

**PLANT CULTURE: SEARCHING QUESTIONS**

## Genetically modified soybeans and food allergies

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### ABSTRACT

Allergenic reactions to proteins expressed in GM crops has been one of the prominent concerns among biotechnology critics and a concern of regulatory agencies. Soybeans like many plants have intrinsic allergens that present problems for sensitive people. Current GM crops, including soybean, have not been shown to add any additional allergenic risk beyond the intrinsic risks already present. Biotechnology can be used to characterize and eliminate allergens naturally present in crops. Biotechnology has been used to remove a major allergen in soybean demonstrating that genetic modification can be used to reduce allergenicity of food and feed. This provides a model for further use of GM approaches to eliminate allergens.

**Key words:** Allergenic reactions, genetic modification, soybeans.

### Allergenicity of plant products is a wide-spread problem

Public awareness and concern about food allergens is growing. Concerns about food allergens have become linked to the application of biotechnology to produce genetically engineered crops and has resulted in many regulatory proposals and regulations (see Anonymous, 2002, for example, by the Royal Society). Food allergies result from the exposure of predisposed individuals to an allergen and, once sensitized, further exposure can result in escalating adverse responses. The most common allergenic responses are atopic reactions in the form of hives and other skin responses and gastric distress, but severe reactions can result in death from anaphylaxis. As the awareness of food allergies has grown so has the public perception of the size of the sensitive population. True food allergies are an immunological response and occur in about 2% of the adult population and 5–8% of young children. The remaining cases of ‘food allergy’ are often due to other dietary difficulties such as lactose intolerance, that is, an enzyme deficiency rather than an allergy. Several different proposals have been presented to explain the apparent perception of the increased incidence of food allergies, among which is the ‘hygiene hypothesis’. This suggests that cleaner modern life-styles result in fewer immunological challenges at an early age which in turn results in increased sensitivity to other immunological challenges leading to food allergies amongst its

effects. Food allergies do have a genetic component exhibited in multiple generations of some families.

Allergies can result from exposure to new immunological challenges from the increased diversity of modern diets containing plant-derived products from throughout the world. One example is the kiwi fruit that became widely available in Western countries relatively recently. Kiwi fruit is allergenic to a significant fraction of consumers causing atopic and other reactions requiring avoidance for sensitive people. Other allergens that have the potential for serious reaction with increasing incidence in Western countries are sesame and sunflower. Allergenic reactions to the storage proteins of tree nuts, sesame, sunflower, and peanuts are responsible for many scores of deaths in the US and Europe on an annual basis, with many more worldwide, but they are not as diligently recorded in the medical literature. Any new and novel food has the potential to introduce new allergens although with the increasing world distribution of agricultural products relatively few of these ‘new’ foods have elicited significant allergies among consumers.

### Allergenicity of GM crops

The potential of any new food to induce allergic reactions has been seized upon by critics of GM crops, which contain foreign or engineered proteins, and has been promulgated as an easily understandable concern that could resonate with consumers. The fear of allergies in GM crops has had broad impact, for example, influencing the leaders of some southern African nations to reject much needed food aid to prevent famine, despite the fact that the food offered by the US was the same GM products consumed safely by most Americans. Genetic engineering of plants for experimental purposes is now commonly undertaken and the results are recorded in journals such as this one. The number of genetic modifications undertaken is now in the thousands. Most of these experiments are conducted in model plants such as *Arabidopsis* or crop plants such as tobacco, tomato and rice with no intent for release or commercialization. The GM plants created so far that have commercial potential, meeting the regulatory standards of the US have been directed at the producer’s level. These contain introduced traits for herbicide resistance and expression of a bacterial-derived insect toxin. In the near future new GM plants will be approved and grown commercially that will possess enhanced traits targeted at the consumers and testing for allergenic potential will be one of the regulatory requirements.

The potential for any protein to be a food allergen is difficult to predict with any accuracy. A decision tree that has been developed

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by the World Health Organization involves asking questions about whether the protein exhibits characteristics that might increase its probability of being an allergen. These include sequence-relationship to other known allergens and the stability of the protein to gastric digestion. Such a decision tree, however, is only a guide and not a good rigorous predictor of allergenic potential or potency. Much has been learned about the protein sequence epitopes of food allergens by using epitope maps constructed with overlapping short peptides encompassing the entire protein. The linear epitopes recognized by IgEs from sensitive people have been mapped and for most food allergens the linear epitopes consist of a few too many non-contiguous sequences that map to the surface of the allergenic proteins. A database of allergenic proteins and epitopes has been compiled and public access is available on the web (<http://www.allergome.org/> and [http://fermi.utab.edu/SDAP/sdap\\_man.html](http://fermi.utab.edu/SDAP/sdap_man.html)). What is striking about these data is that within gene families there are members that vary widely in stimulating an allergenic response. For instance the 2S family of seed storage proteins contains some of the most potent and dangerous of the plant allergens while other members of this gene family do not appear to be allergens.

#### *Soybean biotechnology and allergenicity*

Soybeans have played a central role in concerns about GM introduced allergens and in using GM to remove intrinsic allergens. Soybean protein is widely used in thousands of processed foods throughout the industrialized world and is a staple in Asia. Soybean acreage has been and continues to increase in many nations and is one of the world's great crops. In the US a large fraction of soybeans is GM, almost all of which are herbicide-resistant. Soybeans have great potential to be engineered to produce altered and novel proteins and oils, not only for food and animal feed but also as industrial precursors. Soybean milk and dairy product replacement is growing in acceptance, not only by people sensitive to lactose and/or milk proteins, but also for health considerations. Soybean oil and margarine products do not contain protein and therefore do not elicit allergenic reactions from soybean-sensitive people. Although soybean sensitivity is estimated to occur in 5–8% of children and 1–2% of adults the allergic reaction is only rarely life-threatening with the primary adverse reactions to consumption being atopic (skin) reactions and gastric distress. Biotechnology critics have claimed that an apparent rise in the number of soybean allergic individuals in the UK is correlated with the development of GM soybeans for the American market, however, there is little GM soybean availability in the UK and therefore little exposure. The soybean sensitivity increases are more easily explained by the recent acceptance and wide availability of soybean products and processed foods in the UK marketplace. As a result of consumption, the soybean-sensitive component of the population is being identified that will likely peak with the same population fraction as seen in the US. These people will need to practise avoidance of soybean products in order to minimize adverse reactions. The primary GM soybeans grown in the US are herbicide-resistant. Experiments have directly tested the allergenicity of herbicide-tolerant soybeans using immunological tests with samples from soybean-sensitive people. These assays have shown that herbicide-tolerant GM soybeans do not present any measurable differences in

allergenicity compared with non-GM soybeans (Burks and Fuchs, 1995) and are, therefore, substantially equivalent by allergenic criteria. Sensitive people remain allergic to GM soybeans, but there is no additional allergenic risk to others.

Soybeans are deficient in the essential amino acid, methionine, and in order to provide a balanced composition soybean-based animal feeds are supplemented. Among the earliest studies in altering transgenic plants for a consumer characteristic were those directed at improving seed amino acid composition, adding methionine in legumes and lysine in cereals. Initial studies with model plant seeds such as tobacco showed that genes encoding seed proteins with enhanced methionine content such as the prolamines and 2S albumins could be transferred and expressed so that these foreign proteins could contribute to the overall amino acid content. Tree nut 2S albumins such as that found in Brazil nut is rich in methionine and initial experiments with model plants showed that the gene could be transferred and expressed. The next stage of the experiments to transfer the gene to soybean to enhance methionine content was technically successful, but before any commercialization effort occurred it was recognized that the 2S Brazil nut protein is a potent human allergen with the potential for causing dangerous allergenic reactions (Nordlee et al., 1996). The development of this product was abandoned during development, no product was released and no one was harmed.

With the significant intrinsic allergenicity of soybeans, producing hypoallergenic soybeans would be a desirable commodity given the presence of soybean protein in a large fraction of processed and prepared foods in industrialized countries. The current primary treatment for food allergies is avoidance, but with soybean protein present in thousands of products soybean proteins are difficult to avoid. Hypoallergenic variants of allergenic foods have the potential to reduce the risk of adverse reactions and for less dangerous allergens this offers the prospect of permitting consumption by otherwise sensitive people. Soybeans possess as many as 15 proteins recognized by IgEs from sensitive people (Burks et al., 1988). The immunodominant soybean allergens are the  $\beta$ -subunit of conglycinin and a member of the papain family of cysteine proteases termed P34 or Gly m Bd 30k. The P34/Gly m Bd 30k protein is a unique member of the papain superfamily lacking the catalytic cysteine residue that is replaced by a glycine. These two allergens account for the large majority of the IgE cross-reactivity for soybean-sensitive people with the P34/Gly m Bd 30k protein alone accounting for about two-thirds of the IgE cross-reactivity in the two populations that have been studied, Japanese nationals and babies in the US.

There are several approaches that might be taken to produce a hypoallergenic soybean. Cultivars lacking the allergens could be identified in germplasm collections and then crossed into elite germplasm. An attempt to find soybeans lacking P34/Gly m Bd 30k was made and the results were negative, with the protein found to be present in not only domesticated soybeans but also wild soybean relatives. Immunological assays of P34/Gly m Bd 30k with antibodies from soybean-sensitive people resulted in the identification of 14 contiguous and non-contiguous linear epitopes (Helm et al., 1998). Whether there are any conformational epitopes in addition to the linear epitopes on P34/Gly m Bd 30k is unknown. The presence of so many distinct linear epitopes means that the probability of a naturally occurring variant with a sufficient number of alterations to disrupt the allergenicity is extremely small. Protein engineering could be performed to alter amino acid sequence by

disrupting allergenic sequences. Using linear peptides to test possible modifications, it is straightforward to assay numerous variants and pick one that is not recognized by the IgE population. The epitope modification approach has been studied for peanut allergens and it does seem to be feasible to produce an essentially hypoallergenic variant. While these modifications can be readily engineered *in vitro* and the resulting gene could be expressed in a transgenic plant, the problem with applying this technology in the real world is to remove completely the intrinsic allergen and substitute the 'hypoallergenic' variant in its place. Further, the modification of the protein to remove the allergenic epitopes may alter the protein's folding, that, in turn, may affect the protein's intracellular targeting, stability and accumulation. All these possibilities will need to be tested for experimentally and, finally, the newly produced hypoallergenic variant will need to be tested to ensure that it too is not a new allergen. For these reasons, substituting a hypoallergenic variant of a plant still has a high technological threshold and has yet to be achieved.

The alternative GM approach is to eliminate the allergen by suppression. There have been several attempts to reduce and/or eliminate allergens using gene suppression technology. These have included experiments to use gene suppression to eliminate pollen allergens in ragweed and rye as well as a 5-fold decrease of a rice seed allergen. Plants are not the only subject of gene suppression of allergens. There have been reports of using this approach to suppress an allergen in shrimp that is potentially dangerous to sensitive people, as well as other experiments to suppress the primary allergen of the domestic cat. Using gene-silencing techniques, my colleagues and I have produced transgenic soybeans that eliminated the immunodominant human allergen P34/Gly m Bd 30k (Herman et al., 2003). Using sense gene suppression driven by a soybean seed-specific promoter an essentially complete knockout of the P34/Gly m Bd 30k was obtained. From the initial somatic embryos through the third generation homozygous soybeans, a complete elimination of the P34/Gly m Bd 30k allergen was stably maintained. Suppression of the allergen did not introduce any changes in the pattern of growth and development of the plant or seed at both the gross and subcellular level. In order to compare the P34-suppressed soybeans with the wild type, large-scale proteomic analysis was performed. Imaging of the 2D gels identified over 1400 individual elements. Mass spectrometry analysis of about 140 of these spots confirmed that the only overt changes in composition in the transgenic soybeans was the suppression of the P34/Gly m Bd 30k protein with no other proteins induced or suppressed. Further analysis with sera samples from soybean-sensitive people confirmed a loss of the P34 allergen and no induction of any new allergens. The proteome and immunological analysis together confirms that it is feasible to

suppress an endogenous allergen without introducing adverse effects on the plant or changing the composition of the soybean seed in any way other than the removal of the targeted protein. This result meets the test of 'substantial equivalence' where the GM soybean seed is essentially identical except for the change in the single desired characteristic.

Suppressing P34/Gly m Bd 30k in GM soybeans is a first step and a demonstration in addressing the growing concerns about food allergies and its relationship to the development of GM crops. More detailed studies and approaches should provide the tests needed to gain regulatory approval in nations that are currently cautious about this technology (see Anonymous, 2002, for example, in UK). The fear of allergic reactions has produced much of the concern about the risks of GM crops. There is no evidence that GM crops actually pose risks that are even a small fraction of the risks encountered daily from the naturally occurring plant allergens. In order to broadly apply genetic modification to crops, there is an urgent need for better biochemical and molecular methods, including animal models, to test for food allergens experimentally so that the supporting data can be provided to evaluate newly proposed and actual GM products. To be able to predict allergenicity would be particularly useful as a part of the process of designing transgenes, but, currently, there are no models that would permit accurate assessment of allergenic potential of proteins unrelated to known allergens. Only by developing new tests to ensure the safety of GM products, after they are produced, can the many thousands of experiments that have and will be conducted on gene transfer and protein engineering be used to provide new products.

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