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# Transgenesis affects endogenous soybean allergen levels less than traditional breeding

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ABSTRACT

The regulatory body that oversees the safety assessment of genetically modified (GM) crops in the European Union, the European Food Safety Authority (EFSA), uniquely requires that endogenous allergen levels be quantified as part of the compositional characterization of GM versions of crops, such as soybean, that are considered to be major allergenic foods. The value of this requirement for assessing food safety has been challenged for multiple reasons including negligible risk of altering allergen levels compared with traditional non-GM breeding. Scatter plots comparing the mean endogenous allergen levels in non-GM soybean isoline grain with the respective levels in GM grain or concurrently grown non-GM commercial reference varieties clearly show that transgenesis causes less change compared with traditional breeding. This visual assessment is confirmed by the quantitative fit of the line of identity ($y = x$) to the datasets. The current science on allergy does not support the requirement for quantifying allergen levels in GM crops to support safety assessment.

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## 1. Introduction

The regulatory body that oversees the safety assessment of genetically modified (GM) crops in the European Union, the European Food Safety Authority (EFSA), requires a biochemical compositional assessment of food crops, as do most government regulatory bodies (CODEX, 2009; EFSA, 2013). However, EFSA uniquely requires that endogenous allergen levels be quantified as part of the compositional characterization of GM versions of crops, such as soybean, that are considered to be major allergenic foods. The value of this requirement for assessing food safety has been challenged for multiple reasons including negligible risk of altering allergen levels compared with traditional non-GM breeding (Herman and Ladics, 2011; Graf et al., 2014). Here, we compare the allergen composition profiles of multiple GM soybean events and breeding stacks with a near-isogenic non-GM line (isoline), and contrast this with differences between the same isolines and concurrently grown non-GM commercial reference varieties. Both graphical and statistical approaches are employed that allow the effects of transgenesis and GM-trait stacking on endogenous soybean allergen levels to be placed into the context of traditional breeding.

## 2. Methods and materials

### 2.1. Test entries and field trials

Test entries included four GM lines (DAS-44406-6, DAS-81419-2, DAS-81419-2 x DAS-44406-6, and DAS-68416-4 x MON-89788-1), a matched non-GM near-isogenic line (Maverick), and twenty different non-GM commercial reference varieties. DAS-44406-6 soybean expresses the AAD-12, 2mEPSPS, and PAT proteins that confer tolerance to the herbicides 2,4-D, glyphosate, and glufosinate, respectively; DAS-81419-2 soybean expresses the Cry1F and Cry1Ac insecticidal proteins, and the PAT protein; MON-89778-1 soybean expresses the CP4 EPSPS protein that confers tolerance to glyphosate. Field trials were conducted as previously reported (Herman et al., 2011; Lepping et al., 2013). Briefly, multi-site trials were conducted with a four-replicate randomized complete block experimental design at each site (Hill et al., 2017). Three non-GM

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commercial reference varieties were included at each site. Additional field-trial details can be found in Table 1.

### 2.2. Allergen quantification

Eight endogenous soybean allergens were quantified in grain based on their relevance to allergy in humans (Ladics et al., 2014). These allergens are Gly m 1, Gly m 3, Gly m 4, Gly m 5, Gly m 6, Gly m Bd 28k, Gly m Bd 30K, and Gly m 8. Allergen levels were quantified by LC-MS/MS as previously described (Hill et al., 2017). Briefly, soybean seeds were ground, lyophilized, weighed into 100 (±0.5) mg aliquots and stored at −80 °C until analysis. Each aliquot was defatted with hexane and then extracted with buffer (5 M urea, 2 M thiourea, 50 mM Tris-HCl and 65 mM dithiothreitol) for 1 h in a thermomixer. The samples were centrifuged and diluted 10X with HPLC water to bring allergen concentrations into the peptide calibration range. Aliquots of diluted extracts were denatured and reduced with dithiothreitol at 95 °C for 20 min, followed by refrigeration at 4 °C for 10 min. The denatured extracts were pH buffered with 1 M Tris-HCl followed by overnight incubation (~15 h) at 37 °C with 5 μg trypsin enzyme. Following digestion, a multi-stock of heavy isotope labeled peptide internal standards was added to each digested extract, the digestion reaction was quenched with formic acid/water (50/50, v/v), and centrifuged at 4 °C for 10 min. Following centrifugation, the digested extracts were analyzed along with calibration standards containing both synthetic natural abundance peptides and heavy isotope labeled peptide internal standards by LC-MS/MS.

### 2.3. Data analysis and interpretation

Previously published methods of data analysis were adapted whereby the mean levels of compositional components are profiled in scatter plots (Fast et al., 2016; Herman et al., 2017). Specifically, the mean allergen levels (in the natural and base-10- logarithmic scale) of the GM lines were plotted against their respective isolines (grown concurrently), and the allergen levels of the non-GM commercial reference varieties were also plotted against these same isolines (grown concurrently). Means were calculated across multiple studies when the same GM entry or non-GM reference variety was present (Table 1). The observed level of scatter around the line of identity (y = x) was then used to subjectively assess variability due to each breeding method. To quantify the relationship between the allergen profiles of different test entries, a coefficient of identity ($I^2$) was calculated (Herman et al., 2017). The $I^2$ describes the variability in the data captured by the line of identity in a manner analogous to how the coefficient of determination ($R^2$) describes the variability in data captured by a regression line. Less scatter on the plots and higher $I^2$ values indicate greater identity between allergen levels in the compared soybean lines.

## 3. Results and discussion

Location-matched mean levels of different allergens for the isoline vary more in scatter plots vs. the non-GM reference varieties than in scatter plots vs. the GM lines (greater spread across x-axis; Fig. 1). This reflects differences in the locations used to calculate location-matched isoline means because non-GM reference

**Table 1**
Soybean variety, growing season, and location Information.

| GM events and breeding Stacks in Indicated Study (x's in each column denote entry) | | | | | | |
|---|---|---|---|---|---|---|
| GM Event | Study 110006 | Study 120043 | | | Study 150658 | |
| DAS-44406-6 | | x | | x | x | x |
| DAS-68416-4 | x | | | | | |
| DAS-81419-2 | | | x | x | | x |
| MON-89788-1 | x | | | | | |
| Growing Season | 2011 | 2012 | | | 2015 | |
| Site 1 | Atlantic, IA | Atlantic, IA | | | Atlantic, IA | |
| Site 2 | Richland, IA | Richland, IA | | | Richland, IA | |
| Site 3 | Carlyle, IL | Carlyle, IL | | | Carlyle, IL | |
| Site 4 | York, NE | York, NE | | | York, NE | |
| Site 5 | Germansville, PA | Germansville, PA | | | Germansville, PA | |
| Site 6 | Fisk, MO | Fisk, MO | | | Fisk, MO | |
| Site 7 | Wyoming, IL | Wyoming, IL | | | Stewardson, IL | |
| Site 8 | Sheridan, IN | Sheridan, IN | | | Kirklin, IN | |
| Site 9 | La Plata, MO | Kirksville, MO | | | Kirksville, MO | |
| Site 10 | Brunswick, NE | – | | | Brunswick, NE | |
| Reference Lines (Planting Sites) | | | | | | |
| Reference 1 | DSR 99915 (2, 3, 8, 9, 10) | DSR 3510 (1, 2, 6, 7, 9) | | | Ag Venture AV 39A0 (1, 6, 7) | |
| Reference 2 | HiSoy 38C60 (1, 3, 4, 5, 7) | Dyno-Gro 3410SCN (1, 4, 5, 7) | | | Becks 389N (1, 2, 5) | |
| Reference 3 | Hoffman H387 (1, 2, 4, 5, 8) | Dyno-Gro V388SCN (3, 5, 6, 9) | | | Becks 401 (3, 8, 10) | |
| Reference 4 | IL 3503 (3, 4, 6, 7, 8) | L&M 34 (1, 3, 4, 6, 8) | | | DSR 36Y14Y1 (1, 3, 9) | |
| Reference 5 | LG Seeds C3884N (1, 4, 6, 9, 10) | Pioneer 93Y41 (2, 3, 7, 8) | | | Mark C1438SB (8, 5, 10) | |
| Reference 6 | Williams 82 (2, 5, 6, 7, 9) | Stine 3900-2 (2, 4, 5, 8, 9) | | | Pfister 39C74 (4, 6, 9) | |
| Reference 7 | – | – | | | Stine 3822-2 (2, 4, 9) | |
| Reference 8 | – | – | | | Stine 3900-2 (5, 6, 10) | |
| Reference 9 | – | – | | | Stine 3920-2 (4, 7, 8) | |
| Reference 10 | – | – | | | Williams 82 (2, 3, 7) | |

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