

Assessment of the endogenous allergens in glyphosate-tolerant and commercial soybean varieties

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A gene has been introduced into soybeans to confer tolerance to glyphosate, the active ingredient in the herbicide, Roundup (Monsanto Co., St. Louis, Mo.)^{1,2} Soybean varieties expressing this trait will enable farmers to use Roundup herbicide within the crop during the growing season. Previous studies have confirmed the safety of the introduced protein (5-enolpyruvylshikimate-3-phosphate synthase [EPSPS]), which confers glyphosate tolerance.² Over 1400 analyses have confirmed the compositional and nutritional equivalence of the soybeans produced from the glyphosate-tolerant soybean variety to the parental soybean variety.²

The multiple, endogenous protein allergens previously identified in soybeans³⁻⁵ represent an additional safety concern. The purpose of this study was to qualitatively and quantitatively compare the endogenous allergens in glyphosate-tolerant and commercial soybean varieties and to determine whether the genetic engineering process itself affected these components.

METHODS

Serum from food-sensitive patients

Five patients with atopic dermatitis and a positive immediate skin test response to soybean extracts were confirmed to be sensitive to soybean protein by a positive double-blind, placebo-controlled food challenge.³ Equal volumes of sera were combined to prepare a serum pool containing soybean-specific IgE antibody. A similar serum pool containing peanut allergen-specific IgE antibody was generated from five patients with atopic dermatitis and peanut hypersensitivity.

Abbreviations used

EPSPS:	5-Enolpyruvylshikimate-3-phosphate synthase
SDS-PAGE:	Sodium dodecylsulfate-polyacrylamide gel electrophoresis

Soybean extracts

Preparations of defatted soybean flakes and soy flour were obtained from commercial sources. Defatted soybean flakes from two independent glyphosate-tolerant soybean varieties (lines 40-3-2 and 61-67-1) and the parental control³ were also used in this study. Defatted soybean flakes or flour was ground to a fine powder, then extracted (1:20 wt/vol) at 4° C overnight in extraction buffer (20 mmol/L sodium phosphate, 1 mol/L NaCl, pH 7.2).^{3,5}

Sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting

SDS-PAGE and immunoblotting were performed as described previously.³

RESULTS

Preliminary experiments

Before the levels of endogenous allergens in glyphosate-tolerant soybean varieties were compared with those of the parental variety, immunoblot experiments were conducted to address the potential variability in endogenous allergens in commercial defatted soybean products. The results (Fig. 1, *A* and *B*) showed the presence of a consistent number of distinct IgE-binding proteins in the various products, which were comparable to the results described previously in the literature.³⁻⁵ On the basis of this information, the endogenous allergenic proteins in the glyphosate-tolerant soybeans were compared with the preparations generated from the parental and commercial varieties.

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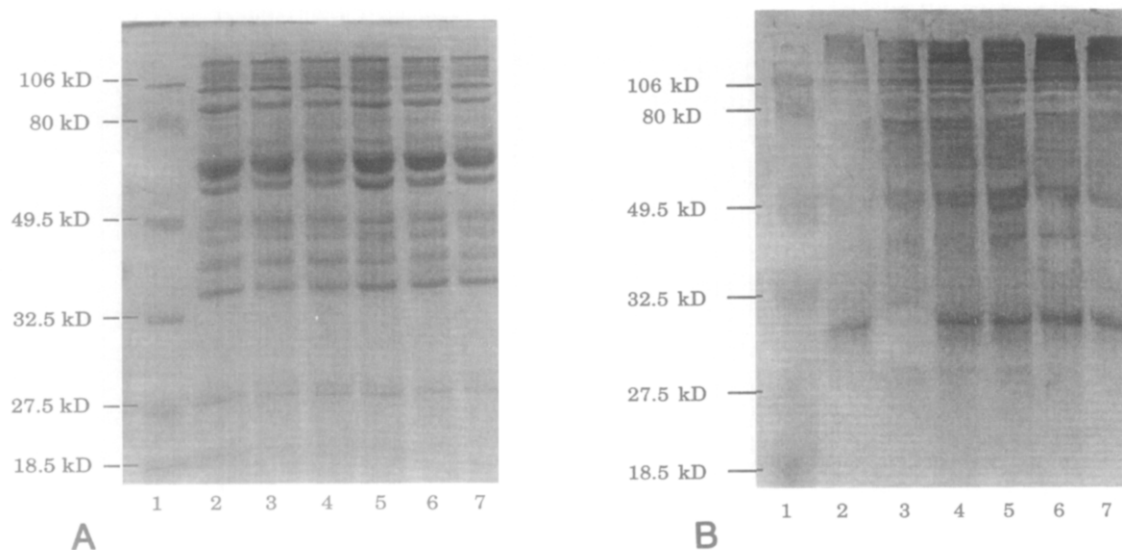


FIG. 1. **A**, Coomassie blue-stained SDS-PAGE gel with soybean extracts. *Lane 1*, Prestained molecular weight standards; *lane 2*, extract from GTS line 61-67-1 (30 μ g); *lane 3*, extract from parental line A5403 (30 μ g); *lane 4*, extract from GTS line 40-3-2 (30 μ g); *lane 5*, extract from Cargill control 1 (30 μ g); *lane 6*, extract from Cargill control 2 (30 μ g); *lane 7*, extract from ADM control (30 μ g). **B**, Immunoblot with serum containing IgE antibodies from soybean-sensitive patients. *Lane 1*, Prestained molecular weight standards; *lane 2*, extract from GTS line 61-67-1 (30 μ g); *lane 3*, extract from parental line A5403 (30 μ g); *lane 4*, extract from GTS line 40-3-2 (30 μ g); *lane 5*, extract from Cargill control 1 (30 μ g); *lane 6*, extract from Cargill control 2 (30 μ g); *lane 7*, extract from ADM control (30 μ g).

Endogenous allergens in glyphosate-tolerant and commercial soybean preparations

Four identical SDS-PAGE gels were run with extracts from each of the soybean preparations. One gel was stained with Coomassie blue (Integrated Separations Systems, Natick, Mass.). The banding patterns for the two glyphosate-tolerant varieties (Fig. 1, *A*, lanes 2 and 4) were identical to that of the parental control (Fig. 1, *A*, lane 3). The three remaining gels were blotted to nitrocellulose and incubated with the serum pools from either the soybean-sensitive, peanut-sensitive, or non-atopic control individuals, respectively. The immunoblots for the different soybean products revealed no differences in IgE-binding soybean protein patterns when the IgE antibody pool from soybean-sensitive individuals was used (Fig. 1, *B*). The areas of the most intense IgE binding were at approximately 30 kD and 50 kD, consistent with previously reported results obtained by this assay method.³ Immunoblots with the IgE antibody pools from either peanut-sensitive or nonatopic individuals showed that a minimal amount of nonspecific binding occurred at approximately 45 kD and 50 kD for all the soybean preparations (data not shown). There were no detectable differences in the banding patterns in any of the soybean samples, includ-

ing those prepared from the glyphosate-tolerant soybean varieties with any of the three pooled sera.

DISCUSSION

Our previous work³ established that there are varying amounts of IgE- and IgG-specific antibodies to multiple proteins present in soybean extracts and that the immunoblot procedure was appropriate for comparing the endogenous allergens in the soybean varieties.

The immunoblots in this investigation revealed IgE binding patterns similar to those described previously.³⁻⁵ Analysis of the protein extracts prepared from the glyphosate-tolerant soybeans showed that both the composition and quantity of the proteins detected by the immunoblot procedure were qualitatively and quantitatively indistinguishable from the results produced with the parental soybean variety, as well as with three commercial soybean preparations. In addition, the immunoblot results with serum from peanut-sensitive and the nonatopic patients showed only limited IgE binding to the soybean proteins from any of these preparations and, as expected, showed no differences between extracts prepared from the glyphosate-tolerant and parental control or commercial soybean varieties.

Our current studies demonstrate that the introduction of the gene encoding the EPSPS protein to confer glyphosate tolerance caused no discernible changes, either qualitatively or quantitatively, in the composition of endogenous soybean allergenic proteins in either of the glyphosate-tolerant varieties analyzed.

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Removal of mite allergens from blankets: Comparison of dry cleaning and hot water washing

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Allergy to house dust allergens is common among patients with bronchial asthma and allergic rhinitis. Two species of *Dermatophagoides* mite, *D. pteronyssinus* and *D. farinae*, are important sources of house dust allergens. Two groups of major allergens, Der 1 and Der 2, have been purified

from each *Dermatophagoides* species.¹ Immunoassays to measure the Der 1 and Der 2 levels have been used in studies of the level of mite allergens in indoor environments.¹

We previously reported that dust specimens obtained from bedding had more mite allergens per weight of dust than those from floors,² indicating that bedding may be a major reservoir of mite allergens. Because a great portion of our lifetime is spent in bed, it is important to reduce the allergen concentration in bedding.

Blankets are a part of bedding. McDonald and Tovey³ reported that dry cleaning of blankets did not reduce the allergen content in the dust, whereas Vandenhove et al.⁴ reported that dry cleaning reduced mite allergens and was an effective means for reducing the mite allergen level. In these studies the dust in blankets was collected with a vacuum cleaner before and after washing,

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