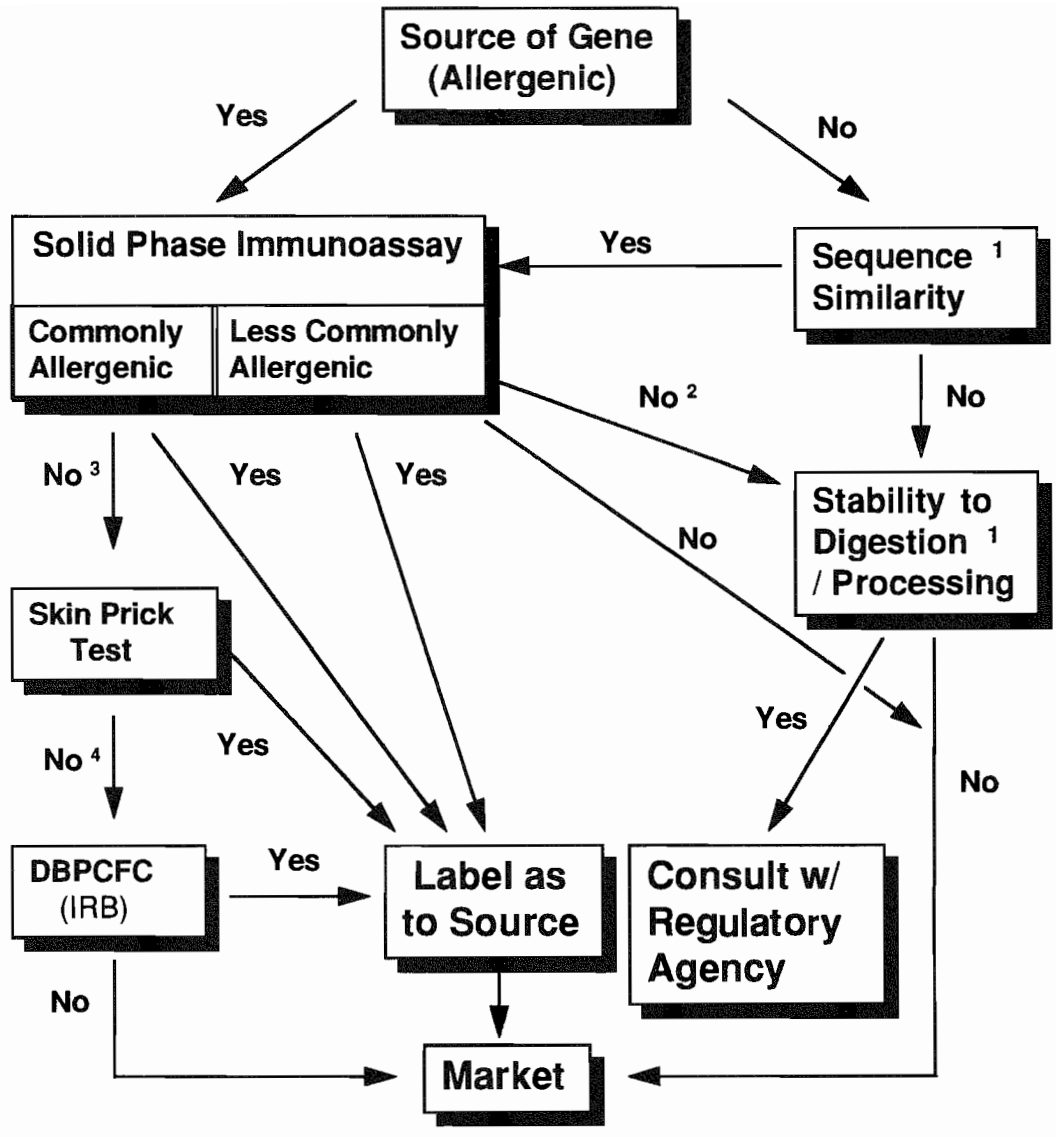
If a gene is obtained from a known allergenic source and the protein encoded is expressed in a food component of the new plant variety, data should be generated to assure that the gene does not encode an allergen. If the gene from an allergenic source is not normally expressed in those

* The FDA has long recognized that certain foods or food ingredients may present risks for some consumers that can be averted. In these cases, the FDA has consistently relied on affirmative labeling declarations identifying the presence of ingredients that possess the potential to trigger allergic reactions in people with sensitivities to such foods or ingredients. Examples include sulfiting agents (21 C.F.R. § 101.100(a)(4)) and color agents like D&C Yellow No. 5 (21 C.F.R. § 74.705 (d)(2)). Another good example, one involving a natural component of food, is gluten. The FDA has required the identification of the source of gluten as either "corn gluten" (21 C.F.R. § 184.1321) or "wheat gluten" (21 C.F.R. § 184.1322) because persons with celiac disease are unable to ingest gluten from wheat without intestinal upset but are able to ingest gluten from corn without side effects. In each case, the required label disclosure alerts consumers to the presence of the ingredient and provides consumers an opportunity to avoid exposure. This use of food labeling ensures that food that is safe for the general population will not provide a risk of harm to comparatively small subpopulations. It is important to note that the FDA has used this labeling authority only when sound science establishes that risk is, in fact, presented.

Under these circumstances, reliance on product-labeling declarations has proven to be an effective and valuable public health mechanism for enabling consumers to choose wisely among foods. Administered in a science-based manner, labeling serves the central purposes of informing, instructing, and warning the consumer.

Moreover, the FDA has also consistently recognized that these goals can only be achieved if consumers can understand and use the information on food labels. For this reason, the agency has limited labeling information to that which is essential about the identity and quality of the subject food or food ingredient.

In addition to being sound as a matter of law and science, this practice of carefully considering whether labeling information is, indeed, justified is reinforced by the very real difficulties that would accompany any attempt to label the plant products of biotechnology. For the most part, these products are fungible crop commodities. Differentiating in the marketplace among such commodities on the basis of biotechnology and traditional breeding techniques would be costly, burdensome, and largely unworkable in light of harvesting, shipping, storage, and processing practices. Moreover, no definitive methodology would be available for distinquishing one type of product from another.



1. It is recommended that an assessment for amino acid sequence similarity to all known allergens and an assessment of stability to digestion be performed for all gene products.
2. Solid phase immunoassay tests depend on availability of sera. Ideally, 14 sera should be used. However, if less than 5 sera are used, then proceed to stability box if results are negative and consult with the appropriate regulatory agency.
3. In the case of equivocal results or suspected false postives, proceed to skin prick tests.
4. DBPCFC's are performed on food products in which there is no evidence of allergenicity based upon solid phase immunoassays and skin prick tests. To assure lack of allergenicity, DBPCFC's should be performed following IRB approval.

FIGURE 1. Assessment of the allergenic potential of foods derived from genetically engineered food crops.

parts of the source organism to which humans are exposed through oral or respiratory routes (e.g., soybean roots or peanut leaves), then the gene need not be considered as coming from an allergenic source. If a known allergen is expressed and restricted to plant parts that are not normally consumed as food, then it should be documented that gene expression and accumulation of the protein

product are limited to the nonfood plant parts before concluding there is no allergenic risk.

In assessing the source from which genes are derived, it is important to distinguish: (a) common allergenic foods; (b) less common allergenic foods and other known allergen sources; and (c) sources with no allergenic histories. For this article, common allergenic foods are defined as the eight food categories mentioned above (also see those listed in Table 1 in "Allergenic Foods"). These foods account for over 90% of the reported food allergies and are those for which clinical reagents, such as patient sera, are likely to be available for the assessments proposed below. This may not be the case for individual species within a food group, such as fish or tree nuts (e.g., swordfish or Brazil nut). Other allergenic sources include the less common allergenic foods listed in Table 2 in "Allergenic Foods" for which clinical reagents may not be readily available, as well as other allergens, for example, pollens, molds, danders, and venoms.

Both common and less common allergenic foods and food groups contain both major and minor allergens. Major and minor allergens are classified according to the frequency with which the allergen is associated with clinical reactions to a specific food. A major allergen is defined as one to which more than 50% of individuals sensitive to that substance react by skin testing (ST) or solid-phase immunoassays. Sensitivity by history or challenge requires signs and symptoms of classic immediate hypersentivity reactions. For example, all individuals with peanut sensitivity react to one or both major allergens in peanuts, whereas the clinical significance of the minor allergens is largely unknown. For these reasons, the majority of concern focuses on the major allergens from both common and less common allergenic foods and food groups.

III. AMINO ACID SEQUENCE SIMILARITY TO KNOWN ALLERGENIC PROTEINS

Assessing the allergenic concerns of foods containing genes from any source should begin with an examination of the amino acid sequence similarity to known allergens. Allergen sources include certain plant- and animal-derived foods (see Tables 1 and 2 in "Allergenic Foods"). Nonfood allergens, such as pollens, fungal spores, insect venoms, and feces, and animal dander and urine,[10] should also be considered. Individuals may experience adverse reactions if they have become sensitized to a protein through the oral, epidermal, or respiratory route, and, subsequently, consume that protein after it has been introduced into a food through recombinant DNA technology. Many respiratory or dermal allergens are labile in the environment or the gastrointestinal tract and are, thus, unlikely to induce sensitivity via that route. However, the oral allergy syndrome (OAS) suggests that even limited exposure in the buccal cavity can induce localized symptoms.

The functions of allergenic proteins *in vivo* are diverse, ranging from enzymes[11] to regulators of the cell cytoskeleton[12] and are of no predictive value in assessing allergenicity. The allergenic proteins of many major sources of allergens, including food allergens, have been characterized by molecular methods (see "Principles and Characteristics of Food Allergens" by Steve L. Taylor and Samuel B. Lehrer in this issue). The important IgE, T-, and B-cell epitopes of some allergens have been mapped.[13–24] The immunologic mechanisms that distinguish atopy from nonpathogenic responses remain obscure.[25] However, from these mapping studies it can be generalized that the optimal peptide length for binding appears to be between 8 and 12 amino acids for T-cell epitopes, and even longer for B-cell epitopes.[26]

Based on this information, it is possible to define a sequence test for comparing the amino acid sequence of an introduced protein with that of known allergens: an immunologically significant sequence identity requires a match of at least eight contiguous identical amino acids. This is well below the level of similarity expected between biologically related (homologous) proteins.[27] The criteria for comparing amino acid sequences may change or evolve over time with additional research and insight into the molecular structure of allergens. The amino acid sequences of allergenic epitopes are known for relatively few allergens, especially food allergens. However, this approach is reasonable in the absence of comprehensive epitope data for allergens in that

no attempt is made to identify matches with known epitopes per se. Instead, the emphasis is on identifying a potential match: failure to find a match of eight contiguous residues anywhere among known allergen amino acid sequences suggests that there is little probability that the introduced protein could possess a shared linear epitope with known allergens. Exact conservation of amino acid sequences of epitopes in homologous pollen allergens of disparate species is occasionally observed. This can explain the IgE cross-reactivity among allergens.[22,28] Furthermore, conservative amino acid substitutions that were introduced into synthetic epitopes[29] or introduced by site-directed mutagenesis[30] reduced IgE-binding efficiency, further supporting the importance of the amino acid sequence of the epitope.

Clearly, this approach is limited in that it cannot identify discontinuous conformational epitopes (see "Food Allergens" by Robert K. Bush and Susan L. Hefle in this issue) that depend on the tertiary structure of the allergen. For example, IgE binding of patient sera to the birch pollen allergen, *Bet v* 3, depends on calcium-regulated conformational changes;[31] *Bet v* 3 contains EF hand motifs, which are calcium-binding domains. IgE binding of *Bet v* 3 depends on the presence of calcium, which presumably changes the conformation so as to allow for recognition of a conformational epitope. The test is also likely to identify conserved sequences that are unrelated to the allergenic potential of the proteins. Therefore, it is used as an indicator and not a determiner of allergenic potential.

Using the FASTA[32] or a similar computer program and the test criteria mentioned above, the amino acid sequences of allergens present in the public domain genetic databases (GenBank, EMBL, PIR, and SwissProt) should be searched for matches to allergenic protein sequences from genetically engineered plants. It is necessary to obtain and retrieve amino acid sequences of allergenic proteins to perform this test. This retrieval method is critical in ensuring that relevant sequences are obtained. A search strategy that only seeks allergen entries on the basis of a key word like "allergen" will not retrieve all relevant food allergens, and may also retrieve nonallergens. For example, many food allergens, such as casein, β-lactoglobulin, and ovomucoid, are not recovered using the key word "allergen." This database should also contain amino acid sequences for all allergenic proteins, not just food allergens. Tables 1 and 2 contain the most comprehensive list of reported amino acid sequences for allergens to date. These tables include food (Table 1) and nonfood allergens (Table 2). Accession numbers are included to facilitate its use. These tables were constructed in May 1995[33] and should be continuously updated to reflect reports of additional amino acid or nucleotide sequences of allergenic proteins.

Searches of this allergen sequence database (or other similar databases) have shown no significant matches for the proteins listed in Table 3, which are examples of proteins expressed in genetically engineered plants. It was concluded from these data that none of these introduced proteins share linear IgE epitopes with known allergenic proteins. Using the information generated from amino acid sequence homology analysis, a different path is taken on the decision tree based on the source from which the gene was derived (Figure 1).

IV. FOOD CONTAINING A GENE DERIVED FROM A COMMONLY ALLERGENIC FOOD

Foods containing a gene derived from an allergenic food, irrespective of the information obtained from the amino acid sequence analysis, must be subjected to immunologic analysis of allergenic potential. Both *in vitro* and *in vivo* assays should be employed. These assays may include the various solid-phase immunoassays such as the RAST or RAST inhibition assay[34,35] or the enzyme-linked immunosorbent assay (ELISA).[36] A positive immunoblot could substitute for a positive solid-phase immunoassay result, but a negative immunoblot would require that a solid-phase immunoassay be performed because of its increased sensitivity. The solid-phase immunoassay employed should allow a calculation of at least a 95% confidence

TABLE 1
Food Allergen Sequences Retrieved from the Public Domain Databases for Proteins and Nucleic Acids*

Species	Common name	Allergen	Synonym/function	Accession
		Food (plant)		
Arachis hypogea	Peanuts	*Ara h* 1	Clone P41b	L34402
			Clone 5A1	L33402
			Clone P17	L38853
		Peanut lectin	Agglutinin	S14765
Bertholletia excelsa	Brazil nut	*Ber e* 1	2S albumin (BE2S1 gene)	X54490
Brassica juncea	Leaf mustard	*Bra j* IE-L	2S albumin large chain	S35592
		Bra j IE-S	2S albumin small chain	S35591
Carica papaya	Papaya	Papain		M15203
Glycine max	Soybean	Glycinin	A1aBx subunit	X02985
			A2B1a subunit	Y00398
			A3B4 subunit	M10962
			A5A4B3 subunit	X02626
			G1 subunit	X15121
			G2 subunit	X15122
			G3 subunit	X15123
		β-Conglycinin	α-Subunit	X17698
			CG4 subunit	S44893
		Soy lectin	Soy agglutinin	K00821
		Kunitz trypsin inhibitor	KTi-s subtype	X80039
			KTi-a subtype	X64447
			KTi-b subtype	X64448
Hordeum vulgare	Barley	*Hor v* 1	α-Amylase/trypsin inhibitor	S26197
		Hor v 1	α-Amylase/trypsin inhibitor	P32360
Malus domestica	Apple	*Mal d* 1	Profilin	X83672
Oryza sativa	Rice	RAP	Rice allergenic protein	X66257
		RAG1	Rice allergen 1	D11433
		RAG2	Rice allergen 2	D11434
		RAG5	Rice allergen 3	D11430
		RAG14	Rice allergen 14	D11432
		RAG17	Rice allergen 17	D11431
Phaseolus vulgaris	Kidney bean	PR-1	Pathogenesis-related protein 1	S11929
		PR-2	Pathogenesis-related protein 2	S11930
Sinapis alba	White mustard	*Sin a* 1.1	2S albumin/amylase inhibitor	S54101
		Sin a 1.2	2S albumin/amylase inhibitor	PC1247
Triticum aestivum		WGA	Wheat germ agglutinin A	M25536
		WGA	Wheat germ agglutinin D	M25537
Triticum durum	Pasta wheat	WGA	Wheat germ agglutinin	J02961
Triticum turgidum	Poulard wheat	16K allergen	α-Amylase inhibitor	S19296
		Food (animal)		
Bos taurus	Cow	BSA	Serum albumin	M73993
		β-Lactoglobulin	Milk globulin (whey)	X14712
		α-Lactalbumin	Milk albumin (whey)	J05147
		Casein	Type α-S1	M33123
			Type α-S1	M38641
			Type α-S2	M16644
			Type β	M15132
			Type κ	M36641
Gadus callarias	Cod fish	*Gad c* 1	β-Parvalbumin, allergen M	A94236
Gallus domesticus	Chicken	*Gal d* 1	Ovomucoid	M10639
		Gal d 2	Ovalbumin Y gene	J00922
		Gal d 2	Ovalbumin	M34352
		Gal d 3	Conalbumin (ovotransferrin)	Y00407
		Gal d 4	Lysozyme	J00885
		Gal d 4	Isolysozyme	X61001
		Vitellogenin II	Lipovitellin/phosvitin	A92941
		Apovitellenin I	Low density lipoprotein II	A91484
Metapenaeus ensis	Shrimp	*Met e* 1	Tropomyosin	U08008

Note: Some of these allergens may be airborne or associated with occupational allergies rather than directly ingested.

* Public domain databases: GenBank/EMBL/Genpept ver 86.0, SWISSPROT ver 30, PIR ver 41.

TABLE 2
Nonfood Allergen Sequences Retrieved from the Public Domain Databases for Proteins and Nucleic Acids*

Species	Common name	Allergen	Synonym/function	Accession
		Pollen		
Agrostis alba	Bent grass	*Agr a* 1	Group I	E37396
Alnus glutinosa	Alder tree	*Aln g* 1	*Bet v* 1 homolog	S50892
Ambrosia artemisiifloia	Ragweed (short)	*Amb a* 1.1	Antigen E	A39099
		Amb a 1.2	Antigen E	B39099
		Amb a 1.3	Antigen E	C39099
		Amb a 1.4	Antigen E	D53240
		Amb a 2	Antigen K	E53240
		Amb a 3	Ra3	P00304
		Amb a 5	Ra5	A03371
Ambrosia trifida	Ragweed (tall)	*Amb t* 5	Ra5 homolog	S39336
		Amb t 5	Ra5 homolog	A23859
Ambrosia psilostachya	Weed	*Amb p* 5 (A2)	Ra5 homolog	L24465
		Amb p 5 (A3)	Ra5 homolog	L24466
		Amb p 5 (B1)	Ra5 homolog	L24467
		Amb p 5 (B2)	Ra5 homolog	L24468
		Amb p 5 (B3)	Ra5 homolog	L24469
Anthoxanthum odoratum	Sweet vernal grass	*Ant o* I	Group I	G37396
Artemisia vulgaris	Mugwort	*Art v* 2	Glycoprotein allergen	A38624
Betula verrucosa	Birch tree	*Bet v* 1	Pathogenesis related (PR)	S05376
		Bet v 1N	*Bet v* 1 isoform	X82028
		Bet v 2	Profilin	B45786
		Bet v 3	Profilin	X79267
Carpinus betulus	Hornbeam tree	*Car b* 1	*Bet v* 1 homolog	C53288
Castanea sativa	European chestnut	*Cas s* 1	*Bet v* 1 homolog	PC2001
Corylus avellana	Hazel tree	*Cor a* 1-5	*Bet v* 1 homolog	S30053
		Cor a 1-6	*Bet v* 1 homolog	S30054
		Cor a 1-11	*Bet v* 1 homolog	S30055
		Cor a 1-16		S30056
Cryptomeria japonica	Japanese cedar	*Cry j* 1-A		D26544
		Cry j 1-B		D26545
		Cry j 2		D29772
Cynodon dactylon	Bermuda grass	*Cyn d* 1		A61226
Dactylis glomerata	Orchard grass	*Dac g* 2		S45354
		Dac g 3		A60359
Festuca elator	Reed fescue	*Fes e* 1-A		C37396
		Fes e 2-B		D37396
Glycine max	Soybean	*Gly m* cim1	Cytokinin-inducible protein	U03860
Holocus lanatus	Meadow velvet	*Hol l* 1	30K allergen	Z27084
Hordeum vulgare	Barley	*Hor v* 9	Group IX	U06640
Lolium perenne	Ryegrass	*Lol p* 1	Group I	M57476
		Lol p 1	Group I	M57474
		Lol p 1b	Group I	M59163
		Lol p 2-A	Group II	A34291
		Lol p 2	Group II	A48595
		Lol p 3	Group III	A33422
		Lol p 4	Group IV	A60737
		Lol p 9	Group IX	L13083
		Lol p 30K	30K group V allergen	S38290
		Lol p 34K	34K group V allergen	S38289
		Lol p 50K	50K allergen	S38288
Lycopersicon esculatum	Tomato	LAT52	*Ole e* 1 homolog	P13447
Olea europea	Olive tree	*Ole e* 1		S36872
Parietaria judaica	Parietaria	*Par j* 1		X77414
Parietaria officinalis	Parietaria	*Par o* 1		A53252

TABLE 2 (continued)
Nonfood Allergen Sequences Retrieved from the Public Domain Databases for Proteins and Nucleic Acids*

Species	Common name	Allergen	Synonym/function	Accession
		Pollen		
Phleum pratense	Timothy grass	*Phl p* 1	Group I	X78813
		Phl p 1		Z27090
		Phl p 2	Group II	X75925
		Phl p 5a	Group V, group IX	X70942
		Phl p 5b	Group V	Z27083
		Phl p 6		Z27082
		Phl p 32K	Group V-like	S38294
		Phl p 38K	Group V-like	S38293
		Phl p 11	Group XI/profilin	P35079
Poa pratensis	Kentucky blue-grass	*Poa p* 1	Group I	F37396
		Poa p 1	Group I	A60372
		Poa p 9 (KBG31)	Group IX	M38342
		Poa p 9 (KBG41)		M38343
		Poa p 9 (KBG60)		M38344
Quercus alba	Oak tree	*Que a* 1	*Bet v* 1 homolog	D53288
Secale cereale	Cultivated rye	*Sec c* 30K	30K group Vallergen	S38292
Triticum aestivum	Bread wheat	*Tri a* 2.1	Profilin	S72384
		Tri a 2.2	Profilin	S72374
		Tri a 2.3	Profilin	S72375
Zea mays	Maize	*Zea m* 1	*Lol p* I homolog	JC1524
		Clone c13	*Ole e* I homolog	P33050
		Mites		
Euroglyphus maynei	House mite	*Eur m* 1	Group I, thiol protease	S21864
Dermatophagoides farinae	House mite	*Der f* 1	Thiol protease	X65196
		Der f 2.1	Antigen 2	D10447
		Der f 2.1	Antigen 2	A61241
		Der f 2.2	Antigen 2	D10448
		Der f 2.2	Antigen 2	B61241
		Der f 2.3	Antigen 2	D10449
		Der f 2.3	Antigen 2	PS0417
Dermatophagoides microceras	House mite	*Der m* 1	Thiol-protease	B27634
Dermatophagoides pteronyssinus	House mite	*Der p* 1	Antigen P_1	U11695
		Der p 1	Antigen P_1	JQ0337
		Der p 2		A60381
		Der p 3	Trypsin	U11719
		Der p 4	Amylase	A61242
		Der p 5	14K allergen	S06734
		Der p 7		X17699
Lepidoglyphus destructor	Feces mite	*Lep d* 1		X81399
		Insect venoms		
Apis mellifera	Honeybee	*Api m* 1	Phospholipase A2	P00630
		Api m 3	Melittin	P01501
Dolichovespula arenaria	Yellow hornet	*Dol a* 5	Antigen 5	M98859
Dolichovespula maculata	Whiteface hornet	*Dol m* 1.02	Phospholipase A1	A44563
		Dol m 2	Hyaluronidase	L34548
		Dol m 5	Antigen 5 clone f5	J03602
		Dol m 5	Antigen 5 clone f10	J03601
Myrmecia pilosula	Bulldog ant	*Myr p* 1		X70256
Polestes annularis	Wasp	*Pol a* 5	Antigen 5	M98857
Polestes exclamans	Paper wasp	*Pol e* 5	Antigen 5	P35759
Polestes fascatus	Paper wasp	*Pol f* 5	Antigen 5	F44522

TABLE 2 (continued)
Nonfood Allergen Sequences Retrieved from the Public Domain Databases for Proteins and Nucleic Acids*

Species	Common name	Allergen	Synonym/function	Accession
		Insect venoms		
Solenopsis invicta	Red fire ant	*Sol i* 2	Phospholipase	A37330
		Sol i 3		B37330
		Sol i 4		C37330
Solenopsis richteri	Black fire ant	*Sol r* 2	Phospholipase	E60727
		Sol r 3		D60727
Vespa crabro	European hornet	*Ves c* 5.0001	Antigen 5	G44522
		Ves c 5.0002	Antigen 5	H44522
Vespula flavopilosa	Yellow jacket	*Ves f* 5	Antigen 5	B44522
V. germanica	German yellowjack	*Ves g* 5	Antigen 5	A44522
V. maculifrons	Eastern yellowjack	*Ves m* 1	Phospholipase A1	A44564
		Ves m 5	Antigen 5	M35760
V. pensylvanica	Western yellowjack	*Ves p* 5	Antigen 5	C44522
V. squamosa	Southern yellowjack	*Ves s* 5	Antigen 5	D44522
V. vidua	Yellow jacket	*Ves vi* 5	Antigen 5	E44522
V. vulgaris	Yellow jacket	*Ves v* 5	Antigen 5	M98858
		Parasitic Nematodes		
Loa loa	Filarial worm	LL20	15K ladder protein	U03103
		Segmented Worms		
Ascaris lumbricoides	Common roundworm	*Asc l* 1	Aba-1	B37188
A. suum	Earthworm	*Asc s* 1	Aba-1	A37188
		Asc s 1	Aba-1	L03211
		Animals		
Felis domesticus	Cat saliva	*Fel d* 1.1	Antigen 4	M74952
		Fel d 1.2	Antigen 4	M74953
		Fel d 1.3	Antigen 4	M77341
Mus masculus	Mouse urine	*Mus m* 1	Major urinary protein (MUP)	M27608
			MUP I	M16355
			MUP II	M16356
			MUP III	M16359
			MUP IV	M16358
			MUP V	M16360
Rattus norvegicus	Rat urine	*Rat n* 1	Hepatic α-2u globulin	J00737
		Fungi (spores)		
Alternaria alternata		*Alt a* 2	Aldehyde dehydrogenase	X78227
		Alt a 6	Ribosomal protein	X78222
		Alt a 7		X78225
Aspergillus fumigatus		*Asp f* 1	Mitogillin toxin/ ribonuclease	M83781
		Asp f 1-A		S39330
Cladosporium herberum		*Cla h* 2	Enolase	X78226
		Cla h 3	Aldehyde dehydrogenase	X78228
		Cla h 4	Ribosomal P2	X78223
		Cla h 5		X78224

* Public domain databases: GenBank/EMBL/Genpept ver 86.0, SWISSPROT ver 30, PIR ver 41.

TABLE 3
Summary of Proteins Introduced Into Crops by Genetic Engineering

Introduced protein[a]	Crop products[b]
ACC deaminase (ACCd)	Delayed ripening tomato
B.t.t. insecticidal protein *(B.t.t.)*	Insect-protected potato
B.t.k. HD-1 insecticidal protein (*B.t.k.* HD-1)	Insect-protected corn and tomato
B.t.k. HD-73 insecticidal protein (*B.t.k.* HD-73)	Insect-protected cotton
CP4 EPSP synthase (CP4 EPSPS)	Herbicide-tolerant canola, cotton, corn, soybean, and sugarbeet
Glyphosate oxidoreductase (GOX)	Herbicide-tolerant canola and corn
β-D-Glucuronidase (GUS)	Herbicide-tolerant soybean
Neomycin phosphotransferase II (NPTII)	Delayed ripening tomato, insect-protected cotton and potato, Flavr Savr™ tomato
Phosphinothricin acetyltransferase (PAT)	Herbicide-tolerant corn

Note: The specific proteins included in this table were based on those proteins for which digestive stability data were available (see Table 4).

[a] ACC, 1-amino-1-cyclopropane-carboxylic acid; *B.t.t.*, *Bacillus thuringiensis* subsp. *tenebrionis*; *B.t.k.*, *Bacillus thuringiensis* subsp. *kurstaki* proteins from strains HD-73 and HD-1, corresponding to the [(Cryla(c)] and [CrylA(b)] proteins according to the nomenclature of Höfte and Whiteley;[103] CP4 EPSPS, 5-enolpyruvylshikimate-3-phosphate synthase from *Agrobacterium* sp. strain CP4.

[b] Adapted from Fuchs et al.,[104] Ciba Geigy,[105] and Noteborn and Kuiper.[85]

limit from the examination of at least 14 sera* from confirmed allergic patients.

The solid-phase immunoassays (described in detail in "Principles and Characteristics of Food Allergens") use IgE fractions of sera from individuals who are confirmed allergic to the food from which the gene was derived. Serum donors should meet rigid clinical criteria, including testing positive in double-blind, placebo-controlled food challenges (DBPCFC),[34,37] or must have a convincing history of severe, IgE-mediated, systemic reactions.[38] A convincing history would consist of an immediate hypersensitivity response following an isolated ingestion/exposure that required medical management, and that was documented in the medical records. Data from one or more of these *in vitro* assays provide strong evidence as to whether the transferred gene encodes an allergen. Sera from at least 14 documented reactors should be tested separately. Any positive result (outside the 95% confidence limit) from the *in vitro* tests should require that any food contain-

* Binomial probability theory (i.e., that a subject in a population will either pass or fail a test) can be used to determine the sample size (N) needed to predict, with a particular level of confidence, the likelihood that a single individual, from a population composed only of individuals sensitive to the food from which a protein is isolated (i.e., the at-risk population, not the general population), will react to a specific protein. To apply the theory, it is necessary to make certain assumptions about the probability (p) that a random subject in the test population is allergic to that particular protein and will test positive (i.e., fail the test).

To be conservative, a predictive incidence of 20% and a 95% level of confidence was used to determine the number of sera to be recommended. These criteria would provide a 95% probability that an allergen to which at least 20% of the sensitive individuals have IgE would be detected if transferred. Using these assumptions and binomial probabilities, a sample of 14 sera (N = 14) would need to be tested and to show no positive reaction with any of the sera (i.e., passed the test) to conclude that an allergen had not been transferred, under the given assumptions. Using the criteria of 14 sera and a predictive incidence of 50% (instead of 20%) would increase the probability of detecting the transfer of a major allergen to >99.9%, compared with a >95% for a minor allergen present at 20% of the sensitive population. This assessment does not exclude the possibility that the transferred protein could represent an allergen that elicits an IgE response from an even less frequent portion of the sensitive population. However, these values provide reasonable assurance to, and protection for, the public.

ing the gene product be labeled as to the source of the transferred gene, as per FDA guidance[9] (Figure 1).

If the *in vitro* test results are negative or equivocal or if a nonspecific cross-reaction is suspected, then the *in vivo* skin prick test[34] should be employed as a further screen for allergenicity. Details on the types of skin tests and the need to verify the quality of the extracts and standards used for these types of tests are critical and are described in this issue (see "Principles and Characteristics of Food Allergens"). At least 14 patients, skin test positive to the source food in question, with a convincing history of sensitivity should be tested. A positive result from this *in vivo* test would raise the same concerns as a positive *in vitro* test and should require labeling as to the source of the transferred gene (Figure 1).

If no positive response is observed in either the *in vitro* or prick/puncture skin tests, a final test could consist of performing double-blind, placebo-controlled food challenges (DBPCFCs) under controlled clinical conditions with patients sensitive to the food in question. The ethical considerations for this type of assessment would include, but not be limited to, factors such as the likelihood of inducing anaphylactic shock in test subjects and the availability of appropriate clinical safety data and procedures. Most, if not all, institutions that perform DBPCFC studies have Institutional Review Boards (IRBs) that review all DBPCFC studies for ethical considerations. This includes a judgement as to whether the risk of performing the procedure places the volunteer at any increased risk over what should take place in the usual assessment of sensitivity in that subject. Obtaining data from an *in vitro* assay and ST should be prerequisites for requesting DBPCFC studies, to minimize any risks to the participants. An earlier article (see "Principles and Characteristics of Food Allergens") describes details for performing DBPCFC studies, including suggested doses and precautions. If there is a positive reaction in one sensitive patient, of a total of at least 14 sensitive individuals tested in the DBPCFC studies, food derived from crops containing the protein should be labeled as to the source of the transferred gene.

If no positive reactions are observed in these three levels of assessments, it can be concluded that the gene obtained from this allergenic food source does not encode one of the allergenic proteins.

An example that illustrates the effectiveness of this assessment approach is the Brazil nut 2S storage protein. The protein was engineered into soybean to increase sulfur-containing amino acid levels to improve the quality of soybean meal as an animal feed. The expression of the 2S protein in soybean represented a significant fraction of total transgenic soybean seed protein.[39,40] The Brazil nut is known to cause anaphylactic reactions in a small number of sensitive individuals.[41,42] A solid-phase immunoassay was used in conjuntion with immunoblotting to assess whether an allergenic protein from Brazil nut had been transferred to soybean.[40,43] A positive response in the immunoassay was observed with sera for eight of the nine Brazil nut-sensitive individuals. The results showed that the gene obtained from Brazil nut probably encoded the major Brazil nut allergen. If a soybean product containing this protein were to be commercialized, any foods containing soybean products derived from this variety should be labeled as containing protein from Brazil nut. This example demonstrates the value and effectiveness of using *in vitro* assays to identify the transfer of known allergenic proteins by genetic modification.

V. FOOD CONTAINING A GENE DERIVED FROM A LESS COMMONLY ALLERGENIC FOOD OR OTHER KNOWN ALLERGEN SOURCE

In the case of a food containing a gene derived from a less common allergenic food or other known allergen source, irrespective of the information obtained from the amino acid sequence analysis, the food should be subjected to immunologic analysis of allergenic potential wherever feasible. *In vitro* assays should be employed. Sera from at least 14 documented reactors should be tested, if reasonably available (e.g., ragweed). In cases where sera are not reasonably available (e.g., maize), the maximum number of sera obtainable

should be used. However, if fewer than five sera* are obtained, the appropriate regulatory agency should be consulted. An examination of the physicochemical properties (see below) should also be undertaken. Any positive result (outside the 95% confidence limit) from the *in vitro* immunologic assays should require that any food containing the gene product be labeled as to the source of the transferred gene, as per FDA guidance[9] (Figure 1). If no positive result is obtained in the *in vitro* immunoassays and at least five sera are analyzed, the product can be marketed without labeling.

In evaluating a gene product transferred from a less commonly known allergenic food or other known allergen source, the gene product should be subjected to physicochemical analysis if less than five sera are tested using a solid-phase immunoassay and all results are negative. The physicochemical and biological characteristics of the gene product can be compared to the characteristics of known allergenic proteins, as a means of predicting allergenic potential. However, at this time, with the exception of identifying known allergens transferred from allergenic sources, there appears to be no single predictive property that can conclusively determine the allergenic potential of a gene product.[9,44,45]

A key prerequisite for food protein allergenicity is its resistance to digestion (e.g., the stability of the protein to proteolytic and acid conditions of the human digestive system). The relative stability of an allergen to conditions encountered during processing operations used for specific food products (e.g., heat denaturation) is also an important property of most food allergens. For example, peanut and soybean allergens retain their allergenic potential through the steps used in processing food products such as peanut butter[46] and soy flour.[47] For these reasons, digestibility and stability during processing should be considered when assessing the potential allergenicity of a protein introduced into a given food. However, an exception to the observation that food proteins tend to resist digestion is seen in oral allergy syndrome (OAS); labile proteins cause the disease but infrequently cause systemic reactions.

Allergenic proteins are also typically 10 to 70 kDa in molecular weight and are often glycosylated. However, these properties are shared by many nonallergenic proteins, and many allergens themselves are exceptions to this generalization. Therefore, these criteria are not included in the decision process.

A. Digestibility

The ability of food allergens to reach and cross the mucosal membrane of the intestinal tract is likely a prerequisite to allergenicity. Intact proteins are capable of crossing the mucosal membrane of the gut and entering the circulatory system.[48] Clearly, a protein that is largely stable to the proteolytic and acidic conditions of the digestive tract has an increased probability of reaching the intestinal mucosa. Many allergens exhibit proteolytic stability,[49-56] although the majority remain directly untested (see "Principles and Characteristics of Food Allergens").

Simulated gastric and intestinal digestive models of mammalian digestion, as described in the U.S. Pharmacopeia,[57] have been employed to compare the relative stability of the proteins engineered into plants with a number of the commonly known allergenic food proteins.[58] These digestion models have also been used to investigate the digestibility of plant[59,60] and animal[61] proteins, and food additives.[62] A similar model has also been used to examine the stability of milk allergens.[63,64]

One study that looked at this issue examined the common food allergens shown in Table 4. Without exception, these food allergens were stable to digestion in the gastrointestinal (GI) digestive model.[58] For the allergens shown in Table 4, either the allergen or a proteolytic fragment of the allergen was stable for at least 2 min in simulated gastric fluid. The major allergens were typically stable for more than 1 h. Similar stability data were reported by other investigators; however, relative stability was not as well defined.[65,66] In contrast to these allergenic food

* Using five sera, there is a ≥67% probability that an allergen present in the sensitive population at a frequency of ≥20% would be detected, if transferred, and there is a ≥95% probability that a major allergen (an allergen present in the sensitive population at a frequency of ≥50%) would be detected, if transferred.

TABLE 4
Summary of Allergen and Protein Stability in a Gastric Model

Protein	% Total protein	Stability (min)[a,b]	
		Whole protein	Fragments
	Egg white allergens[106]		
Ovalbumin (*Gal d* 2)	54	60	—
Ovomucoid (*Gal d* 1)	11	8	—
Conalbumin (*Gal d* 3)	12	0	15
	Milk allergens[106]		
β-Lactoglobulin	9	60	—
Casein	80	2	15
Bovine serum albumin	1	0.5	15
α-Lactalbumin	4	0.5	2
	Soybean allergens		
β-Conglycinin (β-subunit)[107]	18.5[c]	60	—
Kunitz trypsin inhibitor[108]	2–4	60	—
Soy lectin[109]	1–2	15	—
β-Conglycinin (α-subunit)[107]	18.5[c]	2	60
Glycinin[107]	51	0.5	15
Gly m Bd 30K[110]	2–3	0	8
	Peanut allergens		
Ara h II[111]	6[d]	60	—
Peanut lectin[112]	1.3	8	—
	Mustard allergens[113]		
Sin a I	20	60	—
Bra j IE	20	60	—
	Common plant proteins		
Rubisco LSU (spinach leaf)[114]	25[c]	0 (<15 s)	—
Rubisco SSU (spinach leaf)[114]	25[c]	0 (<15 s)	—
Lipoxygenase (soybean seed)[115]	<1	0 (<15 s)	—
Glycolate reductase (spinach leaf)[e]	<1	0 (<15 s)	—
PEP carboxylase (corn kernel)[e]	<1	0 (<15 s)	—
Acid phosphatase (potato tuber)[e]	<1	0 (<15 s)	—
Sucrose synthetase (wheat kernel)[e]	<1	0 (<15 s)	—
β-Amylase (barley kernel)[e]	<1	0 (<15 s)	—
	Introduced proteins[58,100]		
B.t.t. insecticidal protein	<0.01	0 (<30 s)	—
B.t.k. HD-73 insecticidal protein	<0.01	0.5	—
B.t.k. HD-1 insecticidal protein	<0.01	0.5	—
CP4 EPSP synthase	<0.1	0 (<15 s)	—
Glyphosate oxidoreductase	<0.01	0 (<15 s)	—
ACC deaminase	0.4	0 (<15 s)	—

TABLE 4 (continued)
Summary of Allergen and Protein Stability in a Gastric Model

Protein	% Total protein	Stability (min)[a,b] Whole protein	Fragments
	Introduced proteins[58,100]		
β-D-glucuronidase	0.01	0 (<15 s)	—
Neomycin phosphotransferase II	<0.01	0 (<10 s)	—
Phosphinothricin acetyltransferase	n.d.	0	—

[a] After Astwood and Fuchs et al.[116]
[b] Gastric digests were perfomed as described previously[80] with the following modifications: 170 ng/μl of protein was digested in 200-μl aliquots of simulated gastric fluid composed of 0.3% (w/v) pepsin, 0.03 *M* NaCl, pH 1.2. Digests were quenched by neutralization with 75 μl 0.16 *M* Na_2CO_3 at the following times: 0, 15, and 30 s; and 1, 2, 4, 8, 15, and 60 min. Digestion of proteins was evaluated by SDS-PAGE (10 to 20% acrylamide with tricine buffers[117]) and visualized by Coomassie Brilliant Blue colloidal staining.[118] 500 ng protein was loaded per lane. Stability represents the last time point at which the protein or a proteolytic fragment was observed; (n.d.) = not detectable; (—) = no fragments.
[c] Total amount of protein for combined subunits.
[d] Reported as % crude extract.
[e] Values estimated from the literature.

proteins, common food proteins with no allergenic history rapidly degraded under similar conditions. All eight of the common food proteins shown in Table 4 rapidly degraded within 15 s, the first time point analyzed.

Rapid proteolytic degradation of proteins greatly minimizes the likelihood that proteins could be absorbed by the intestinal mucosa, and should limit the opportunity for sensitization. The human digestive system provides an effective mechanism to remove these proteins before they have the opportunity to reach the intestinal mucosa. Therefore, the simulated gastric model provides a method to assess allergenic potential of proteins introduced into food plants.

B. Stability to Processing

The stability of a protein to various food-processing activities is also an important factor when assessing the allergenic potential of an introduced protein. Food allergens, particularly those present in processed food products like peanuts and soybeans, tend to be stable to processing conditions (see "Principles and Characteristics of Food Allergens"). This is expected because the processed food products derived from these foods maintain their allergenicity. If a protein is being engineered into fresh market products such as tomatoes, squash, or lettuce, processing stability is irrelevant because the product will be consumed fresh. If, on the other hand, a protein is engineered into soybeans, wheat, or rice, which are processed in one or more ways prior to consumption, the stability of the protein to processing conditions should be taken into account and suitable tests to investigate stability should be designed and conducted. If the product used for human consumption is free of protein (e.g., oils or carbohydrates), there is no significant human exposure and the allergenic potential of the expressed protein is greatly minimized or eliminated. Studies using direct food challenges with a limited number of subjects, with oils derived from several different crops, including soybean, peanut, and sunflower, showed no allergic reaction in patients who are allergic to these foods.[67–69] This is not surprising because there is an extremely low or negligible level of protein in hot-processed crop-derived oils.[70]

If a protein derived from a less commonly allergenic food or other allergenic source is highly

susceptible to digestion and/or is otherwise extensively degraded or removed by processing (if all food products derived from that plant are processed), no labeling should be required. If the protein is resistant to processing and/or digestion, the appropriate regulatory agency should be consulted.

VI. FOOD CONTAINING A GENE DERIVED FROM A SOURCE THAT HAS NO HISTORY OF ALLERGENICITY

If the gene is derived from a source that has no history of allergenicity, a comparison of the amino acid sequence identity between the gene product and known allergens should be conducted as described in Section III. If significant amino acid similarity exists between the gene product and a known allergen and sera from individuals that are sensitive to that allergen are reasonably available, the gene product should be assessed for immunologic reactivity using a solid-phase immunoassay. This should be performed as described for genes encoding proteins derived from less common allergenic foods or other known allergenic sources.

If there is no significant amino acid similarity, the digestibility and stability of the gene product should be assessed as described in Sections V.A and V.B. If the protein shows no significant amino acid similarity and is rapidly degraded or removed by processing, the product should be marketed without labeling. For example, comparison of the amino acid sequence of the nine proteins introduced into the number of different genetically modified plant products listed in Table 3 demonstrated that these proteins did not show any significant amino acid similarity to known allergens. These same nine proteins were rapidly degraded in the digestibility assay described above (Table 4). Based on these data, the products containing these proteins should be marketed without labeling.

VII. ADDITIONAL CONSIDERATIONS

A. Prevalence in Food

Many food allergens, especially those in the common allergenic foods, are present as major protein components, typically ranging between 1.0 and 80% of total protein. Examples of highly abundant allergens (Table 4) include those in milk,[54,55,71,72] soybean,[73,74] and peanuts.[75–78] Therefore, if a protein is expressed in the food at a level exceeding 1% of the total protein, this should also be taken into account in the allergenicity assessment. In contrast to the food allergens shown in Table 4, which are typically present at less than 1% of the total protein, the proteins expressed in the initial genetically engineered plants targeted for market introduction (Table 3) range from approximately <0.001 to 0.03% of the raw product on a fresh weight basis or <0.01 to 0.4% of the protein content,[79–85] and therefore do not trigger this concern.

B. The Host

Patients who are already sensitized to foods derived from hosts that contain endogenous allergens will likely still avoid the food derived from genetically engineered varieties of the same host (e.g., soybean or peanut). However, significant increases in the level of an endogenous allergen(s) that inadvertently resulted from the genetic modification could result in more individuals becoming sensitized to the allergen(s). Therefore, if the host being genetically modified is known to contain specific endogenous allergenic proteins, and sera from sensitive patients are readily available (e.g., for commonly allergenic foods), the food derived from the new plant variety should be analyzed to assure that the level of endogenous allergens was not increased during the modification process beyond natural differences that occur in the plant. It may not be necessary to evaluate the levels of endogenous allergens in all genetically modified, common allergenic host plants such as soybeans. The nature of the desired change should be determinant. If an antisense gene is introduced to turn off the production of a key allergen in soybeans, the levels of all endogenous soybean allergens in the resultant recombinant crop should be checked. However, these levels would not need to be checked if there were no reason to expect the introduced genetic material would influence the level of endogenous soybean allergens.

When an analysis of endogenous proteins is desired and feasible, immunoblotting and/or ELISA methods could be implemented for this assessment.[86] However, this assessment cannot be performed if the plant has no history of causing allergy or a limited history that precludes the availability of sera.

C. Animal Models

Although animal models provide important information for understanding the mechanisms of allergenicity, these models have not been validated for assessing the allergenic potential of specific proteins in humans. Examples of animal models include (1) mouse models to evaluate IgE responses to modified recombinant allergens;[87] (2) IgE-mediated rat anaphylaxis models;[88] (3) guinea pig models of anaphylaxis;[89–91] (4) dog models to study asthma[92,93] and food allergy;[93] and (5) mouse models to study possible immunotherapeutic peptide epitopes[24] and immunoprophylactic strategies.[94]

Animal models provide opportunities to study fundamental questions and mechanisms of allergenicity. However, none of these models have been shown to predict the allergenic potential of introduced proteins.[95,96] In all cases, these models have been used to study the biological or molecular mechanisms of immunopathogenesis of established allergenic responses. In the absence of data, these models cannot be extrapolated to humans. Variable responses from allergen to allergen, animal to animal, species to species, and even within the same animal over time[95,96] suggest that it will be extremely difficult to develop a reliable animal model that will be predictive for human allergenicity.

An example of an instance in which one animal model did not predict allergenicity is the Brazil nut 2S globulin protein, which was assessed by passive cutaneous anaphylaxis in mice fed the antigen orally.[97] This study reported that the 2S albumin protein did not elicit an IgE response in the mouse strains used under specific conditions. The authors came to the conclusion that the 2S gene was a strong candidate for genetic engineering into crop plants to enhance the nutritional quality of derived foods.[97] This does not mean that a more appropriate animal model would not have predicted allergenicity.

VIII. NATIONAL AND INTERNATIONAL CONSENSUS

The recommendations in this article are consistent with and expand other suggested approaches. The FDA provided guidance for allergenicity assessment in their 1992 "Food Policy" document.[9] The Environmental Protection Agency (EPA) provided some guidance in their November 1994 draft guidelines for pesticidal plants.[98] The FDA, EPA, and the U.S. Department of Agriculture (USDA) cosponsored a symposium in April 1994[97] focused specifically on assessing the allergenic potential of foods derived from genetically engineered plants. Recent workshops sponsored by the Organization for Economic Cooperation and Development (OECD)[99] and the World Health Organization (WHO)[100] also provided guidance on allergenicity assessment.

IX. FUTURE PROSPECTS

Genetic engineering can also provide an important tool to reduce the levels of specific allergenic proteins in the food supply. By suppressing gene expression, for example, by introducing genes in the antisense orientation (the opposite orientation required to produce a protein), the levels of specific proteins can be dramatically reduced. This is the technique used to produce the delayed-softening, Flav-Savr™ tomato. Inhibiting the production of the polygalacturonase enzyme, which causes the tomato to soften, extended the shelf life of the tomato.[101] This same approach has been used to significantly reduce the primary allergen in rice. Tada et al.[102] cloned the gene encoding the 16-kDa allergenic protein from rice and introduced the gene encoding this protein in the antisense orientation. The levels of the 16-kDa protein were significantly reduced in the rice seed in a number of the progeny. However, this protein was not completely eliminated in these plants. Further studies are underway to achieve greater reductions in this allergenic protein.

This approach could be used in other crops containing known allergens, such as peanuts and soybeans, to selectively reduce or eliminate the levels of specific allergenic proteins. The presence of multiple allergens in foods like peanuts and soybeans, however, greatly complicates this challenge. Furthermore, a protein that is an allergen and which also serves a critical structural or functional role cannot be removed without a negative impact on the plant.

X. FUTURE RESEARCH NEEDS

The cornerstone of allergenicity assessment is the accumulation of physicochemical, immunologic, and biochemical knowledge concerning food allergens. Assessment depends on validated assays (simulated digestion models), the availability of immunologic reagents (patient sera for assessing proteins from allergenic sources), and information on newly characterized allergens (i.e., an evolving database of allergen amino acid sequences). A reliable animal model may also be desirable.

The amino acid and/or nucleotide sequences of additional allergenic proteins, especially food allergens, and the mapping of the major B- and T-cell epitopes on known allergens would provide valuable information to expand the existing data base. Generating the physicochemical and biological data that are recommended in this article on a much larger collection of allergenic and nonallergenic proteins, as well as proteins introduced into genetically engineered plants, would help to validate the use of these criteria in the decision tree approach. The generation of easily accessible serum banks would greatly facilitate the assessments described.

A greater understanding of the molecular basis of immunopathogenesis (what makes certain food proteins allergenic) and the requirements for sensitization and elicitation of allergenic reactions is needed. Such research not only facilitates the assessment of the allergenic potential of foods derived by genetic engineering, it also serves as a basis for the development of new approaches to treat or prevent the development of allergies.

XI. SUMMARY

This article provides a science-based, decision tree approach to assess the allergenic concerns associated with the introduction of gene products into new plant varieties. The assessment focuses on the source from which the transferred gene was derived. Sources fall into three general categories: common allergenic food proteins; less common allergenic foods or other known allergen sources; and sources with no history of allergenicity. Information concerning the amino acid sequence identity to known allergenic proteins, *in vitro* and/or *in vivo* immunologic assays, and assessment of key physiochemical properties are included in reaching a recommendation on whether food derived from the genetically modified plant variety should be labeled as to the source of the transferred gene.

In the end, a balanced judgement of all the available data generated during allergenicity assessment will assure the safety of foods derived from genetically engineered crops. Using the approaches described here, new plant varieties generated by genetic modification should be introduced into the marketplace with the same confidence that new plant varieties developed by traditional breeding have been introduced for decades.

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