



Investigation of endogenous soybean food allergens by using a 2-dimensional gel electrophoresis approach

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ABSTRACT

As part of the safety assessment of genetically modified (GM) soybean, 2-dimensional gel electrophoresis analyses were performed with the isoxaflutole and glyphosate tolerant soybean FG72, its non-GM near-isogenic counterpart (Jack) and three commercial non-GM soybean lines. The objective was to compare the known endogenous human food allergens in seeds in the five different soybean lines in order to evaluate any potential unintended effect(s) of the genetic modification. In total, 37 protein spots representing five well known soybean food allergen groups were quantified in each genotype. Qualitatively, all the allergenic proteins were detected in the different genetic backgrounds. Quantitatively, among 37 protein spots, the levels of accumulation of three allergens were slightly lower in the GM soybean than in the non-GM counterparts. Specifically, while the levels of two of these three allergens fell within the normal range of variation observed in the four non-GM varieties, the level of the third allergen was slightly below the normal range. Overall, there was no significant increase in the level of allergens in FG72 soybean seeds. Therefore, the FG72 soybean can be considered as safe as its non-GM counterpart with regards to endogenous allergenicity. Additional research is needed to evaluate the biological variability in the levels of endogenous soybean allergens and the correlation between level of allergens and allergenic potential in order to improve the interpretation of these data in the safety assessment of GM soybean context.

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1. Introduction

Tolerance to the herbicides isoxaflutole and glyphosate has been conferred to soybean by inserting the genes encoding the HPPD W336 and 2mEPSPS proteins, respectively, into the soybean genome. The resulting genetically modified (GM) soybean is called FG72 soybean. As for all GM crops, a robust evaluation of the safety of FG72 soybean has been performed according to the regulatory recommendations (e.g. Codex Alimentarius, 2009; EFSA, 2006). This assessment includes an evaluation of potential unintended effects. In particular, it addresses the possibility that the genetic modification could modulate directly or indirectly the level of accumulation of endogenous allergens and consequently could increase the allergenic potential of the GM crop when compared to its non-GM counterpart.

Soybean (*Glycine max* L. Merr.) is described as one of the main allergenic food crops in the labeling regulation in many continents (e.g. European Union, United States of America, Japan). Clinical

allergic reactions to soybean can be systemic, but typically have more localized effects including the skin, the gastrointestinal tract, or the respiratory tract (Savage et al., 2010). The severity of the reaction ranges from mild rashes up to anaphylaxis. The prevalence of soybean allergy is estimated at 0.4% in children and 0.3% in adults North America (Sicherer and Sampson, 2010). The meta-analysis from Zuidmeer et al. (2008) found soybean allergy prevalence in Europe ranging from 0.04% to 0.2%. These estimates are consistent with the first results of the EuroPrevall observations (communication from Dr. C. Mills at the 2010 EAACI congress). However, because soybean is widely used as a source of protein in the food and beverage industry, traces of soybean might represent hidden allergens (Ballmer-Weber and Vieths, 2008). Therefore, despite the relatively low prevalence of soybean allergy in the Western hemisphere, labeling regulations (e.g. from Codex Alimentarius, US Food and Drug Administration, European Union) incorporated soybean into the major allergenic food lists (EU, 2000, 2003, 2005, US-FDA, 2004).

Many soybean allergenic proteins have been identified, characterized and recorded in multiple allergen databases. The publicly available allergen database AllergenOnline (www.allergenonline.org), contains the highest number of allergens that have been

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sequenced. In total, this database contains 38 protein sequences of soybean allergens. The present work was focused on 25 soybean allergens, corresponding to food allergens expressed in soybean seeds that were unequivocally resolved with our protein separation technique. These 25 allergens belong to five allergen groups: Gly m 5, Gly m Bd30 K, Gly m Bd28 K, Gly m 6 and Gly m TI (see Table 1). These groups have conserved structural features related to their biological activity, which may explain the wide immunochemical cross-recognition observed among members of the legume family e.g. garden pea, chick pea, green pea, lima bean, string bean and peanut (Ballmer-Weber and Vieths, 2008; Barnett and Howden, 1987; Bernhisel-Broadbent and Sampson, 1989; Eigenmann et al., 1996; Fries, 1971; Hagner et al., 1998).

To evaluate the levels of known endogenous food allergens in soybean, 2-dimensional gel electrophoresis (2D-GE) is a method of choice. The applicability of such a proteomic approach for the safety assessment of GM crops was evaluated with success in several projects (FAO/WHO, 2001; GMOCARE, 2002; Ruebelt et al., 2006). The 2D-GE constitutes a high resolution and powerful technique to investigate the soybean proteome (Natarajan et al., 2007; Selgrade et al., 2009). Proteins are separated according to their isoelectric point (pI) in the first dimension and molecular weight (MW) in the second dimension. Coupled with Coomassie blue staining, it allows the simultaneous detection and quantification of several hundreds of individual proteins present in complex protein mixtures (Righetti et al., 2004), including known soybean allergens.

In the present study, the levels of endogenous known food allergens (25 allergen sequences represented in 37 protein spots) of FG72 soybean was investigated. After identification of the protein spots corresponding to the food allergenic polypeptides in the 2D gels by mass spectrometry, the FG72 soybean allergenic polypeptides were individually compared to those from its non-GM counterpart, at the qualitative and quantitative levels. In addition, these polypeptides were measured in three commercial non-GM soybean lines (Stine® 3000-0, Stine® 2686-6 and Stine® 2788), which provided an insight into the biological variability of endogenous soybean allergens.

2. Material and methods

2.1. Selection of the allergenic polypeptides

AllergenOnline database is a free, publicly available, archived resource list of known and putative allergenic sequences, including all known soybean food allergens. This database was developed by the Food Allergy Research and Resource Program (FARRP). The database is updated annually by searching National Center for Biotechnology Information (NCBI) and International Union of Immunological Societies (IUIS) annotated sequences and by evaluating the candidate entries for evidence of protein allergenicity (i.e. IgE binding test) and food allergy (e.g. clinical test). A peer review panel of international food allergy experts is in charge of this curation. The pre-defined criteria for curation and the list of experts are reported on the website (<http://www.allergenonline.org>; version 9.2; updated in 2009). This database includes 1386 unique sequences that are clustered into 502 allergen groups based on species ($n = 236$) and sequence identities (Thomas et al., 2008). According to this database, 38 soybean sequences have been identified (25 soybean food allergens plus isoforms). A limited number of these allergens are responsible for a majority of the allergenic responses observed in the allergic patients exposed to soybean foods. Table 1 summarizes the list of the 37 protein spots, with their respective identification (ID) and gi numbers investigated in the study.

2.2. Seed material

The FG72 soybean is genetically engineered to be tolerant to the herbicides isoxaflutole and glyphosate. Its non-GM near-isogenic counterpart soybean (Jack) was used as the comparator. Three non-GM commercial lines (Stine® 3000-0, Stine® 2686-6 and Stine® 2788) were also included for estimating the biological variability of endogenous soybean allergens. The seeds were collected from a field trial conducted in Marcus, Iowa, USA, which is one of the typical soybean production areas in North America. Three randomized plots were planted with the FG72 variety and three randomized plots with the Jack variety. Three additional plots were planted with the three commercial lines (one plot each). The plants were grown under conditions typical of production practices for Group II maturity soybeans. In addition, the FG72 soybean plots were sprayed with isoxaflutole herbicide at a target rate of 70 g active ingredient/Ha and with glyphosate herbicide at a target rate of 1060 g active ingredient/Ha.

The presence or absence of the DNA sequence containing the genes responsible for the tolerance to isoxaflutole and glyphosate was verified in the FG72, Jack, and in the three commercial soybean lines by a validated event-specific PCR protocol. As expected, a 263-bp long PCR amplified fragment was obtained with FG72, while no amplification was obtained with non-GM soybean varieties (data not shown).

3. 2D-GE

After harvesting, 100 g of FG72 or Jack seeds from each plot were pooled before grinding. For the three commercial lines, samples of seeds from each plot were used directly. Frozen soybean seed powders were used for total soybean protein extraction (Friry-Santini et al., 2007). Frozen soybean seed powders were homogenized with an Ultraturax in urea/thiourea buffer (7 M urea, 2 M thiourea, 4% 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS) (w/v), 0.24% Triton X100 (v/v)) supplemented with 20 mM spermine, and 50 mM dithiothreitol. After incubation for 1 h at room temperature, 100 mM iodoacetamide was added to the samples, and the mixtures were incubated for 3 h at room temperature in the dark. After centrifugation (15,000 rpm for 30 min at 20 °C), supernatants were removed, aliquoted and kept frozen at approximately –74 °C until further use. Protein concentration was determined by the modified Bradford protein assay using bovine serum albumin (BSA) as the standard.

For each genotype, the 2D-GE analysis was performed in triplicate, and with two protein loading amounts. Isoelectric focusing (IEF) was carried out using a Protean IEF Cell (Bio-Rad, France). Precast immobilized pH gradient (IPG) ReadyStrips™ (pH range 4–7, 24 cm) were rehydrated with 460 µl urea/thiourea buffer supplemented with 100 mM 2-hydroxyethyl disulfide (2-HED, Acros Organics, Belgium) and 0.001% bromophenol blue as a tracking dye (approximately 9 h of passive rehydration at 20 °C). Using a cup-loading system (Bio-Rad), either 600 µg (q1) or 60 µg (q2) of total soybean proteins in 75 µl urea/thiourea buffer supplemented with 100 mM 2-HED were loaded onto IPG strips. Loading order of each sample on Precast IPG ReadyStrips™ was randomly defined. The proteins were focused successively for 15 min at 250 V, followed by a slow voltage ramping to 10,000 V. Focusing was continued at 10,000 V up to 300,000 Vh at 20 °C under low viscosity oil. After IEF, Precast IPG ReadyStrips™ were stored at approximately –74 °C until Sodium dodecyl sulfate–polyacrylamide gels electrophoresis (SDS–PAGE) was run. After thawing, prior to SDS–PAGE, the IPG strips were incubated at room temperature for 30 min in an equilibration buffer (0.375 M Tris–HCl [pH 8.8], 6 M urea, 20% glycerol, 4% SDS) containing 65 mM dithiothreitol. A second

Table 1

List of the soybean endogenous food allergens investigated with the corresponding identification names in the 2D maps, the allergen group the protein name the Genbank accession number, the score value, the number of matched peptides and the percentage of sequence coverage.

Allergen group (common name)	Protein ID on 2D gels	Protein Name	gi number	Score value	Matched peptide number	Sequence coverage (%)
Gly m 5 (Conglycinin)	GLCA_14	Beta-conglycinin, alpha chain [Precursor]	18536	1046	52	37
	GLCA_15	Beta-conglycinin, alpha chain [Precursor]	18536	195	14	18
	GLCA_16	Beta-conglycinin, alpha chain [Precursor]	18536	328	18	18
	GLCA_17	Beta-conglycinin, alpha chain [Precursor]	18536	558	21	24
Gly m Bd30 K (P34)	Bd30_K_19	Gly m Bd 30 K	1199563	417	17	18
	Bd30K_20	Gly m Bd 30K	1199563	339	16	18
Gly m Bd28 K (Vicilin)	Bd28_K_12	Gly m Bd 28K	12697782	292	19	13
Gly m 6 (11S-globulin)	GY1A1a_26	Glycinin G1 [Precursor A1a subunit]	121276	807	38	34
	GY1A1a_27	Glycinin G1 [Precursor A1a subunit]	121276	1095	42	37
	GY1A1a_28	Glycinin G1 [Precursor A1a subunit]	121276	1291	66	42
	GY1A1a_29	Glycinin G1 [Precursor A1a subunit]	121276	1574	74	42
	GY1A1a_30	Glycinin G1 [Precursor A1a subunit]	121276	1834	87	44
	GY1A1a_31	Glycinin G1 [Precursor]	18635	405	13	30
	GY1A1a_32	Glycinin G1 [Precursor]	18635	231	13	22
	GY1A1a_33	Glycinin G1 [Precursor]	18635	255	14	29
	GY1A1a_34	Glycinin G1 [Precursor]	18635	97	8	20
	GY2B1a_9	Glycinin G2 [Precursor]	18637	422	35	31
	GY2B1a_10	Glycinin G2 [Precursor]	18637	579	33	23
	GY2A2_13	Glycinin G2 [Precursor]	18637	163	5	10
	GY2A2_23	Glycinin G2 [Precursor]	121277	1737	69	31
	GY2A2_24	Glycinin G2 [Precursor]	121277	1965	73	29
	GY2A2_25	Glycinin G2 [Precursor]	18637	749	58	34
	GY3B1b_11	Glycinin G3 [Precursor]	18639	192	8	12
	GY4B3_8	Glycinin G4 [Precursor]	18641	226	5	29
	GY4A5A4_18	Glycinin G4 [Precursor]	732706	479	13	19
	GY4A4_21	Glycinin G4 [Precursor]	18641	1600	64	28
	GY4A4_22	Glycinin G4 [Precursor]	18641	2690	115	29
	GY5A3_35	Glycinin G5 [Precursor]	736002	1444	56	46
	GY5A3_36	Glycinin G5 [Precursor]	736002	1782	66	41
	GY5A3_37	Glycinin G5 [Precursor]	169969	641	26	31
Gly m TI (Kunitz soybean trypsin inhibitor)	KTI3_1	Trypsin inhibitor A [precursor]	125020	1142	36	47
	KTI3_2	Trypsin inhibitor A [precursor]	125020	2541	98	64
	KTI3_3	Trypsin inhibitor A [precursor]	125020	594	44	47
	KTI (1,2,3)_4	Trypsin inhibitor A [precursor]	125020	374	18	50
	KTI (1,2,3)_5	Trypsin inhibitor KTI 1 [precursor]	125722	892	31	54
	KTI (1,2,3)_6	Trypsin inhibitor A [precursor]	125020	725	37	50
	KTI (1,2,3)_7	Trypsin inhibitor KTI 1 [precursor]	125722	374	26	48

equilibration step was carried out for 40 min under the same conditions, except that dithiothreitol was replaced by 135 mM iodoacetamide and 0.001% bromophenol blue was added as a tracking dye. SDS–PAGE was carried out by using the Ettan™ DaltSix and Ettan™ DaltTwelve Electrophoresis Units (GE Healthcare, France). Briefly, the equilibrated strips were loaded onto homemade 0.1%SDS–12.5% polyacrylamide gels, with 0.1%SDS–4% polyacrylamide on top as a stacking gel. IPG strips were sealed with 1% low melting point agarose to ensure good contact between the IPG strips and the SDS–polyacrylamide gel. The electrophoresis was performed at approximately 15 °C with Tris–glycine buffer (25 mM trizma base, 192 mM glycine, 0.1% SDS) at a constant power of 5 W per gel for 30 min followed by 12.5 W per gel until the tracking dye reached the bottom of the gel. The gels were stained with colloidal Coomassie blue dye as recommended by the manufacturer (SafeStain, Invitrogen, France) and digitized using a high-resolution scanner (GS-800™ calibrated densitometer, BioRad).

3.1. Protein spot identification

The protein spots of interest were manually excised from the 2-D gel. Each protein sample was further processed by enzymatic digestion with trypsin to generate peptide fragments (Cayatte et al., 2006). Mass spectra were recorded with a matrix-assisted laser desorption/ionization time of flight (MALDI-ToF) spectrometer (Voyager DE-PRO, PerSeptive Biosystems, Framingham, MA) set in positive reflectron mode. Peptide mass profiles obtained by MALDI-MS were analyzed using Protein Prospector. Peptide masses were compared with the theoretical masses derived from the sequence contained in SWISS-PROT/TrEMBL databases or deduced from the nrNCBI Data Bank.

3.2. Image analysis

To compare soybean food endogenous allergens qualitatively (presence/absence of spots) and quantitatively (spot intensity), 24 images for each protein concentration (q1 and q2) were analyzed in parallel. The scanned images of the gels were analyzed with the PDQuest image analysis system (version 7.2, BioRad). For each gel, the raw intensity (quantity) of each protein spot was normalized with regard to the total intensity of all the spots in each gel. As described above, a total of 37 spots were analyzed on the 2D-PAGE maps.

For each type of soybean seeds, two protein extract amounts of 600 µg (q1) and 60 µg (q2) were used. In order to ensure an accurate measurement of the level of accumulation of all polypeptides, the highly abundant soybean seed proteins (18 polypeptides in total) were quantified in the gels loaded with 60 µg of proteins (q2 gels) whereas those which were less abundant (19 polypeptides in total) were quantified in the gels loaded with 600 µg of proteins (q1 gels). For each spot, the value of intensity was obtained in three replicates, then mean and standard deviation were calculated.

3.3. Statistical comparison

Means of the FG72 replicates were compared to means of the Jack replicates by using the *t*-test (2-sided) at the 5% level of significance. Statistical analyses were carried out using the software Spotfire version 9.1.1 module functional genomics (Tibco).

4. Results

4.1. Qualitative analysis

As expected, 37 spots corresponding to the known endogenous allergenic polypeptides were identified in the FG72 and its

near-isogenic counterpart (Jack) soybean seed groups. Fig. 1A is a representative image of gel obtained with FG72. Fig. 1B is a representative image of gel obtained with its non-GM counterpart (Jack).

In order to have a better understanding of the biological variability of known endogenous soybean allergens, the same allergens found in the FG72 soybean were also compared to those of the three commercial non-GM varieties Stine® 3000-0, Stine® 2686-6 and Stine® 2788 lines) from the same maturity group (group II) by using the 2D-GE technique. All spots were identified in the three commercial soybean varieties (data not shown).

There was no qualitative difference between the number of allergens in FG72 soybean and the non-GM counterpart. In addition, the three commercial lines contained the same allergens that were found in the FG72 soybean. These observations were confirmed in all replicates in all groups.

4.2. Quantitative analysis

The intensities of each FG72 soybean spot were further compared to those found in its non-GM counterpart (Jack). Among the 37 allergenic protein spots investigated, only three polypeptides were found to be statistically different between the FG72 soybean and Jack soybean. Mean intensities of the KTI3_3, KTI (1,2,3)_5 and GLCA_17 polypeptides were lower in the FG72 group than in the Jack group by –43%, –29% and –21%, respectively (Fig. 2A and B).

KTI3_3 and KTI (1,2,3)_5 belong to the Kunitz trypsin inhibitor family (among seven Kunitz trypsin inhibitor polypeptides studied). Mean intensities of KTI3_3 and KTI (1,2,3)_5 allergens in the FG72 group fell within the range of biological variation observed in non-GM soybean groups (Jack and commercial varieties) (Fig. 2A and B).

GLCA_17 belongs to the β-conglycinin family (one of the four β-conglycinin polypeptides studied). The mean intensity of the GLCA_17 allergen in the FG72 group was slightly below the range of biological variation observed in non-GM soybean groups (Fig. 2C).

Overall, there was no significant modification of the level of endogenous allergens investigated in the FG72 soybean seeds.

5. Discussion

For crops known to be allergenic (such as soybean), a possible consequence of the transgene insertion is the modification of the level of endogenous allergens. This modification has the potential to increase the allergenicity of the engineered soybean (EFSA, 2006). To address this issue, the relative levels of accumulation of known endogenous allergens have to be investigated in the GM plant and compared to its non-GM counterpart. In addition, similar analysis of the levels of endogenous allergens in non-GM commercial varieties would allow the evaluation of the biological variability of these allergens in soybeans.

Variability of endogenous allergen expression can result from the genetic background of soybean varieties and/or from environmental conditions (Natarajan et al., 2007). More specifically, it has been reported that in 18 commercial lines of soybean, the content of β-conglycinin and glycin subunits could be found statistically different between the different lines, with differences ranging from 47% to 157% (Yaklich, 2001). Although these allergen level variations may appear significant at the statistical level, all marketed soybean varieties (from different maturity groups, genetic backgrounds, seasons, or growing conditions) have a good history of safe use.

To quantify the level of accumulation of known endogenous soybean food allergens, the Coomassie blue-stained 2D-GE

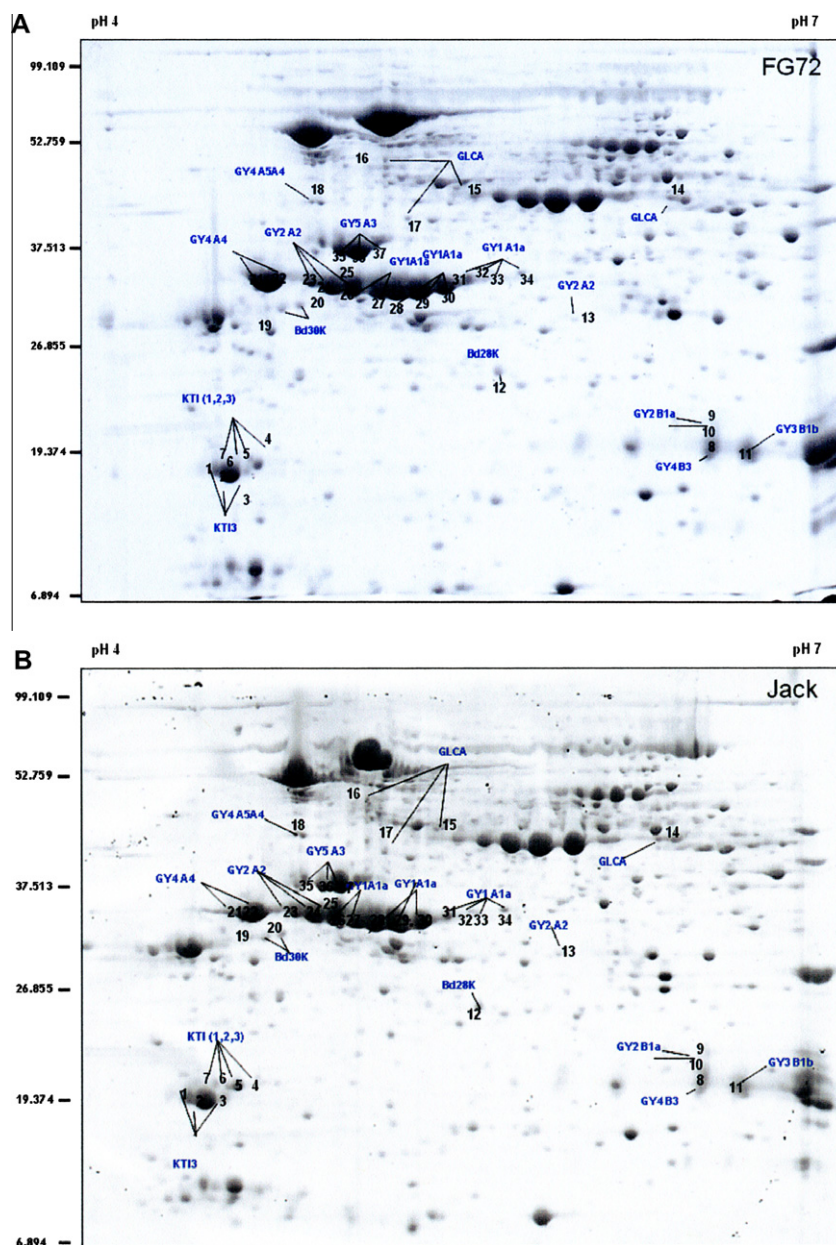


Fig. 1. Representative 2D maps of FG72 (A) and Jack (B) soybean seed protein extracts. The first dimension was run using a pH gradient from 4 to 7 and the second dimension is a 12% SDS-PAGE. Food allergen proteins are numbered with their corresponding ID name (see Table 1).

technique is one of the methods of choice. Indeed, this technique allows for the simultaneous quantification of several hundreds of proteins from a complex mixture of soybean seed protein extract. In this study, the use of 2D-GE analysis allowed a qualitative and quantitative investigation of the polypeptides known to cause allergenic reactions in human patients.

The current 2D-GE analyses of FG72 soybean, Jack soybean and 3 commercial soybean lines showed that the investigated 37 known major soybean food allergenic protein spots were detectable in all varieties. Therefore, neither the transgenic modification nor the genetic background of the soybean groups used in this study impacted the qualitative expression of known soybean food allergens.

Moreover, a quantitative comparison of these 37 protein spots showed that a few statistically significant differences could be detected between FG72 and its near-isogenic line Jack. Two Kunitz trypsin inhibitors were decreased by 43% and 29%, and were within biological range of variation observed in commercial lines. One

β -conglycinin allergen was decreased by 21%, and was slightly below the biological range of variation observed in commercial lines.

Again, including commercial varieties in the comparison is critical for assessing the biological variability of allergen expression. In the study with FG72 soybean, among the three polypeptides found statistically lower in the FG72 soybean than in the non-GM counterpart, only the β -conglycinin subunit was found out of the range of quantities observed in the commercial lines. As the decrease was minor (–21%), this difference was considered as not biologically significant. It can be hypothesized that such level of accumulation could be found in conventional soybeans as long as sufficient number of conventional soybean lines is evaluated. It is worth noting here that these three polypeptides were part of the allergens that accumulate at a very low level in soybean seeds (Fig. 1). We believe that, to improve the interpretation of the results of these analyses, additional data should be generated on the biological variability of allergens in a wide range of non-GM soybean varieties. This biological variability information would help to determine which

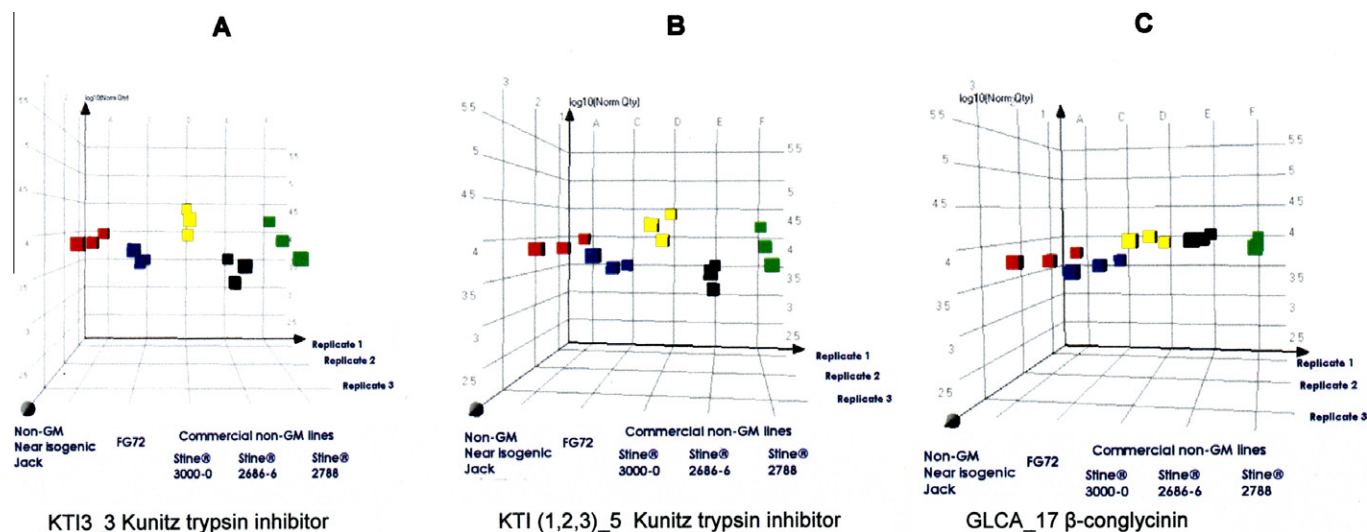


Fig. 2. Graphical representations of the levels of accumulation of KTI3_3 Kunitz trypsin inhibitor (A), KTI (1,2,3)_5 Kunitz trypsin inhibitor (B) and GLCA_17 β -conglycinin (C) proteins. For each genotype investigated, three technical replicates were performed.

alteration in the content of endogenous allergens in GM soybean is biologically significant and specifically induced by the genetic modification.

We conclude from our current results that no biologically significant modification of the level of endogenous allergens was observed in the FG72 soybean seeds and the allergenic potential of the transgenic FG72 soybean was found similar to that of non-transgenic soybeans, namely the near-isogenic Jack variety and the three soybean commercial lines. These results are in agreement with previous reports based on skin prick tests with protein extracts prepared from transgenic soybean and IgE immunoblot using sera from patients with food allergy to soybean, showing that the allergenic profile of another GM soybean (Roundup Ready soybean) was similar to that of conventional soybeans (Batista et al., 2007). Similarly, a soybean genetically engineered to prevent expression of the major food allergen Gly m Bd 30 K showed no significant changes in polypeptide pattern except for the intended suppression of the Gly m Bd 30 K protein (Herman, 2003).

Furthermore, due to limited data on quantitative thresholds for sensitizing individuals to most allergens, it is important to discuss the value of these results for assessing the safety of GM crops. It is well admitted by the vast majority of food allergy experts that it is not possible at this time to correlate allergen expression with food allergy clinical relevance, and to differentiate the levels of change that would be (un)acceptable (Goodman et al., 2008). In other words, an increase in the quantity of allergen(s) cannot be directly correlated with an increase in allergenicity potential of soybean, and similarly, a decrease in quantity of allergen(s) cannot be correlated with a decrease in allergenicity potential. More generally, the relationship between the level of endogenous allergens in soybean and the ability of soybean to sensitize patients or to elicit an allergic reaction needs to be understood before interpreting these data. In particular, the determination of a threshold level for eliciting a clinical reaction in patients will help to interpret the differences in allergen contents (Goodman et al., 2008; Selgrade et al., 2009; Thomas et al., 2008).

Overall, the investigation of the endogenous allergens in the allergenic crops is part of a more global assessment of unintended effects potentially resulting from the genetic modification (Codex Alimentarius, 2009; EFSA, 2006). These 2D-GE data are part of the weight of evidence approach. In particular, 2D-GE data corroborate that the sites of insertion of the transgenes were not located in regions regulating the investigated soybean allergens (data not

shown). Furthermore, none of the agronomic, compositional and nutritional studies showed any pleiotropic effects (data not shown).

6. Conclusion

To conclude, we showed that the levels of accumulation of 37 known food soybean protein spots were not significantly different in the herbicide-tolerant FG72 soybean when compared to its non-GM counterpart and other commercial lines of soybeans. Therefore, the FG72 soybean can be considered as safe as its non-GM counterpart with regards to allergenicity. In order to better interpret this type of data in the context of safety assessment of the allergenic potential of GM soybeans, future research should be conducted to appreciate the biological variability of endogenous soybean allergens in different varieties grown in different environmental conditions. Moreover, the correlation between the level of allergens and the allergenic potential of soybean should be further explored. Overall, the results have to be interpreted by using a weight-of-evidence approach in accord with the Codex alimentarius recommendations.

Conflict of interest

The authors declare that they do not have any conflict of interest with regards to the data reported in this scientific paper.

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