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Full Length Research Paper

Comparison of chemical composition of different transgenic insect-resistant cotton seeds using Fourier transform infrared spectroscopy (FTIR)

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The comparison of chemical composition of genetic modification (GM) crops to their conventional counterparts forms the basis of safety assessment process for GM crops. In this study, we used Fourier transform infrared spectroscopy (FTIR) to detect the chemical and conformational changes between transgenic cotton seeds and their non-transgenic counterparts. The assignment of absorption bands and comparison in band areas of four regions from original FTIR spectra indicated that the contents of the compounds did not differ significantly between transgenic cotton seeds and their non-transgenic counterparts ($P > 0.05$). The comparison of Fourier self-deconvolution (FSD) and after peak-smoothing spectra in the region between 2000 and 1000 cm^{-1} showed that the differences in band pattern can be observed obviously between transgenic cotton seeds and its counterpart, depending on the varieties. The changes in the protein profile of transgenic *Bacillus thuringiensis* (*Bt*) cotton seeds Z30 were significant, both in multi-peaks-fitted protein structures and its ratios, compared with non-transgenic cotton seeds Z16 ($P < 0.05$). However, transgenic *Bt* + cowpea trypsin inhibitor (CpTI) cotton seeds SGK321, did not show significant changes, in comparison with non-transgenic cotton seeds SY321. These results indicated that both the indigenous and exogenous proteins structural changes in genetically modified organism (GMO) are worth being detected in detail for research related to its' safety assessment.

Key words: Transgenic, cotton seeds, safety assessment, Fourier transform infrared spectroscopy (FTIR).

INTRODUCTION

Cotton (*Gossypium hirsutum* L.) is one of the most major crops in China and worldwide, and genetic modification (GM) has been used to produce *Bacillus thuringiensis* (*Bt*) toxin and/or cowpea trypsin inhibitor (CpTI) in order to enhance resistance against insect damage. Cotton is not only a valuable fiber, but it is also used for the production of cotton seeds oil. Besides, the cotton meal is used as a protein source for human and animal diets (Elangovan et al., 2006; Jiang et al., 2008).

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Transgenic insect-resistant cotton hybrids express *Bt* toxin and/or CpTI constitutively. Since, the *Bt* toxin is constitutively expressed, safety assessment of transgenic insect-resistant cotton seeds should be paid more attention.

The 'substantial equivalence' concept that is based on the comparison of GM product with a traditionally bred parent with a history of safe use is usually the first step in safety supervision (Millstone et al., 1999). As such, comparison of chemical composition of GM crops to their conventional counterparts forms the basis for a series of toxicological and nutritional safety assessment process for GM crops (Millstone et al., 1999; Rischer and Oksman

-Caldentey, 2006; Kier and Petrick, 2008). Because the current methods of plant transformation do not offer control over the insertion site, for the number of genes transferred, or the integrity of the gene site, unintended effects can arise in GM crops through disruption of host gene functions or through somaclonal variation at the tissue culture stage of the transformation process (Kohli et al., 2003; Cellini et al., 2004; Shrawat and Lőrz, 2006). In order to assess the potential effect or secondary effect of the genetic insertion on plant biochemistry, analytes, including a certain number of important nutrients, antinutrients, and toxicants used for crops, were selected to speculate this effect (Momma et al., 1999; Vasconcelos et al., 2003; Wang et al., 2003; Kier and Petrick, 2008). However, there is a difficulty with predefined or targeted analyses that some unforeseen, unintended effects of the genetic modification on metabolism outside those specific compounds may escape detection. In the attempt to increase the chance of detecting unintended effects deriving from transformation events, non-targeted profiling techniques including genomics, proteomics, and metabolomics are currently tested as analytical tools complementary to the existing safety assessment methods (Hollingworth et al., 2003; Cellini et al., 2004; Tengs et al., 2010).

Spectroscopic methods have been used for the detection of genetically modified organisms (GMOs) and the characterization of crop varieties (Surewicz and Mantsch, 1988; McCann et al., 1992; Roussel et al., 2001; Emura et al., 2006; Rischer and Oksman-Caldentey, 2006). Given the complex biological matrix, the changes of a specific functional group cannot be assigned easily to a particular molecule in the cells. However, the changes in chemical composition reflect the overall changes in the metabolic process, and can be detected more sensitively and effectively than the traditional methods (Yang and Yen, 2002). A nondestructive analysis using Fourier transform infrared spectroscopy (FTIR) and FT-Raman has successfully characterized the changes of lignin structure in transgenic tobacco (Stewart et al., 1997). Keymanesh et al. (2009) also studied the safety of transgenic rice at metabolite level using FTIR. However, FTIR methods for the characterization of transgenic cotton seeds have not been found in the literature. In this study, we examined the foreign gene insertion on the chemical composition and structural details of transgenic cotton seeds using FTIR technology, to verify whether nonspecific alterations of the chemical composition in transgenic cotton seed can be detected by FTIR.

MATERIALS AND METHODS

Plant

Two types of indigenous Chinese commercial transgenic cotton including the transgenic *Bt* cotton Z30, the transgenic *Bt* + CpTI cotton SGK321, and their non-transgenic parental counterparts Z16 and SY321 were used in this experiment, respectively. Cotton seeds of each variety were kindly provided by the Germ Plasm Resources Centre, Institute of Cotton, Chinese Academy of Agricultural Sciences (Anyang, Henan).

FTIR sample preparation and data analysis

The seed coats of the cotton seeds were carefully removed and powdered in Pestle and Mortar with liquid nitrogen for analysis. Each sample for FTIR analysis was prepared by mixing the fine ground powder of cotton seeds with 2% of KBr. The FTIR spectra of each variety were recorded on a Nicolet 380 spectrometer (Thermo Electron Corporation, USA) in the range of 4000 to 400 cm^{-1} at a resolution of 4 cm^{-1}. The spectral data of each sample were collected, corrected for background spectrum, displayed in the absorbance mode and analyzed using OMNIC software 7.0. Protein amide I ratio were determined, and chemical functional groups were identified according to published reports (Golovina et al., 1997; Surewicz and Mantsch, 1988; Wolkers et al., 1998; Yang and Yen, 2002; El-Bahy, 2005; Stehfest et al., 2005). Mathematical approaches such as the Fourier self-deconvolution (FSD) in OMINIC and a multi-peaks-fitting program with Gaussian function in ORIGIN 7.5 were generally applied to extract information from the raw FTIR spectra to resolve the overlapping band components. The relative amount of model-fitted α-helices and β-sheets based on modeled peak areas was calculated according to the report generated by the software. Statistical analyses among varieties were performed using Statistical Package for Social Sciences (SPSS) 11.0. Significance was declared at $P < 0.05$.

RESULTS

Comparison of chemical composition between transgenic and non-transgenic cotton seeds based on FTIR and FSD analysis

The feasibility of FTIR spectrometry for obtaining the chemical information from cotton seeds was investigated, and typical FTIR spectra obtained from different cotton seeds as shown in Figure 1. There were no distinct differences in FTIR absorption spectra among transgenic and non-transgenic cotton seeds, indicating their absorption bands had the same structural assignments (Table 1). Assayed cotton seeds consist of proteins, carbohydrates, lipids, nucleic acids, etc (Table 1 and Figure 1), and there are no major alternations in the original FTIR spectra of the assayed transgenic cotton seed and non-transgenic cotton seed. In the presence of high relative content of storage proteins, carbohydrates and lipids, the changes of other minor components may not be visible. After carefully evaluating the FTIR spectra generated from the dried cotton seeds, four absorption regions, namely, 1800 to 1720, 1720 to 1580, 1580 to 1480 and 1200 to 1130 cm^{-1} were chosen and analysed for the characteristics of original FTIR absorption spectral regions. The band positions corresponding to lipids (at 1746 cm^{-1}) were identical in all cotton seeds, indicating that the chemical structure of lipids was stable in both transgenic and non-transgenic cotton seeds. Compared

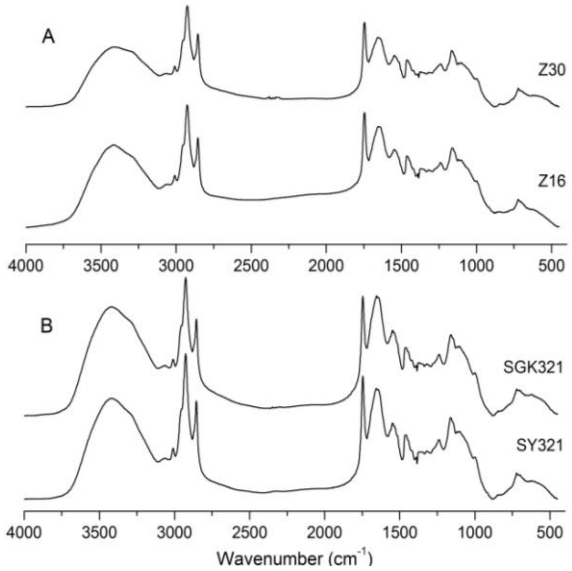

Figure 1. FTIR absorption spectrum of transgenic cotton seeds (A, Z30; B, SGK321) and their non-transgenic counterparts (A, Z16; B, SY321).

Table 1. Band assignments for FTIR.

Wavenumber (cm^{-1})	Assignment
3400	vO-H and vN-H, mainly from proteins and carbohydrates
3000 - 2850	vC-H, mainly from lipid and carbohydrates
1746	vC=O of ester groups, primarily from lipids and fatty acids
1655	vC=O of amide I from proteins
1550	δN-H of amide II from proteins
1460	δC-H, mainly from proteins
1239	vP=O of phosphodiester groups of nucleic acids and phospholipids
1200 - 900	vC-O, mainly from carbohydrates

with non-transgenic cotton, transgenic cotton had slight shifts of band positions corresponding to amides and carbohydrates, respectively. The results also suggested that in the cotton seeds of two transgenic varieties, theaccumulation of lipids and carbohydrates were less than that in their non-transgenic counterparts, as indicated by the comparison of their band area (Table 2). Among those alternations, the obvious decrease of carbohydrates in transgenic cotton Z30 was lowered to 39% than that in non-transgenic cotton Z16, but the alternations were not significant ($P > 0.05$). There was no large difference in total protein content because the absorption peak area of transgenic cotton seed showed no statistical difference ($P > 0.05$) when compared with the non-transgenic cotton seed neither within amide I peak areas and amide II peak areas, nor within their amide I to II area ratios.

To distinguish the small difference in patterns caused by overlapping bands, which was not revealed using the original spectrum, a deconvolution technique was used, which can enhance the resolution of small bands that were buried under other bands in the original spectra. The results of FSD and after peak-smoothing spectra in the region between 2000 and 1000 cm^{-1} are as shown in Figure 2. Closer examination revealed the presence of considerably more minor peaks, although, not all peaks were fully resolved. The significant observation in the FSD peak shape of transgenic cotton Z30 (Figure 2A and B) was extensively broader than the spectrum obtained from non-transgenic cotton Z16 (Figure 2C and D). This result suggested that new molecules were produced during seed development and/or that the interactions between the existing molecules became stronger. Band patterns in this region of transgenic cotton SGK321 (Figure 2E and F) and non-transgenic counterpart SY321 (Figure 2G and H) were fairly similar. FSD spectrum after

Table 2. Characteristics of four typical regions in FTIR spectra from transgenic cotton seeds (Z30 and SGK321) and their non-transgenic counterparts (Z16 andSY321).

	Z30	Z16	SGK 321	SY321
Peak region[a] (cm^{-1}) and area (area units, ±SD[b])				
1800 - 1720	4.43 ± 1.05	4.69 ± 1.19	4.29 ± 0.41	5.19 ± 1.46
1720 - 1580	15.02 ± 4.75	18.70 ± 11.42	22.12 ± 12.50	19.28 ± 10.65
1580 - 1480	2.71 ± 0.52	3.75 ± 1.43	4.84 ± 1.74	3.26 ± 0.63
1200 - 1130	1.41 ± 0.36	2.30 ± 0.65	1.78 ± 0.75	2.57 ± 1.49
Total amide I and II (±SD)	17.73 ± 4.22	22.45 ± 12.86	26.96 ± 14.24	22.54 ± 11.28
Ratio of amide I to II (±SD)	5.92 ± 2.95	4.64 ± 1.37	4.31 ± 1.11	5.63 ± 2.22

[a]The region of 1800 to 1720, 1720 to 1580, 1580 to 1480 and 1200 to 1130 cm^{-1} were assigned to lipids, amide I, amide II and carbohydrates, respectively. [b]SD = standard deviation.

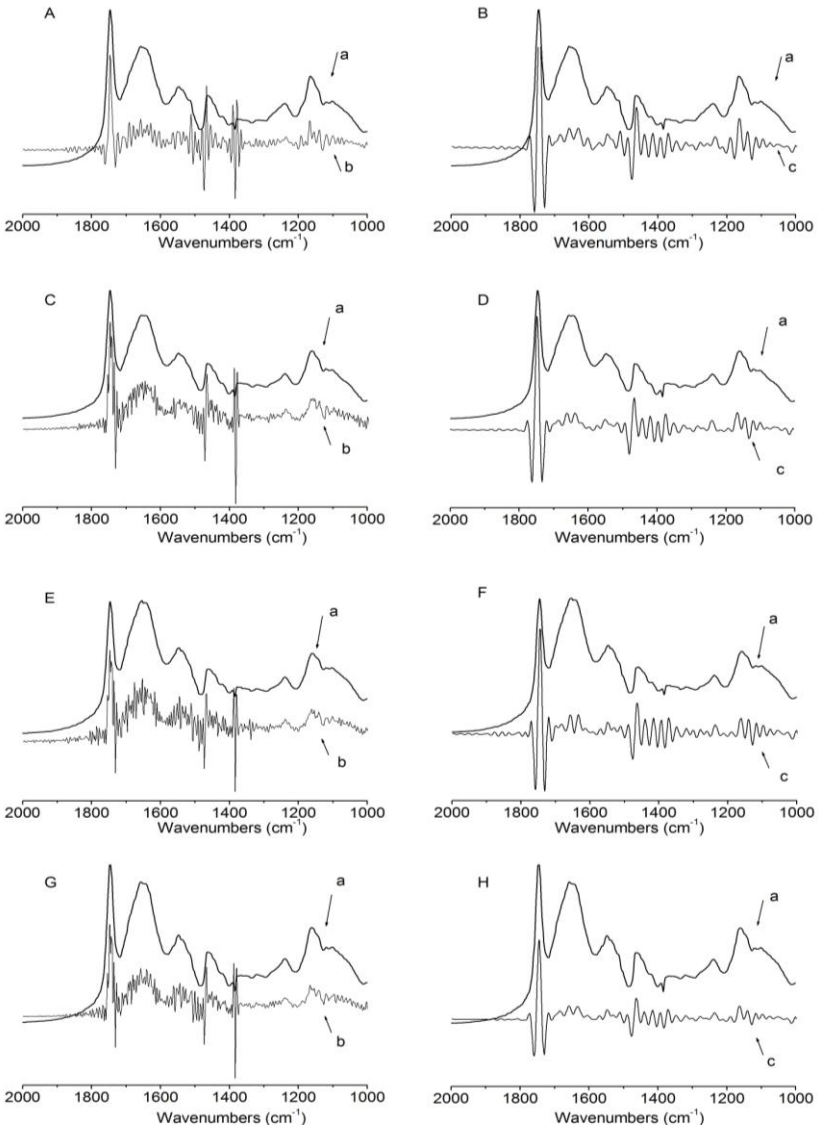

Figure 2. FTIR spectra, FSD and FSD spectra after peak-smoothing for transgenic cotton seeds (Z30, A, B; SGK321, E, F) and their non-transgenic counterparts (Z16, C, D; SY321, G, H). Line a, original spectrum; line b, FDS spectrum; and line c, FSD spectrum after smoothing.

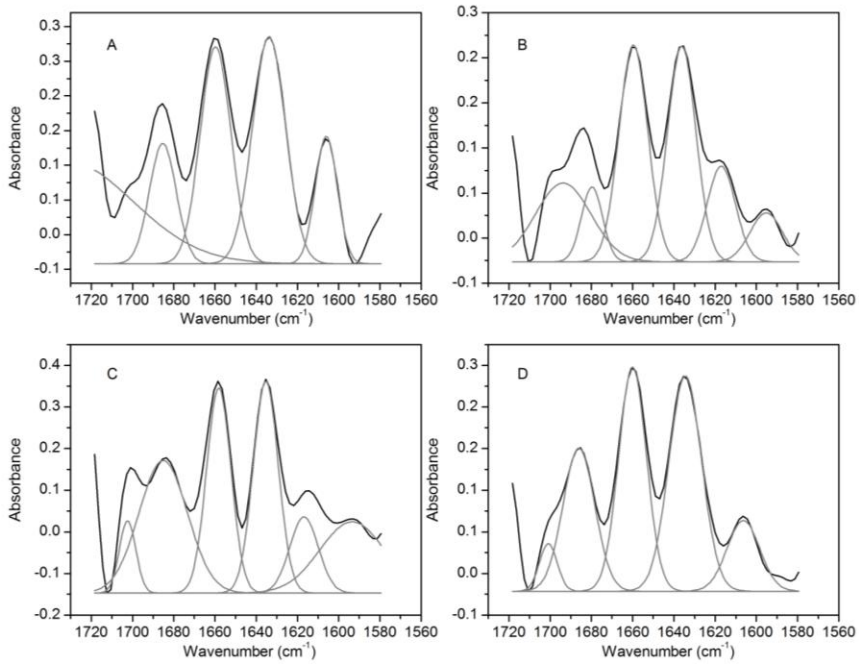

Figure 3. Typical multi-peaks fitting of FSD spectrum after smoothing in the protein amide I region of transgenic cotton seeds (Z30, A; SGK321, C) and their non-transgenic counterparts (Z16, B; SY321, D).

applying a smoothing factor, showing the most prominent difference between transgenic cotton and non-transgenic counterparts was associated with amide bands, which are sensitive for protein secondary structure analysis.

Comparison of protein secondary structure of transgenic and non-transgenic cotton seeds based on multi-peaks fitting analysis

Two important absorption bands for protein analysis was located around 1650 and 1550 cm^{-1}, which were assigned as amide I and II bands, respectively, were confirmed by FSD and after peak-smoothing. The amide I vibration arose mainly from the C=O stretching vibration with minor contributions from the out-of-phase C-N stretching vibration, the C-N deformation and the N-H in-plane bend. The amide I vibration is hardly affected by the nature of the side chain. It depends, however, on the secondary structure of the backbone and is, therefore, most commonly used for secondary structure analysis. The amide II band is predominantly an N-H bending vibration (60%) coupled to significant C-N stretching (40%) and is also used to assess protein conformation. But utility of the amide II band for protein structure prediction is lower than the amide I band, since the former arises from complex vibrations involving multiple functional groups. So, the amide I band of different cotton seeds will be discussed in detail subsequently.

To quantitatively analyze the changes in intensities of the amide I band, a curve-fitting procedure was applied over the wave number range from 1725 to 1580 cm^{-1}, and a typical curve-fitting result is as shown in Figure 3. As can be seen in this figure, the spectra can be decomposed into several bands with high fitness. Protein contains a mix of secondary structures, including a large amount of α-helices and β-sheets and a small amount of random coils and β-turns. Figures 3 showed that the major component of the amide I region had one band around 1658 cm^{-1}, which can be assigned to α-helical structures, and the other band around 1637 cm^{-1} reflected β-sheet structure. Then, multi-peaks fitting were tested using Gaussian modeling functions to best approximate the areas of multi-peaks-fitting within the protein amide I band region. The differences in relative proportion of the assigned secondary structures are illustrated in Table 3. There is need to mention that using multi-peaks modeling method to study protein secondary structure was not an accurate method but can be used for comparison purpose among varieties.

As can be seen in Table 3, the α-helical structures increased significantly from an average of 25% of the total protein secondary structures in non-transgenic cotton Z16 to 33% in transgenic *Bt* cotton Z30, and the differences of the β-sheet structures, other structures, the ratio of α-helix to other structures, and the ratio of β-sheet to other structures between transgenic *Bt* cotton Z30 and its non-transgenic counterpart were also significant ($P <$

Table 3. Comparison of model-fitted protein secondary structure in transgenic cotton seeds (Z30, SGK321) and non-transgenic counterparts (Z16, SY321).

	Z30	Z16	SGK 321	SY321
Model-fitted protein structure profile				
α-helices (±SD[a], %)	33 (±4)[a]	25 (±6)[b]	28 (±6)[b]	27 (±6)[b]
β-sheets (±SD, %)	35 (±2)[a]	25 (±8)[b]	27 (±7)[b]	27 (±8)[b]
Others (±SD, %)	32 (±6)[b]	50 (±14)[a]	45 (±13)[a]	46 (±14)[a]
Protein structure ratio profile				
Ratio of α-helix to β-sheet (±SD)	1.0 (±0.1)[a]	1.0 (±0.1)[a]	1.0 (±0.1)[a]	1.1 (±0.1)[a]
Ratio of α-helix to others (±SD)	1.1 (±0.3)[a]	0.6 (±0.3)[b]	0.7 (±0.3)[b]	0.7 (±0.3)[b]
Ratio of β-sheet to others (±SD)	1.1 (±0.2)[a]	0.6 (±0.3)[b]	0.7 (±0.4)[b]	0.7 (±0.4)[b]

[a]SD= standard deviation. Means with the different letter in the same line are significantly different ($P < 0.05$).

0.05). But in transgenic *Bt* + CpTI cotton SGK321, the significant changes of model-fitted protein structure and protein structure ratio were not observed when compared with its non-transgenic counterpart SY321 ($P > 0.05$).

DISCUSSION

In order to make regulations for the use, growth, and development of GMO, reliable analytical methods should be used to evaluate GMO in raw materials and food products. In this study, we used FTIR to detect chemical and conformational changes of different transgenic cotton seeds. Compared with non-transgenic counterparts, no major alterations regarding original FTIR spectra was observed for transgenic cotton seeds, indicating that the synthesis and accumulation of main storage materials, such as lipids, carbohydrates and proteins were not obviously pleiotropically affected by the genetic modification. However, when FSD, peak-smoothing and multi-peaks fitting procedures were applied, more detailed changes, especially in protein structures were revealed. Traditionally, we usually determine total protein or amino acid content to study changes of proteins. However, such test approach has a significant disadvantage because protein quality relies not only on total protein and amino acid content, but also on protein inherent structures such as protein secondary structures (McAllister et al., 1993; Yu, 2008). FTIR, based on mathematical approach analysis has been widely used for the analysis of protein conformation (Surewicz and Mantsch, 1988). Although, the normal FTIR spectroscopy may not be accurate for protein secondary structure analysis, because the spectrum provided, information only on the average protein secondary structure, and the averaging character of FTIR made it difficult to directly link changes in the overall protein secondary structure to the synthesis of specific proteins (Wolkers et al., 1998). However, our result showed that protein structure profiles obtained from the whole seed could be useful to detect unintended effects between non-transgenic and transgenic crops using FTIR based on mathematical approaches analysis such as deconvolution and curve fitting.

Takahashi et al. (2005) reported that in transgenic rice plants, over expression of a single gene (YK1) was observed, the unrelated proteins and metabolites in rice cells was found, whereas in another transgenic rice, a considerable fraction of the storage proteins turned out to be strongly under expressed (Islam et al., 2005). Similar results have been reported for transgenic soybean leaves, where genes involved cysteine protease inhibitor activity and dihydroflavonlo-4-reducatase activity were down regulated (Cheng et al., 2008). Zolla et al. (2008) reported that the insertion of a single gene did not result in a unique newly expressed protein, but rather in a total of 43 differently expressed protein spots in transgenic *Bt* maize seed with respect to non-transgenic control by comparing theirs' proteomics profiles. They also found out that a number of seed storage proteins exhibited truncated forms having molecular masses significantly lower than the native ones. These reports are similar to our result, that is, significant changes in the protein profile of transgenic *Bt* cotton seeds Z30 can be observed, both in protein structure, such as α-helix, β-sheet and other structures, and in the ratio of protein structures. The composition changes of protein in transgenic seed may be as a result of secondary or pleiotropic effects of the transgene expression and insertion, just as Koornneef et al. (2004) have pointed out in a review that a point mutation of DNA, for example, could result in missense mutation, nonsense mutation leading to protein elongation or truncation, loss of phosphorylation or glycosylation sites, and alteration of the degradation stability of the resulting protein. Otherwise, alterations in metabolic pathways in leaves that act as source tissues would have an impact on the general state of a plant and so could have an effect on the final seed composition (Cheng et al., 2008). Another study showed that both transgenic *Bt* maize and non-transgenic counterpart had

a similar N partitioning pattern, but the *Bt* hybrid produced greater dry matter in leaves and kernels than its non-*Bt* counterpart. Besides, it also accumulated more than 11% N in kernels and on a whole-plant (Subedi and Ma, 2007). Although further investigations are required, quantitative variations between transgenic *Bt* cotton seed storage protein structure may result from the *Bt* gene insertion directly or indirectly. We cannot ignore the fact that, for transgenic *Bt* + CpTI cotton seeds SGK321, no significant changes were found for protein structures, indicating that it depended on the varieties. The probable cause was that the expression of *Bt* coupled with CpTI in transgenic cotton seed was more complicated than that of single gene of *Bt* (Kang et al., 2005). In addition, similar results were also found in some literature, indicating that the expression of transgenes has little impact on the GMO (Ouakfaoui and Miki, 2005; Gregersen et al., 2005; Baudo et al., 2006). Such discrepancies remind us that a case-by-case assessment of the GMO should be carried out in order to have a wide knowledge of its feature.

Protein is one of the most important nutrients and it also could be toxins, antinutrients and allergens in seeds. As a macronutrient, protein is an essential component of the human diet. So, an important component of safety assessment of agricultural products, produced through biotechnology by the expression of transgenic proteins is the evaluation of the safety of the proteins (Delaney et al., 2008). Its assessment, generally, includes the biological and chemical characteristics of the exogenous proteins. But, if neither new nor chimeric proteins could be revealed during safety assessment, the changes of endogenous protein structures in seed are also worth being detected in detail. Besides, the change of protein profile of transgenic and non-transgenic cotton seeds has to be assessed at the time of seed germination, which would give a clear picture about the shift in protein profile. Because of the changes in protein structure, such as from β-sheet to α-helix ratios, the protein nutritive value in the feed or food may be affected (Yu, 2008).

Protein is the direct product of genome transcription and translation; therefore, if a genetic modification affects the genome of a plant by changing its metabolic pathways or producing new proteins, the resulting proteome will be altered (Zolle et al., 2008). Stabilization of the structure of proteins and membranes is crucial for desiccation tolerance and long-term survival of the seed in the dry state (Golovina et al., 1997). Wolkers et al. (1998) also pointed out that based on the amide I absorption band, an increased ratio of α-helix/β-sheet was found to be associated with the increase of desiccation tolerance in developing seed embryo. So, the research of protein structure in transgenic seed is also of great significance in terms of seed physiology.

Conclusively, FTIR is not yet a routine practice when assessing the safety of GM products, but it has the potential of reducing uncertainty by providing many more data on crop composition than those obtained with targeted analysis alone. Especially, this approach reveals that the molecular structural chemical differences of protein will provide basic information to the food and feed safety assessment.

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REFERENCES

Baudo MM, Lyons R, Powers S, Pastori GM, Edwards KJ, Holdsworth MJ, Shewry PR (2006). Transgenesis has less impact on the transcriptome of wheat grain than conventional breeding. J. Plant Biotech., 4: 369-380.

Cellini FA, Chesson I, Colquhoun I, Constable A, Davies HV, Engel KH, Gatehouse AM, Karenlampi S, Kok EJ, Leguay JJ, Lehesranta S, Noteborn HP, Pedersen J, Smith M (2004). Unintended effects and their detection in genetically modified crops. Food Chem. Toxicol., 42: 1089-1125.

Cheng KC, Beaulieu J, Iquira E, Belzile FJ, Fortin MG, Strömvik MV (2008). Effect of transgenes on global gene expression in soybean is within the natural range of variation of conventional cultivars. J. Agr Food Chem., 56: 3057-3067.

Delaney B, Astwood JD, Cunny H, Conn RE, Herouet-Guicheney C, Macintosh S, Meyer LS, Privalle L, Gao Y, Mattsson J, Levine M (2008). Evaluation of protein safety in the context of agricultural biotechnology. Food and Chem. Toxicol., 46: S71-S97.

EI-Bahy GMS (2005). FITR and Raman spectroscopic study of fenugreek (*Trigonella foenum graecum* L.) seeds. J. Appl. Spectros, 72: 111-116.

Elangovan AV, Tyagi Praveen K, Shrivastav AK, Tyagi Pramod K, Mandal AB (2006). GMO (*Bt-Cry1Ac* gene) cotton seeds meal is similar to non-GMO low free gossypol cotton seeds meal for growth performance of broiler chickens. Anim. Feed Sci. Tech., 129: 252-263.

Emura K, Yamanaka S, Isoda H, Watanabe KN (2006). Estimation for different genotypes of plants based on DNA analysis using near infrared and Fourier-transform infrared spectroscopy. Breed. Sci., 56: 399-403.

Golovina EA, Wolkers WF, Hoekstra FA (1997). Long-term stability of protein secondary structure in dry seeds. Comp. Biochem. Physiol., 117: 343-348.

Gregersen PL, Brinch-Pedersen H, Holm PB (2005). A microarray-based comparative analysis of gene expression profiles during grain development in transgenic and wild type wheat. Transgenic Res., 14: 887-905.

Hollingworth RM, Bjeldanes LF, Bolger M, Kimber I, Meade BJ, Tayor SL, Wallace KB (2003). The safety of genetically modified foods produced through biotechnology. Toxicol. Sci., 71: 2-8.

Islam N, Upadhyaya NM, Campbell PM, Akhurst R, Hagan N, Higgins TJV (2005). Decreased accumulation of glutelin types in rice grains constitutively expressing a sunflower seed albumin gene. Phytochemistry, 66: 2534-2539.

Kang BS, Zhang R, Pan DK, Wang Y, Guo SD (2005). Synchronous expression of the *Cry1Ac* and CpTI genes in transgenic cotton. Cotton Sci., 17: 131-136.

Keymanesh K, Darvishi MH, Sardari S (2009). Metabolome comparison

of transgenic and non-transgenci rice by statistical analysis of FTIR and NMR spectra. Rice Sci., 16: 119-123.
Kier LD, Petrick JS (2008). Safety assessment considerations for food and feed derived from plants with genetic modifications that modulate endogenous gene expression and pathways. Food Chemi. Toxicol., 46: 2591-2605.
Kohli A, Twyman RM, Abranches R, Wegel E, Stoger E, Christou P (2003). Transgene integration, organization and interaction in plants. Plant Mol. Biol., 52: 247.
Koornneef M, Alonso-Blanco C, Vreugdenhil D (2004). Naturally occurring genetic variation in *Arabidopsis thaliana*. Annu. Rev. Plant Biol., 55: 141-172.
McAllister TA, Phillippe RC, Rode LM, Cheng KJ (1993). Effect of the protein matrix on the digestion of cereal grains by ruminal microorganisms. J. Anim. Sci., 71: 205-212.
McCann MC, Hammouri M, Wilson R, Belton P, Roberts K (1992). Fourier transform infrared microspectroscopy is a new way to look at plant cell wall. Plant Physiol., 100: 1940-1947.
Millstone E, Brunner E, Mayer S (1999). Beyond 'substantial equivalence'. Nature, 401: 525-526.
Momma K, Hashimoto W, Ozawa S, Kawai S, Katsube TL, Takaiva F, Kito M, Utsmi S, Murata K (1999). Quality and safety evaluation of genetically engineered rice with soybean glycinin: analyses of the grain composition and digestibility of glycinin in transgenic rice. Biosci. Biotech. Biochem., 63: 314-318.
Ouakfaoui SE, Miki B (2005). The stability of the *Arabidopsis* transcriptome in transgenic plants expressing the marker genes nptII and uidA. J. Plant, 41: 791-800.
Rischer H, Oksman-Caldenter KM (2006). Unintended effects in genetically modified crops: revealed by metabolomics? Trends Biotech., 24: 102-104.
Roussel SA, Hardy CL, Hurburgh CR, Rippke GR (2001). Detection of Roundy Ready™ soybeans by near-infrared spectroscopy. Appl. Spectros., 55: 1425-1430.
Shrawat AK, Lőrz H (2006). Agrobacterium-medialed transformation of cereals: A promising approach crossing barriers. J. Plant Biotech., 4: 575-603.
Stehfest K, Toepel J, Wilhelm C (2005). The application of micro-FTIR spectroscopy to analyze nutrient stress-related changes in biomass composition of phytoplankton algae. Plant Physiol. Biochem., 43: 717-726.
Stewart D, Yahiaoui N, McDougall GJ, Myton K, Marque C, Bouder AM, Haigh J (1997). Fourier-transform infrared and Raman spectroscopic evidence for the incorporation of cinnamaldehydes into the lignin of transgenic tobacco plants with reduced expression of cinnamyl alcohol dehydrogenase. Planta, 201: 311-318.
Subedi KD, Ma BL (2007). Dry matter and nitrogen partitioning patterns in *Bt* and non-*Bt* near-isoline maize hybrids. Crop Sci., 47: 1186-1192.
Surewicz WK, Mantsch HH (1988). New insight into protein secondary structure from resolution-enhance infrared spectra. Biochim. Biophys. Acta, 952: 115-130.
Takahashi H, Hotta Y, Hayashi M, Kawai-Yamada M, Komatsu S, Uchimiya H (2005). High throughput metabolome and proteome analysis of transgenic rice plants (*Oryza sativa* L.). Plant Biotech., 22: 47-50.
Tengs T, Kristoffersen AB, Zhang H, Berdal KG, Løvoll M, Holst-Jensen A (2010). Non-prejudiced detection and characterization of genetic modifications. Food Anal. Methods, 3: 120-128.
Vasconcelos M, Datta K, Oliva N, Khalekuzzaman M, Torrizo L, Krishnan S, Oliveira M, Goto F, Datta S (2003). Enhanced iron and zinc accumulation in transgenic rice with the ferritin gene. Plant Sci., 164: 371-378.
Wang Z, Yant Y, Cui H, Xia Y, Altosaar I, Shu G (2003). Toxicological evaluation of transgenic rice flour with a synthetic *Cry 1Ab* gene from *Bacillus thuringiensis*. J. Sci. Food Agric., 82: 738-734.
Wolkers WF, Bochicchio A, Selvaggi G, Hoekstra FA (1998). Fourier transform infrared microspectroscopy detects changes in protein secondary structure associated with desiccation tolerance in developing maize embryos. Plant Physiol., 116: 1169-1177.
Yang J, Yen HE (2002). Early salt stress effects on the changes in chemical composition in leaves of ice plant and *Arabidopsis*. A Fourier transform infrared spectroscopy study. Plant Physiol., 130: 1032-1042.
Yu P (2008). Molecular chemistry of plant protein structure at a cellular level by synchrotron-based FTIR spectroscopy: Comparison of yellow (*Brassica rapa*) and brown (*Brassica napus*) canola seed tissues. Infrared Phys. Tech., 51: 473-481.
Zolla L, Rinalducci S, Antonioli P, Righetti PG (2008). Proteomics as a complementary tool for identifying unintended side effects occurring in transgenic maize seeds as a result of genetic modifications. J. Proteome Res., 7: 1850-1861.