



## Commentary

## Measurement of endogenous allergens in genetically modified soybeans – Short communication



Gregory S. Ladics<sup>a,\*</sup>, Gregory J. Budziszewski<sup>b</sup>, Rod A. Herman<sup>c</sup>, Corinne Herouet-Guicheney<sup>d</sup>, Saurabh Joshi<sup>e</sup>, Elizabeth A. Lipscomb<sup>b</sup>, Scott McClain<sup>f</sup>, Jason M. Ward<sup>e</sup>

<sup>a</sup> DuPont Pioneer Agricultural Biotechnology, DuPont Experimental Station, 200 Powder Mill Road, Wilmington, DE 19803-0400, USA

<sup>b</sup> BASF Plant Science, 26 Davis Drive, Research Triangle Park, NC 27709, USA

<sup>c</sup> Dow AgroSciences, 9330 Zionsville Rd, Indianapolis, IN 46077, USA

<sup>d</sup> Bayer SAS, Bayer CropScience, 355 rue Dostoievski, CS 90153 Valbonne 06906 Sophia Antipolis, France

<sup>e</sup> Monsanto Company, Product Characterization Center, 800 North Lindbergh Blvd., St. Louis, MO 63167, USA

<sup>f</sup> Syngenta Crop Protection, LLC, 3054 E. Cornwallis Road, Research Triangle Park, NC, USA

## ARTICLE INFO

## Article history:

Received 30 May 2014

Available online 16 June 2014

## Keywords:

Genetically modified crops

Soybean

Endogenous allergens

Analytical methods

## ABSTRACT

The measurement of endogenous allergens is required by the European Commission (EC) as part of the compositional analysis for GM products from host plants that are common causes of food allergy, such as soybean (EC Implementing Regulation No. 503/2013). In each case, the EC Implementing Regulation indicates that analysis be conducted on identified allergens as specified in the Organization of Economic Cooperation and Development (OECD) consensus documents on compositional considerations for new plant varieties. This communication discusses the methods available to measure endogenous allergens as well as the endogenous soybean allergens that should be analyzed. It is suggested herein that in conjunction with the 2012 OECD consensus document on soybean, any list of soybean allergens should be based on clinically relevant data among publicly available allergen databases and peer-reviewed scientific publications, and the ability to measure the identified allergen. Based on a detailed analysis of the scientific literature, the following key points are recommended: (1) the acceptance of serum-free, quantitative analytical method data as an alternative to traditional IgE reactivity qualitative or semi-quantitative data for evaluation of endogenous soybean allergen content; (2) eight of the 15 potential allergens listed in the OECD soybean consensus document (Gly m 3, Gly m 4, Gly m Bd28K, Gly m Bd30K, Gly m 5, Gly m 6, Gly m 8, and Kunitz trypsin inhibitor) have both appropriate supporting clinical data and sufficient sequence information to be evaluated in comparative endogenous soybean allergen studies; and (3) the remaining seven proteins (Gly m 1, Gly m 2, unknown 50 kDa protein, unknown 39 kDa protein, P-22-25, lipoxygenase and lectin) lack sufficient data for clear classification as confirmed allergens and/or available sequence information and should not be currently included in the measurement of endogenous soybean allergens in the compositional analysis for the EU.

© 2014 Elsevier Inc. All rights reserved.

The comparative composition analysis between the edible tissues of a genetically modified (GM) crop and those of non-GM comparators has been a recommended component of the safety assessment of GM crops for over 20 years (Organization of Economic Development and Cooperation (OECD), 1993). Over these two decades, many studies have been conducted to evaluate crop composition, and results consistently indicate that transgenesis affects the composition much less so than conventional

breeding methods (DiLeo et al., 2014; Herman and Price, 2013; Ricroch, 2011, 2013). Our understanding of the molecular mechanisms that affect crop composition have also increased and are consistent with the finding that the insertion of a small number of genes via transgenesis is less impactful on crop composition compared with traditional breeding methods (Herman et al., 2009; Herman and Price, 2013; Parrott et al., 2010). However, analysis of crop composition continues to be a key regulatory requirement for gaining approval to commercialize GM crops, and additional regulatory requirements in this area have been formulated, including a recent requirement from the European Union (EU) Commission to measure endogenous allergen levels in soybean as part of the compositional assessment of GM crops

\* Corresponding author. Address: DuPont Pioneer Agricultural Biotechnology, DuPont Experimental Station, 200 Powder Mill Road, Wilmington, DE 19803-0400, USA. Fax: +1 302 695 3075.

E-mail address: [Gregory.s.ladics@usa.dupont.com](mailto:Gregory.s.ladics@usa.dupont.com) (G.S. Ladics).

(European Commission Implementing Regulation No. 503/2013). This new requirement has been implemented in the EU even though allergens have not been included in the list of compounds required to be measured in compositional analyses of GM soybeans (OECD, 2012).

### 1. Should endogenous allergens be measured as part of the risk assessment of GM crops?

The measurement of endogenous allergens is mentioned as part of the 'compositional analysis of key components' in GM crops including nutrients, anti-nutrients, and toxicants in the Codex Alimentarius document (Codex, 2009). This recommendation, however, has generated much debate on the relevance of such data in the risk assessment process (Panda et al., 2013; Fernandez et al., 2013; Herman and Ladics, 2011; Doerr et al., 2010; Goodman et al., 2008). The main reasons why the measurement of endogenous allergens in GM crops does not add to the risk assessment include the following: (1) allergic individuals will attempt to avoid offending foods completely, whether GM or non-GM. Given that soybean containing products are labeled, based on labeling regulations including the regulation in the EU, this risk is manageable by allergic patients. Therefore, the level of allergen(s) is irrelevant when the food is already known to be allergenic and is regulated as such; (2) measurement of endogenous allergens in non-GM crops indicates a wide range in seed concentrations due to differences in the genetics of commercial varieties (Houston et al., 2011) and the interactions of those varieties with the environment (i.e., temperature, moisture, nutrients, plant pathogens, insect loads) (Stevenson et al., 2012; Doerr et al., 2010; Goodman et al., 2008; Sancho et al., 2006 a,b). The outcome is very similar to what has been observed with other seed analytes where it has been reported that environmental and genetic differences have a much greater effect on expression of proteins, metabolites, etc. (Panda et al., 2013; Harrigan et al., 2010; Natarajan et al., 2010; Sancho and Mills, 2010; Davies et al., 2010; Bradford et al., 2005) compared with the insertion of a small number of genes via transgenesis (Ricroch et al., 2011); (3) lack of threshold data on individual proteins for either the sensitization or elicitation phase of an allergic reaction prevent the interpretation of any differences between the GM and non-GM crop (Crevel, 2010; Chassy, 2010; Taylor and Hourihane, 2008; Taylor et al., 2002). Furthermore, there is no quantitative association between the exposure to an individual amount of a protein allergen(s) and the risk for sensitization and/or clinically relevant reaction (Panda et al., 2013). In other words, although the capability to measure small differences in allergens among crop varieties is growing, it will remain a challenge to place these data within the context of assessing food safety risk. For these reasons, it is not clear what relevance the measurement of endogenous allergens has to the risk assessment of GM crops (Rouquie et al., 2010). Any guidance associated with a risk assessment process should have defined acceptance criteria to allow assessment of outcomes.

### 2. What methods are available to measure endogenous allergens?

Despite the lack of a well-defined hypothesis, the measurement of endogenous allergens is required by the European Commission as part of the compositional analysis for GM products from host plants that are common causes of food allergy, such as soybean (European Commission Implementing Regulation No. 503/2013). Prior to this new regulation in the EU, endogenous allergen data for GM soybean (versus its non-GM counterpart control and a few commercially available non-GM reference varieties) were

generated using IgE immunoblots and/or IgE ELISA or similar techniques (e.g., radioallergosorbent test) that employ sera from well-characterized soybean allergic individuals. The sera's IgE antibody reactivity to soybean allergens was measured either qualitatively or quantitatively to assess the allergen content.

The European Commission Implementing Regulation (No. 503/2013) indicates that generation of endogenous allergen data is "Generally performed by analytical methodologies such as proteomics in association with the use of allergic human sera probes. Sera from clinically well-characterized allergic individuals that are the reference material for IgE binding studies may be available in limited number, volume, and quality." The latter point is an important consideration as several hundred samples are measured for compositional analysis in support of EU submissions. Conducting IgE serum screening studies (i.e., immunoblots) to evaluate endogenous allergens on such a large number of samples is difficult due to the lack of availability of well-characterized, clinically relevant serum samples from a sufficient number of allergic subjects since the prevalence of individuals with food allergies in general is quite low (i.e., approximately 1–2% of adults and 4–6% of children) (Ladics et al., 2008). For specific food allergies the prevalence is even lower. For example, the prevalence of soybean allergy is estimated at 0.4% in children and 0.3% in adults in North America and even lower in the EU ranging from 0.04% to 0.2% (Sicherer and Sampson, 2010; Zuidmeer et al., 2008). It should also be noted that although access to any given serum sample does not take away from validating an ELISA (or other methods) using that serum for quantitative purposes. There is the inherent variability in serum IgE content from each donor patient (Shek et al., 2004; Yunginger et al., 2000) that limits the ability to repeatedly source serum as a reproducible reagent with the same IgE binding characteristics. Furthermore, with the new EC Implementing Regulation No. 503/2013, there is a need to have quantitative and comparative data from appropriately designed field trials for all compositional analytes including allergens and use of IgE containing sera to evaluate endogenous allergens by immunoblots is qualitative.

Several analytical tools, such as quantitative mass spectrometry (Stevenson et al., 2010, 2012; Houston et al., 2011) and ELISAs (Liu et al., 2012; Ma et al., 2010; You et al., 2008), are now available as alternative methods. These methods are commonly used to quantitatively measure protein expression level, and with proper validation can accurately and reproducibly measure allergen(s) with reliable specificity and sensitivity. Therefore, to comply with the new EU regulation, it is recommended that analytical tools such as ELISAs and/or mass spectrometry be employed to quantitatively measure allergens. Both of these approaches offer the opportunity for validating robust analytical methods.

### 3. Which endogenous soybean allergens should be measured?

The European Commission Implementing Regulation (No. 503/2013) indicates that "...the compositional analysis shall be carried out on an appropriate range of compounds. In each case, the applicant shall provide at least analysis on ... already identified allergens ... as referred to in the Organization of Economic Cooperation and Development (OECD) consensus documents on compositional considerations for new plant varieties". The scientific guidance for selecting key allergens for measurement is based on the identification of proteins with sufficient allergenic potency and abundance in foods to be important for public health (Bjorksten et al., 2008; EFSA, 2010). Therefore, as part of the overall recommendations to the endogenous allergen assessment, it is suggested herein that in conjunction with the OECD consensus document on soybean, any list of endogenous soybean allergens should be based on clinically relevant data among publicly available allergen databases

(e.g., [www.allergenonline.org](http://www.allergenonline.org)) and peer-reviewed scientific publications, and the ability to measure the identified allergen. Endogenous allergens should only include those proteins with appropriate clinical evidence of allergy and confirmation of an available sequence allowing the development of an analytical method (Table 1).

To this end, in this short communication the peer-reviewed literature was evaluated for 15 potential soybean allergens listed in the OECD soybean consensus document on compositional considerations for new varieties of soybean (OECD, 2012). The list of potential soybean allergens includes Gly m 1 (lipid transfer protein), Gly m 2 (defensin; storage protein), Gly m 3 (profilin), Gly m 4 (pathogenesis related protein; Bet v 1 homolog), Gly m 5 ( $\beta$ -conglycinin; storage protein), Gly m 6 (glycinin; storage protein), Gly m Bd30K (protease), Gly m Bd28K, Gly m 8 (2S albumin), Kunitz trypsin inhibitor (protease inhibitor), soybean lectin; lipoxygenase, unknown 50 kDa, and unknown 39 kDa proteins, and P22-25 protein (OECD, 2012; L'Hocine and Boye, 2007). The following criteria were used to determine if these proteins had sufficient scientific evidence to be classified as allergens: (1) the protein has been demonstrated to specifically bind IgE using sera from individuals with a clinically relevant allergy to the source of the gene/protein and/or (2) the protein causes basophil activation or histamine release, skin test reactivity and/or challenge test reactivity using subjects allergic to the soybean source.

Eight of the 15 putative OECD soybean allergens have clinically relevant data indicating that they are well-characterized food allergens (Gly m 3, Gly m 4, Gly m Bd28K, Gly m Bd30K, Gly m 5, Gly m 6, Gly m 8, and Kunitz trypsin inhibitor) (Ebisawa et al., 2013; Klemans et al., 2013; Julka et al., 2012; Fukutomi et al., 2012; Holzhauser et al., 2009; Hiemori et al., 2000; Helm et al., 1998; Burks et al., 1994). Two peer-reviewed papers suggested that Gly m 3 might be a soybean food allergen (Rihs et al., 1999; Mittag et al., 2004). Rihs et al. (1999) used serum from patients that did not have clinically diagnosed soybean induced allergic symptoms, but instead had allergic symptoms to several other foods. These patients had IgE antibodies that bound Gly m 3; however, since they did not have symptoms when eating soybean, it was not clear that these IgE antibodies, which recognized Gly m 3, had any clinical significance for soybean allergic reactions. In a more recent paper, Mittag et al. (2004) reported that 25% (6/22) of soybean allergic subjects with a positive double blind placebo controlled food challenge and/or positive case histories of soybean allergy had IgE binding to Gly m 3. Although the data is limited, given the described reactivity to soybean allergic patients, Gly m 3 is considered a putative soybean allergen.

By comparison, Gly m 1, Gly m 2, unknown 50 kDa protein, unknown 39 kDa protein, P-22-25, lectin, and lipoxygenase lacked sufficient data for clear classification as confirmed allergens and/or available sequence information. Gly m 1 is a protein present in soybean hulls which are removed during soybean processing and used for animal feed. Gly m 1 may be a possible respiratory or occupational allergen that sensitizes some workers in soybean handling facilities (Codina et al., 1999). Although there was a report of IgE binding to Gly m 1, it has also been reported that the respiratory allergy associated with soybean handling was due to contamination of the soybean by potent allergenic molds (Codina and Lockey 1998). Therefore, it is not evident that Gly m 1 is responsible for the observed occupational allergies.

Gly m 2 and the unknown 50 kDa protein are also expressed in the hull and considered as possible respiratory or occupational allergens (Codina et al., 2002; Codina et al., 1999). However, like in the Gly m 1 report described above, it was not clear if the allergic symptoms reported were associated with soybean handling or was due to contamination of the soybean by potent allergenic molds (Codina and Lockey 1998). In addition to the lack of information to conclude that they are true allergens, Gly m 2 and the unknown 50 kDa protein have only been identified by short N-terminal sequences in the scientific literature. Since there are no full-length sequences currently available for Gly m 2 and the unknown 50 kDa protein, currently it is not technically feasible to identify and quantify these proteins.

The unknown 39 kDa protein belongs to a family of plant-specific proteins of unknown function but has been found to be strongly associated with oil bodies. IgE binding was assessed for recombinant proteins that were fused to thioredoxin, a known fungal allergen (Xiang et al., 2008). The thioredoxin protein alone was also shown to bind IgE, indicating that the IgE binding might be to the thioredoxin protein and not to the unknown 39 kDa soybean protein. For this reason, the authors cautioned against interpreting the allergenic potential of the unknown 39 kDa protein (Xiang et al., 2008).

The P22-25 is a crude protein fraction with no publications demonstrating IgE reactivity to soybean allergic subject serum. Furthermore, there are no full-length or even partial protein sequences available. Since there are no full-length sequences currently available for these proteins, it is not technically feasible to identify and quantify them.

Soybean lectin is included in the compositional analysis as an anti-nutrient; however, there are no peer-reviewed publications that indicate soybean lectin could be an allergen.

**Table 1**

Summary of endogenous soybean proteins listed as allergens and the availability of their sequences and clinical data in the peer-reviewed literature.

| Soybean proteins <sup>a</sup>               | Allergen nomenclature | Clinically relevant data available? | Sequence available? | Family                                      |
|---------------------------------------------|-----------------------|-------------------------------------|---------------------|---------------------------------------------|
| Hydrophobic protein                         | Gly m 1               | No                                  | Yes                 | Lipid transfer protein                      |
| Defensin                                    | Gly m 2               | No                                  | No                  | Storage protein                             |
| Profilin                                    | Gly m 3               | Yes                                 | Yes                 | Profilin                                    |
| SAM22                                       | Gly m 4               | Yes                                 | Yes                 | Pathogenesis related protein PR-10          |
| P34                                         | Gly m Bd 30K          | Yes                                 | Yes                 | Protease                                    |
| Unknown asn-linked glycoprotein             | Gly m Bd 28K          | Yes                                 | Yes                 | Unknown                                     |
| $\beta$ -Conglycinin (vicilin, 7S globulin) | Gly m 5               | Yes                                 | Yes                 | Storage protein                             |
| Glycinin (legumin, 11S globulin)            | Gly m 6               | Yes                                 | Yes                 | Storage protein                             |
| 2S albumin                                  | Gly m 8               | Yes                                 | Yes                 | Prolamin                                    |
| Lectin                                      | Not assigned          | No                                  | Yes                 | Lectin                                      |
| Lipoxygenase                                | Not assigned          | No                                  | Yes                 | Enzyme                                      |
| Kunitz trypsin inhibitor                    | Gly m TI              | Yes                                 | Yes                 | Protease inhibitor                          |
| Unknown 39 kDa                              | Not assigned          | No                                  | Yes                 | Unknown                                     |
| Unknown 50 kDa                              | Not assigned          | No                                  | No                  | Homology to chlorophyll A-B binding protein |
| P22-25                                      | Not assigned          | No                                  | No                  | Unknown                                     |

<sup>a</sup> Soybean proteins listed as allergens in the OECD soybean consensus document (2012).

Lipoxygenases are enzymes that catalyze the deoxygenation of polyunsaturated fatty acids in lipids. These enzymes are found in plants, animals and fungi and yet are not known to be allergenic (FARRP, 2014). To date, only one peer-reviewed publication (Baur et al., 1996) indicated soybean lipoxygenases as potential allergens. However upon closer review of the study, the authors likely used a contaminated test substance. Multiple bands around the molecular weight of glycinin were observed in the SDS-PAGE of the soybean lipoxygenase commercially purchased for performing the study (Lakshmi et al., 2012).

#### 4. Recommendations and conclusion

Based on a detailed analysis of the 2012 OECD soybean consensus document and the scientific literature, the following key points are recommended:

1. The acceptance of serum-free, quantitative analytical method data as an alternative to traditional IgE reactivity qualitative or semi-quantitative data for evaluation of endogenous soybean allergen content.
2. The OECD soybean consensus document on compositional considerations for new varieties of soybean (2012) is used in conjunction with publicly available allergen data bases and the peer-reviewed literature, along with the confirmation of available sequence, to formulate the list of endogenous soybean allergens to measure for the purpose of GM crop allergenicity assessments. Based on our review of the peer-reviewed scientific literature, eight of the potential allergens listed in the 2012 OECD soybean consensus document (Gly m 3, Gly m 4, Gly m Bd28K, Gly m Bd30K, Gly m 5, Gly m 6, Gly m 8, and Kunitz trypsin inhibitor) have both appropriate supporting clinical data and sufficient sequence information to be evaluated in comparative endogenous soybean allergen studies. The remaining seven proteins (Gly m 1, Gly m 2, unknown 50 kDa protein, unknown 39 kDa protein, P-22-25, lipoxygenases and lectin) lack sufficient data for clear classification as confirmed allergens and/or available sequence information and should not be included in the measurement of endogenous soybean allergens in the compositional analysis (Table 1). Also, the annual re-evaluation of this list of soybean allergens and proteins as additional scientific information becomes available is supported.

#### References

- Baur, X., Pau, M., Czuppon, A., Fruhmann, G., 1996. Characterization of soybean allergens causing sensitization of occupationally exposed bakers. *Allergy* 51, 326–330.
- Bjorksten, B., Crevel, R., Hitchenhuber, C., Lovik, M., Samuels, F., Strobel, S., Taylor, S.L., Wal, J.M., Ward, R., 2008. Criteria for identifying allergenic foods of public health importance. *Regul. Toxicol. Pharmacol.* 51, 42–52.
- Bradford, K.J., Van Deynze, A., Gutterson, N., Parrott, W., Strauss, S.H., 2005. Regulating transgenic crops sensibly: lessons from plant breeding, biotechnology, and genomics. *Nat. Biotechnol.* 23, 439–444.
- Burks, A.W., Cockrell, G., Connaughton, C., Guin, J., Allen, W., Helm, R.M., 1994. Identification of peanut agglutinin and soybean trypsin inhibitor as minor legume allergens. *Int. Arch. Allergy Immunol.* 105, 143–149.
- Chassy, B.M., 2010. Can -omics inform a food safety assessment? *Regul. Toxicol. Pharmacol.* 58, S62–S70.
- Codex Alimentarius Commission, 2009. *Foods Derived from Modern Biotechnology*. FAO/WHO, Rome, pp. 1–85.
- Codina, R., Arduoso, L., Lockey, R.F., Crisci, C.D., Jaen, C., Bertoya, N.H., 2002. Identification of the soybean hull allergens involved in sensitization to soybean dust in a rural population from Argentina and N-terminal sequence of a major 50 KD allergen. *Clin. Exp. Allergy* 32, 1059–1063.
- Codina, R., Lockey, R.F., 1998. Possible role of molds as secondary etiologic agents of the asthma epidemics in Barcelona, Spain. *J. Allergy Clin. Immunol.* 102, 318–320.
- Codina, R., Lockey, R.F., Fernandez-Caldas, E., Rama, R., 1999. Identification of the soybean hull allergens responsible for the Barcelona asthma outbreaks. *Int. Arch. Allergy Immunol.* 119, 69–71.
- Crevel, R., 2010. In: Boye, J.I., Godefroy, S.B. (Eds.), *Allergen Management in the Food Industry*. John Wiley & Sons, Inc., New York, pp. 421–451.
- Davies, H.V., Shepherd, L.V.T., Stewart, D., Frank, T., Rohlig, R.M., 2010. Metabolome variability in crop plant species – when, where, how much and so what? *Regul. Toxicol. Pharmacol.* 58, S54–S61.
- Dileo, M.V., Bakker, M.D., Chu, E.Y., Hoekenga, O.A., 2014. An assessment of the relative influences of genetic background, functional diversity at major regulatory genes, and transgenic constructs on the tomato fruit metabolome. *Plant Gen.* 7. <http://dx.doi.org/10.3835/plantgenome2013.06.0021>.
- Doerrer, N., Ladics, G., McClain, S., Herouet-Guichene, C., Poulsen, L.K., Privalle, L., Stagg, N., 2010. Evaluating biological variation in non-transgenic crops: executive summary from the ILSI Health and Environmental Sciences Institute workshop, November 16–17, 2009, Paris, France. *Regul. Toxicol. Pharmacol.* 58, S2–S7.
- Ebisawa, M., Brostedt, P., Sjölander, S., Sato, S., Borres, M.P., Ito, K., 2013. Gly m 2S albumin is a major allergen with a high diagnostic value in soybean-allergic children. *J. Allergy Clin. Immunol.* 132 (4), 976–978.
- EFSA (European Food Safety Authority), 2010. EFSA panel on Genetically Modified Organisms (GMO). Scientific opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed. *EFSA J.* 8, 1–168.
- European Commission Implementing Regulation (EU) No. 503/2013 of 3 April 2013 on applications for authorization of genetically modified food and feed in accordance with regulation (EC) No. 1829/2003 of the European Parliament and of the council and amending commission regulations (EC) No. 641/2004 and (EC) No. 1981/2006. *Off. J. EU L157*, 1–48.
- FARRP, 2014. University of Nebraska, Lincoln, NE <http://www.allergenonline.org>.
- Fernandez, A., Mills, E.N.C., Lovik, M., Spoek, A., Germini, A., Mikalsen, A., Wal, J.M., 2013. Endogenous allergens and compositional analysis in the allergenicity assessment of genetically modified plants. *Food Chem. Toxicol.* 62, 1–6.
- Fukutomi, Y., Sjölander, S., Nakazawa, T., Borres, M.P., Ishii, T., Nakayama, S., Tanaka, A., Taniguchi, M., Saito, A., Yasueda, H., Nakamura, H., Akiyama, K., 2012. Clinical relevance of IgE to recombinant Gly m 4 in the diagnosis of adult soybean allergy. *J. Allergy Clin. Immunol.* 129, 860–863.
- Goodman, R.E., Vieths, S., Sampson, H.A., Hill, D., Ebisawa, M., Taylor, S.L., van Ree, R., 2008. Allergenicity assessment of genetically modified crops – what makes sense? *Nat. Biotechnol.* 26, 73–81.
- Harrigan, G.G., Glenn, K.C., Ridley, W.P., 2010. Assessing the natural variability in crop composition. *Regul. Toxicol. Pharmacol.* 58, S13–S20.
- Helm, R., Cockrell, G., Herman, E., Burks, A.W., Sampson, H., Bannon, G., 1998. Cellular and molecular characterization of a major soybean allergen. *Int. Arch. Allergy Immunol.* 117, 29–37.
- Herman, R.A., Chassy, B.M., Parrott, W., 2009. Compositional assessment of transgenic crops: an idea whose time has passed. *Trends Biotechnol.* 27, 555–557.
- Herman, R.A., Ladics, G.S., 2011. Endogenous allergen upregulation: transgenic vs traditionally bred crops. *Food Chem. Toxicol.* 49, 2667–2669.
- Herman, R.A., Price, W.D., 2013. Unintended compositional changes in genetically modified (GM) crops: 20 years of research. *J. Agric. Food Chem.* 61, 11695–11701.
- Hiemori, M., Bando, N., Ogawa, T., Shimada, H., Tsuji, H., Yamanishi, R., Terao, J., 2000. Occurrence of IgE antibody-recognizing N-linked glycan moiety of a soybean allergen, Gly m Bd 28K. *Int. Arch. Allergy Immunol.* 122, 238–245.
- Holzhauser, T., Wackermann, O., Ballmer-Weber, B.K., Bindslev-Jensen, C., Scibilia, J., Perono-Garoffo, L., Utsumi, S., Poulsen, L.K., Vieths, S., 2009. Soybean (*Glycine max*) allergy in Europe: Gly m 5 (beta-conglycinin) and Gly m 6 (glycinin) are potential diagnostic markers for severe allergic reactions to soy. *J. Allergy Clin. Immunol.* 123, 452–458.
- Houston, N.L., Lee, D.G., Stevenson, S.E., Ladics, G.S., McClain, S., Privalle, L., Stagg, N., Herouet-Guichene, C., Thelen, J.J., 2011. Relative and absolute quantitation of ten allergens from twenty conventional soybean varieties using tandem mass spectrometry. *J. Proteome Res.* 10, 763–773.
- Julka, S., Kuppannan, K., Karnou, A., Dielman, D., Schafer, B., Young, S.A., 2012. Quantification of gly m 4 protein, a major soybean allergen, by two-dimensional liquid chromatography with ultraviolet and mass spectrometry detection. *Anal. Chem.* 84, 10019–100130.
- Klemans, R.J., Knol, E.F., Michelsen-Huisman, A., Pasmans, S.G., de Kruijf-Broekman, W., Bruijnzeel-Koomen, C.A., van Hoffen, E., Knulst, A.C., 2013. Components in soy allergy diagnostics: Gly m 2S albumin has the best diagnostic value in adults. *Allergy* 68, 1396–1402.
- Ladics, G.S., 2008. Current codex guidelines for assessment of potential protein allergenicity. *Food Chem. Toxicol.* 46, S20–S23.
- Lakshmi, M.C., Madhusudhan, M.C., Raghavarao, K.S.M.S., 2012. Extraction and purification of lipoxygenase from soybean using aqueous two-phase system. *Food Bioprocess Technol.* 5, 193–199.
- L'Hocine, L., Boye, J.I., 2007. Allergenicity of soybean: new developments in identification of allergenic proteins, cross-reactivities and hypoallergenization technologies. *Crit. Rev. Food Sci. Nutr.* 47, 127–143.
- Liu, B., Teng, D., Wang, X., Yang, Y., Wang, J., 2012. Expression of the soybean allergenic protein P34 in *Escherichia coli* and its indirect ELISA detection method. *Appl. Microbiol. Biotechnol.* 94 (5), 1337–1345.
- Ma, X., Sun, P., He, P., Han, P., Wang, J., Qiao, S., Li, D., 2010. Development of monoclonal antibodies and a competitive ELISA detection method for glycinin, an allergen in soybean. *Food Chem.* 121 (2), 546–551.
- Mittag, D., Vieths, S., Vogel, L., Becker, W.M., Rihs, H.P., Helbling, A., Wuthrich, B., Ballmer-Weber, B.K., 2004. Soybean allergy in patients allergic to birch pollen:

- clinical investigation and molecular characterization of allergens. *J. Allergy Clin. Immunol.* 113, 148–154.
- Natarajan, S.S., 2010. Natural variability in abundance of prevalent soybean proteins. *Regul. Toxicol. Pharmacol.* 58, S26–S29.
- OECD, 1993. Safety evaluation of foods derived by modern biotechnology: concepts and principles.
- OECD, 2012. Revised consensus document on compositional considerations for new varieties of soybean [*Glycine max* (L.) Merr.]: key food and feed nutrients, antinutrients, toxicants and allergens. Series on the Safety of Novel Foods and Feeds No. 25.
- Panda, R., Ariyaratna, H., Amnuaycheewa, P., Tetteh, A., Pramod, S.N., Taylor, S.L., Ballmer-Weber, B.K., Goodman, R.E., 2013. Challenges in testing genetically modified crops for potential increases in endogenous allergen expression for safety. *Allergy* 68, 142–151.
- Parrott, W., Chassy, B., Ligon, J., Meyer, L., Petrick, J., Zhou, J., et al., 2010. Application of food and feed safety assessment principles to evaluate transgenic approaches to gene modulation in crops. *Food Chem. Toxicol.* 48, 1773–1790. <http://dx.doi.org/10.1016/j.fct.2010.04.017>.
- Ricroch, A.E., Berge, J.B., Kuntz, M., 2011. Evaluation of genetically engineered crops using transcriptomic, proteomic and metabolomic profiling techniques. *Plant Physiol.* 155, 1752–1761. <http://dx.doi.org/10.1104/pp.111.173609>.
- Ricroch, A.E., 2013. Assessment of GE food safety using ‘-omics’ techniques and long-term animal feeding studies. *New Biotechnol.* 30, 349–354.
- Rihs, H.P., Chen, Z., Rueff, F., Petersen, A., Rozynek, P., Heimann, H.X., Baur, X., 1999. IgE binding of the recombinant allergen soybean profilin (rGly m 3) is mediated by conformational epitopes. *J. Allergy Clin. Immunol.* 104, 1293–1301.
- Rouquié, D., Capt, A., Eby, W.H., Sekar, V., Herouet-Guicheney, C., 2010. Investigation of endogenous soybean food allergens by using a 2-dimensional gel electrophoresis approach. *Regul. Toxicol. Pharmacol.* 58, S47–S53.
- Sancho, A.I., Foxall, R., Browne, T., Dey, R., Zuidmeer, L., Marzban, G., Waldron, K.W., van Ree, R., Hoffmann-Sommergruber, K., Laimer, M., Mills, E.N.C., 2006a. Effect of postharvest storage on expression of the apple allergen Mal d 1. *J. Agric. Food Chem.* 54, 5917–5923.
- Sancho, A.I., Foxall, R., Rigby, N.M., Browne, T., Zuidmeer, L., van Ree, R., Waldron, K.W., Mills, E.N.C., 2006b. Maturity and storage influence on the apple (*Malus domestica*) allergen Mal d 3, a nonspecific lipid transfer protein. *J. Agric. Food Chem.* 54, 5098–5104.
- Sancho, A.I., Mills, E.N.C., 2010. Proteomic approaches for qualitative and quantitative characterization of food allergens. *Regul. Toxicol. Pharmacol.* 58, S42–S46.
- Shek, L.P.C., Soderstrom, L., Ahlstedt, S., Beyer, K., Sampson, H.A., 2004. Determination of food specific IgE levels over time can predict the development of tolerance in cow's milk and hen's egg allergy. *J. Allergy Clin. Immunol.* 114, 387–391.
- Sicherer, S.H., Sampson, H.A., 2010. Food allergy: primer on allergic and immunologic diseases. *J. Allergy Clin. Immunol.* 125, S116–S125.
- Stevenson, S.E., Houston, N.L., Thelen, J.J., 2010. Evolution of seed allergen quantification – from antibodies to mass spectrometry. *Regul. Toxicol. Pharmacol.* 58, S36–S41.
- Stevenson, S.E., Woods, C.A., Hong, B., Kong, X., Thelen, J.J., Ladics, G.S., 2012. Environmental effects on allergen levels in commercially grown non-genetically modified soybeans: assessing variation across North America. *Front. Plant Sci.* 3, 1–13.
- Taylor, S.L., Hefle, S.L., Bindslev-Jensen, C., Bock, S.A., Burks Jr., A.W., Christie, L., Hill, D.J., Host, A., Hourihane, J.O.B., Lack, G., Metcalfe, D.D., Moneret-Vautrin, D.A., Vadas, P.A., Rance, F., Skrypec, D.J., Trautman, T.A., Yman, I.M., Zeiger, R.S., 2002. Factors affecting the determination of threshold doses for allergenic foods: how much is too much? *J. Allergy Clin. Immunol.* 109, 24–30. <http://digitalcommons.unl.edu/foodsciefacpu>.
- Taylor, S.L., Hourihane, J.O.B., 2008. Food allergen thresholds of reactivity. In: Metcalfe, D.D., Sampson, H.A., Simon, R.A. (Eds.), *Food Allergy: Adverse Reactions to Foods and Food Additives*, fourth ed. Wiley, Hoboken, NJ, pp. 82–89.
- Xiang, P., Baird, L.M., Jung, R., Zeece, M.G., Markwell, J., Sarath, G., 2008. P39, a novel soybean protein allergen, belongs to a plant-specific protein family and is present in protein storage vacuoles. *J. Agric. Food Chem.* 56, 2266–2272.
- You, J., Li, D., Qiao, S., Wang, Z., He, P., Ou, D., Dong, B., 2008. Development of a monoclonal antibody-based competitive ELISA for detection of  $\beta$ -conglycinin, an allergen from soybean. *Food Chem.* 106 (1), 352–360.
- Yunginger, J.W., Ahlstedt, S., Eggleston, P.A., Homburger, H.A., Nelson, H.S., Ownby, D.R., Platts-Mills, T.A.E., Sampson, H.A., Sicherer, S.H., Weinstein, A.M., Williams, P.B., Wood, R.A., Zeiger, R.S., 2000. Quantitative IgE antibody assays in allergic diseases. *J. Allergy Clin. Immunol.* 105, 1077–1084.
- Zuidmeer, L., Goldhahn, K., Rona, R.J., Gislason, D., Madsen, C., Summers, C., Sodegren, E., Dahlstrom, J., Linder, T., Sigurdardottir, S.T., McBride, D., Keil, T., 2008. The prevalence of plant food allergies: a systematic review. *J. Allergy Clin. Immunol.* 121, 1210–1218.