Regulatory Toxicology and Pharmacology 89 (2017) 70–73

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

Transgenesis affects endogenous soybean allergen levels less than traditional breeding

Ryan C. Hill*, Brandon J. Fast, Rod A. Herman

Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, IN 46268, United States

ARTICLE INFO

Article history:
Received 7 June 2017
Received in revised form
13 July 2017
Accepted 14 July 2017
Available online 15 July 2017

Keywords:
Surrogate peptide
Multiplexing method
Method validation
Endogenous allergens
Soybean
Genetically engineered
LC-MS/MS
Mass spectrometry
Food safety
Glycine max

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.

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

* Corresponding author.
E-mail address: rhill1@dow.com (R.C. Hill).

http://dx.doi.org/10.1016/j.yrtph.2017.07.013
0273-2300/

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)	

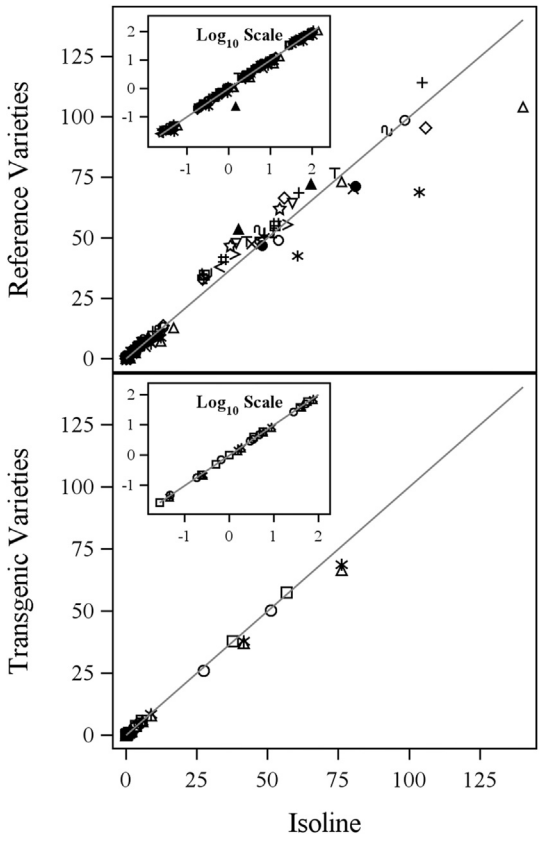

Fig. 1. Soybean allergens. Isoline vs. location-matched reference varieties (upper panel) and GM lines (lower panel). Analytes from left to right: Gly m 3, Gly m 4, Gly m 1, Gly m Bd 28 K, Gly m Bd 30 K, Gly m 8, Gly m 5, and Gly m 6. (μg/mg). Line of identity (y = x) shown. The symbols representing each GM and non-GM variety are listed in Table 2.

varieties were included in only a subset of locations while GM lines were present at all locations within each study (Table 1), and because soybean allergen levels are known to be more affected by environment than genotype (Geng et al., 2017). This difference in spread among the isoline means between these comparison groups will necessarily inflate the I^2 values for the isoline vs. non-GM reference varieties compared with the isoline vs. GM varieties. This artifact is mitigated by transformation to the base-10 logarithmic scale where the spread of isoline values is equivalent between comparison groups (Fig. 1 insets).

Allergen levels were relatively consistent across non-GM soybean varieties ($I^2 = 0.66$ to 0.997; Fig. 1, Table 2) as expected based on previous reports that environment impacts soybean allergen levels more than genotype (Geng et al., 2017; Hill et al., 2017; Stevenson et al., 2012). However, scatter plots comparing the mean endogenous allergen levels in the soybean isoline grain with the respective levels in GM grain or concurrently grown non-GM commercial reference-variety grain show more scatter around the line of identity for comparisons with non-GM reference varieties, even over the shared range of isoline values (x-axis, Fig. 1). The uniformly high coefficients of identity between the isoline and the GM lines (>0.97) compared with the isoline and non-GM commercial reference varieties (>0.66) provides quantitative confirmatory evidence of this (Table 2). However, comparisons in I^2 values calculated in the base-10 scale are more appropriate in this situation since the spread of values along the x-axis is more similar in this scale (Fig. 1 insets). In the base-10 logarithmic scale, the distribution of I^2 values for the isoline vs GM lines is clearly

Table 2
Comparison of coefficients of identity between GM and non-GM soybean.

Entry Type	Entry Name	Plot Symbol	I^2 (I^2 Log_{10} Scale)
Transgenic Event or Breeding Stack	DAS-81419-2	○	0.9986 (0.9997)
	DAS-444Ø6-6	△	0.9732 (0.9971)
	DAS-81419-2 × DAS-444Ø6-6	✱	0.9843 (0.9986)
	DAS-68416-4 × MON-89788-1	□	0.9997 (0.9997)
Non-Transgenic Reference Variety	AgVenture AV 39A0	○	0.9973 (0.9983)
	Beck's 389N	△	0.8813 (0.9855)
	Beck's 401	✱	0.6637 (0.9758)
	DSR 3510	□	0.9816 (0.9967)
	DSR 36Y14Y1	◇	0.9767 (0.9987)
	DSR 99915	☆	0.9642 (0.9961)
	Dyna-Gro 3410SCN	▽	0.9865 (0.9986)
	Dyna-Gro V388SCN	↓	0.9777 (0.9976)
	HiSOY 38C60	▽	0.9751 (0.9969)
	Hoffman H387	>	0.9930 (0.9993)
	IL3503	<	0.9893 (0.9948)
	LG Seeds C3884N	#	0.9846 (0.9970)
	L&M 34	I	0.9885 (0.9984)
	Mark C1438SB	+	0.9877 (0.9933)
	Pfister 39C74	T	0.9920 (0.9938)
	Pioneer 93Y41	U	0.9802 (0.9978)
	Stine 3822-2	∾	0.9949 (0.9966)
	Stine 3900-2	×	0.9778 (0.9962)
	Stine 3920-2	●	0.9797 (0.9981)
	Williams 82	▲	0.9570 (0.9331)

elevated compared with the isoline vs. non-GM reference varieties. The chances of randomly seeing this pattern of I^2 values can be conservatively calculated using probability theory (binomial distribution) from the observation that only eight of the twenty I^2 values comparing the isoline to the non-GM reference line exceed the minimum I^2 value comparing the isoline to the GM lines (0.9971). The chance of randomly selecting a set of four reference varieties all from the eight of twenty varieties with the highest I^2 values is less than 1.5%. This indicates less impact of transgenesis and stacking of GM traits on soybean allergen levels compared with non-GM variety development (Fig. 2).

4. Conclusion

With limited resources available to evaluate risks, judicious regulation of technologies by government agencies should focus resources proportionally based on the magnitude of risk. Results clearly indicate that the risk of altering endogenous allergen levels

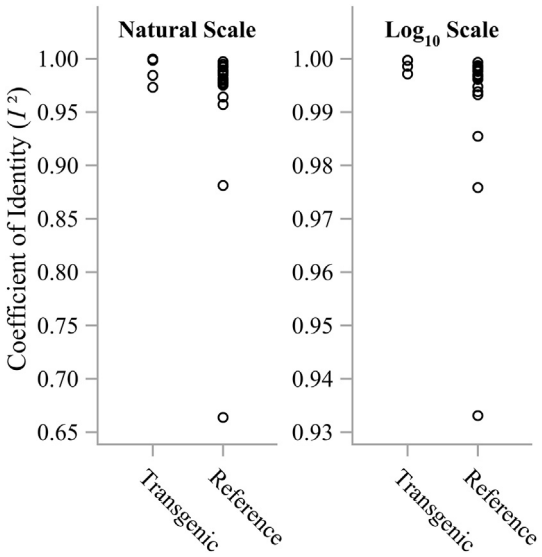

Fig. 2. Coefficient of identity (I^2) for allergen levels in isoline soybean predicting levels for transgenic lines and non-GM commercial reference varieties determined from results in both the natural and base-10 logarithmic scales.

through transgenesis is less than that of traditional breeding. This is particularly noteworthy because genotype within non-GM soybean varieties has been shown to influence endogenous allergen levels much less than growing environment, such that genotype differences among non-GM varieties is not the main factor contributing to allergen variation (Geng et al., 2017). In addition, it is not clear if increased or decreased exposure to allergens would reduce sensitization rates in a population (Herman and Ladics, 2011; Du Toit et al., 2015). The current science on allergy does not support the requirement for quantifying allergen levels in GM crops to support safety assessment.

Acknowledgements

We thank Jeff Gilbert, Guomin Shan, Nick Storer, Chandra Aradhya, Barry Schafer, and Kathryn Clayton from Dow AgroSciences and Kevin Glenn from Monsanto for reviewing a draft of this manuscript.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.yrtph.2017.07.013.

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