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BRIEF COMMUNICATION

Gene expression profiles of MON810 and comparable non-GM maize varieties cultured in the field are more similar than are those of conventional lines

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Abstract Maize is a major food crop and genetically modified (GM) varieties represented 24% of the global production in 2007. Authorized GM organisms have been tested for human and environmental safety. We previously used microarrays to compare the transcriptome profiles of widely used commercial MON810 versus near-isogenic varieties and reported differential expression of a small set of sequences in leaves of in vitro cultured plants of AristisBt/Aristis and PR33P67/PR33P66 (Coll et al. 2008). Here we further assessed the significance of these differential expression patterns in plants grown in a real context, i.e. in the field. Most sequences that were differentially expressed in plants cultured in vitro had the same expression values in MON810 and comparable varieties when grown in the field; and no sequence was found to be differentially regulated in the two variety pairs grown in the field. The differential expression patterns observed between in vitro and field culture were similar between MON810 and comparable varieties, with higher divergence between the two conventional varieties. This further indicates that MON810 and comparable non-GM varieties are equivalent except for the introduced character.

Keywords GMO (Genetically Modified Organism) · MON810 · Maize · Field · Unintended effects · Expression profile

Abbreviations

cDNA	Complementary DNA.
CRM	Certified reference material.
EFSA	European Food Safety Authority.
EU	European Union.
GM	Genetically modified.
GMO	Genetically modified organism.
IRMM	Institute for Reference Materials and Measurements.
ISAAA	International Service for the Acquisition of Agri-biotech Applications.

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mRNA	Messenger RNA.
OD	Optical density.
Real-time RT-PCR	Reverse transcription coupled to real-time polymerase chain reaction.
V2	Vegetative two-leaf stage.
VT	Tasseling stage.

Introduction

Genetically modified (GM) crops are subjected to different legislation worldwide to cover aspects of consumer safety and protection. An increasing number of publications show the equivalence of transformed and non-transformed lines of the same species [see reviews in (Cellini et al. 2004; Shewry et al. 2007)]. Some unpredicted differences have been shown between transgenic and conventional lines, but these do not significantly alter overall gene expression and fall within the range of natural variation (Baker et al. 2006; Baudo et al. 2006; Catchpole et al. 2005; Cheng et al. 2008; Coll et al. 2008; Dubouzet et al. 2007; Ioset et al. 2007; Kristensen et al. 2005; Ruebelt et al. 2006; Shewry et al. 2007).

Plant varieties have a high degree of diversity, due in part to the genetic fluidity of plant genomes (Parrott 2005), with extensive variation within a species. Conventional breeding includes the use of techniques known to cause genome alteration for incorporating new diversity into crop varieties, e.g. interspecies crosses, tissue culture, chemical or irradiation mutagenesis and the use of transposons (Batista et al. 2008). Unintended variation between GM and comparable non-GM plants has very little impact when compared to the large differences observed between lines produced by conventional breeding (Baudo et al. 2006; Catchpole et al. 2005; Ioset et al. 2007; Lehesranta et al. 2005; Shepherd et al. 2006). In this context, recent literature on comparative safety assessment of conventional breeding and GM crops (Bradford et al. 2005; Cellini et al. 2004; Chassy et al. 2008; Kok et al. 2008) suggests an adaptation of the current legislative frame to the present knowledge.

Maize is the second most widespread GM crop, after soybean, with a global area of 37.3 million Ha in 2008 [ISAAA, (James 2008)]; and the maize transgenic event MON810 (YieldGard®) is widely cultured worldwide and the only GM crop grown in the European Union (EU). In March 2007, 47 MON810 varieties were inscribed in the Common EU Catalogue of Varieties of Agricultural Plant Species and can now be marketed and grown in Member States (GMO-Compass, http://www.gmo-compass.org/eng/gmo/db/). These varieties are genetically diverse but they all harbor the same insert at the same chromosomal position.

Particular MON810 and non-GM comparable varieties have been described as having unexpected differences. For example, higher lignin levels and composition were found in stems of the MON810 Novelis T and Valmont T varieties than the respective near-isogenic lines (Poerschmann et al. 2005), and unforeseen metabolic variations involving the primary nitrogen pathway were observed when comparing La73-Bt (MON810) and La73 (non-GM) (Manetti et al. 2006). Differences between MON810 and near-isogenic varieties are generally not conserved among the different variety pairs analyzed, which suggests they are not a direct consequence of the transgene. The carbon to nitrogen ratio was found to be different in shoots of two transgenic versus conventional varieties but similar in six other variety pairs (Griffiths et al. 2007). Statistical differences have been reported in enantiomeric amino acid composition of Aristis Bt/Aristis (% *D* content of Arg, Ser, and Asp) and PR33P67/PR33P66 (% *D* content of Arg, Ser, and Ala) but not of Tietar Bt and Tietar (Herrero et al. 2007).

We recently described a number of genes with altered expression levels in leaves of two variety pairs (Coll et al. 2008). They corresponded to 282 genes in Aristis Bt versus Aristis and 24 genes in PR33P67 versus PR33P66 (around 2.10 and 0.18% analyzed sequences, respectively). Such differences were less significant than those found among varieties produced by conventional breeding and most were variety-specific. Only 14 sequences (corresponding to 13 genes) were down-regulated in both variety pairs. A subset of 38 differentially expressed sequences was further analyzed in other pairs of commercial MON810 versus near-isogenic varieties [DKC6575/Tietar (DeKalb, Monsanto Agricultura), Beles Sur/Sancia (Limagrain Ibérica) and Helen Bt/Helen (Advanta)]. None were differentially regulated in all five varieties but most were repressed in just one or two pairs.

These analyses were performed on in vitro cultured plantlets to reduce variability due to external and developmental factors that are known to cause considerable transcriptome changes in plants. However, as abiotic and biotic stress, light and nutrient levels are factors which fluctuate greatly in agricultural fields; it is not clear whether the differential expression patterns observed under highly controlled experimental conditions will be significant in plants grown in real-world environments. A number of recent studies highlight the importance of carrying out real-world tests to complement the results obtained under highly controlled experimental conditions (EFSA GMO Panel 2008; Roles and Conner 2008). Because of the major agricultural interest of MON810 maize we further assessed the significance of the differential expression patterns found between MON810 and near-isogenic varieties by analyzing comparable plants grown in the field.

The main objective of this study was to assess to what extent the differential expression patterns previously observed between in vitro grown MON810 and comparative non-GM varieties were maintained in plants cultivated in a natural environment following common agricultural practices.

Materials and methods

Plant material

Seeds from the following MON810 varieties (company, date of authorization in the Spanish official publication BOE) were used: Aristis Bt (Nickerson Sur/Senasa, 11/03/2003, now commercialized by Limagrain Ibérica) and PR33P67 (Pioneer Hi-Bred, 11/03/2003). The corresponding near-isogenic varieties (Aristis and PR33P66) were from the same companies. They were initially analyzed to confirm they were MON810, using powdered certified reference material (CRM, ref#ERM-BF413A, B, D, F), purchased from Fluka (Fluka-Riedel, Geel, Belgium), as control. Genomic DNAs were isolated from 0.2 g of plant material using the Nucleospin Food Kit (Macherey-Nagel Int, Easton, PA) and then subjected to event specific real-time PCR (Hernández et al. 2003) using *hmg* as the endogenous control (Hernández et al. 2005).

The seeds were grown in La Tallada d'Empordà (Girona), Catalonia, Spain (42°05′N, 3°E), where transgenic insect resistant (MON810) and conventional maize are commercially grown. Close to the sea and with a Mediterranean climate, the soil type in this area is Xerofluvent oxiaqüic, coarse-loamy, mixed, calcareous, thermic. The field under study was divided into 24 m^2 micro-plots, 4 rows wide (row spacing 0.75 m) and 8 m long. They were sown at a density of 80,000 plants/ha (25 April 2007) and were treated following standard agricultural practices in the region. About 100 kg N/ha, 100 kg P/ha and 100 kg K/ha were applied before sowing and an additional 150 kg N/ha were side-dressed at the V8 (vegetative eight-leaf) stage. Weeds were controlled with pre-emergence application of 5 l/ha of Trophy Super (Dow Agrosciences, Indianapolis, IN, USA) (35% acetochlor + 15% atrazine + 5.8% Diclormid) and with post-emergence application of 1.25 l/ha of Samson (Syngenta, Basel, Switzerland) (4% nicosulfuron). Meteorological conditions were recorded in the region (Mas Badia agro-meteorological station) from sowing to flowering dates (silk emergence, 11 July 2007). Mean temperatures were 14.3, 16.6, 20.8 and 22.3°C in April, May, June and July, respectively, i.e. similar to the temperatures recorded between 1984 and 2008 (13.0, 16.9, 20.5 and 23.2°C, respectively). The recorded rainfall values were 137.2, 41.0, 3.4 and 1.6 l/m^2 in April, May, June and July, with mean rainfall values for the same months in 1984–2008 of 61.8, 58.5, 45.1 and 27.1 l/m^2. When necessary, the fields under study were irrigated following conventional agricultural practices.

Maize plantlets were harvested at the vegetative two-leaf (V2) and the tasseling (VT) stages at the same time of day, immediately frozen in liquid nitrogen and stored at −80°C. Each sample consisted of two leaves of each of three plantlets. For VT plants, only 10 cm-long leaf portions were collected, discarding the 5 cm apical portion and removing the central vein from the usable section of the leaf. Plants were carefully checked for the absence of infections and other lesions. Three biological replicates were sampled per maize variety and growth stage, each grown in a different micro-plot.

In addition, 3 replicates of PR33P67 and PR33P66 V2 plants were sampled with a different strategy: 2 leaves from each of 15 plantlets, without lesions.

Total RNA extraction

Total RNA was extracted using the Qiagen RNeasy Plant Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. RNA concentration was quantified by UV absorption at 260 nm using a NanoDrop ND1000 spectrophotometer (Nanodrop technologies, Wilmington, DE, USA). Integrity and purity of the RNA samples were determined by agarose gel electrophoresis and OD 260/280 nm absorption ratios. All RNA samples had appropriate values.

Reverse transcription and real-time PCR amplifications

The expression of the 38 selected sequences, 3 maize housekeeping genes and the *cryIA*(*b*) transgene were assayed by Reverse transcription coupled to Real-time polymerase chain reaction (real-time RT-PCR) with SYBR Green technology as previously described (Coll et al. 2008). Reverse transcription was on 500 ng total RNA, previously treated with Turbo DNase (Ambion, Austin, TX, USA) using 50 U of MultiScribe Reverse Transcriptase (Applied Biosystems, Foster City, CA, USA) and random hexamer primers (Applied Biosystems) according to the manufacturer's protocol. For each sample, cDNA was prepared at least in duplicate and the 38 sequences were analyzed with all cDNA preparations. The absence of remaining DNA targets was demonstrated by real-time PCR analyses of DNase-treated RNA samples. Real-time PCR assays targeting the housekeeping genes β-actin, α-tubulin (developed at Consorci CSIC-IRTA) and 18S ribosomal RNA (Coll et al. 2008) were performed on all cDNA samples to normalise gene expression data. The suitability of the housekeeping genes as internal standards was confirmed in our samples through the geNORM v3.4 statistical algorithm, with M values below 0.5 in all cases.

Bioinformatics analysis

Normalization data and statistical analyses (t-test) were performed using the Genex software v.4.3.1 (MultiDAnalyses). The Benjamini and Hochberg False Discovery Rate multiple testing correction was applied (Benjamini and Hochberg 1995). The SPSS for Windows v.8 software (SPSS Inc. Chicago, Illinois, USA) was used for ANOVA and residual variance analyses.

Results and discussion

Differential gene expression between MON810 and non-GM comparable varieties grown in agricultural conditions

The present study was carried out using 38 sequences differentially expressed in Aristis Bt versus Aristis and/or PR33P67 versus PR33P66 leaves of V2 plants grown under highly controlled conditions (Coll et al. 2008). Thirty-six sequences were differentially regulated in Aristis Bt/Aristis and 11 in PR33P67/PR33P66, while 9 were in the two pairs (Table 1). Note that this included more than 10% of the sequences differentially expressed in Aristis Bt/Aristis and nearly every sequence differentially expressed in PR33P67/PR33P66 (as analyzed by transcriptomics). Here, we monitored the expression of these sequences in leaves of MON810 and comparative non-GM plants cultivated in a natural environment. We focused on two different growth stages: vegetative two leaves (V2, the same as analysed in vitro) and tasseling (VT) stages. VT is initiated when the last branch of the tassel is completely visible. At that time the plant reaches maturity, it is almost its full height and pollen shed begins. VT begins approximately 2–3 days before silk emergence.

Maize is a major crop in the region and about half that grown are MON810 varieties. Aristis Bt, Aristis, PR33P67 and PR33P66 plants were grown in the field following the normal agricultural practices in the region, with leaves being sampled at the V2 and VT stages. Sampled plants were checked for the absence of corn-borer infection, even though the incidence in the 2007 season was very low, with a mean of around 0.1 larvae per plant (Salvia et al. 2008).

Comparing plants grown in vitro and in a natural environment, a small proportion of sequences were equally regulated in the same V2 stage (Table 1). Student t pair wise comparison ($P < 0.05$) showed that only three sequences were differentially expressed in Aristis Bt versus Aristis cultured in the field, which corresponds to 10% of the analyzed sequences. Two of them were repressed 5.3- and 4.3-fold in Aristis Bt, and *ai1* (trypsin inhibitor gene) had the highest level of

Table 1 Differential expression of a total of 38 sequences between MON810 and near-isogenic varieties (36 for Aristis Bt versus Aristis; and 11 for PR33P66 versus PR33P67)

Candidate sequences	GenBank Accession Number	Aristis Bt vs. Aristis			PR33P67 vs. PR33P66		
		V2 in vitro*	V2 field	VT field	V2 in vitro*	V2 field	VT field
ar6	AY108935.1	*0.015*	0.599	0.646	*0.007*	0.609	0.270
ar10	BM382651	*0.007*	0.311	0.673	*0.000*	0.66	0.722
pr3	AF297044.1	*0.047*	0.699	0.452	*0.008*	0.623	0.616
pr4	CO518420	*0.037*	0.916	0.741	*0.011*	0.329	0.841
pr5	CF635310	*0.009*	0.368	0.451	*0.007*	0.941	0.499
pr6	U33318.1	*0.010*	0.163	0.687	*0.008*	0.454	0.617
pr7	CD438478	*0.050*	0.061	0.457	*0.022*	0.591	0.99
pr8	AW927712	*0.047*	0.447	0.746	*0.008*	0.617	0.934
pr9	CK144500	*0.018*	0.952	*0.005*	*0.009*	0.621	0.925
ai1	AF057184.1	*0.000*	*0.000*	0.235			
ai2	BI431120	*0.027*	0.066	0.458			
ai3	U17351.1	*0.022*	0.055	0.404			
ai4	BM335222	*0.008*	0.259	0.488			
ai6	U17350.1	*0.018*	0.098	0.842			
ai7	CK371178	*0.016*	0.059	0.501			
ai8	BM378406	*0.007*	0.167	0.448			
ai9	CF638013	*0.008*	0.644	0.734			
ai10	CD219268	*0.000*	0.086	0.511			
ai11	AY639018.1	*0.009*	0.058	0.738			
ai12	M33103.1	*0.027*	0.092	0.735			
ai13	D45402.1	*0.007*	0.067	0.48			
ai14	CK985533	*0.000*	0.055	0.715			
ai15	CD435044	*0.010*	0.227	0.456			
ai16	CO519322	*0.000*	0.172	0.745			
ar1	BM379705	*0.030*	0.615	0.855			
ar2	AF056326.1	*0.005*	0.217	0.844			
ar3	CO528265	*0.005*	0.288	0.69			
ar4	CF623731	*0.007*	0.217	0.938			
ar5	AF133840.1	*0.006*	0.066	0.282			
ar7	AI666020	*0.006*	*0.000*	0.959			
ar8	CF624123	*0.016*	0.061	0.844			
ar9	CK827218	*0.006*	0.068	0.842			
ar11	X54076.1	*0.048*	0.216	0.856			
ar12	CF632382	*0.010*	0.166	0.728			
ar13	AY105790.1	*0.006*	*0.035*	0.593			
ar14	BM896110	*0.006*	0.059	0.860			
pi1	CF625331				*0.027*	0.548	0.958
pi2	AF297046.1				*0.007*	0.694	0.698

T-test significance levels are indicated for each sequence, developmental stage and growth conditions. *T*-test values with statistical significance ($P < 0.05$) are italicized. * Results obtained by Coll et al. (2008). Only statistically significant values are indicated

differential expression under controlled conditions, with a 25-fold induction in transgenic plants (Coll et al. 2008). None of the 11 sequences analyzed were differentially expressed in PR33P67 versus PR33P66 in field conditions. None of the nine sequences previously identified as being up- or down-regulated in the two variety pairs grown in vitro (*ar6, ar10* and *pr3–pr9*), were regulated in any of the two pairs when plants of the same V2 stage were grown in natural conditions. Recently, major differences were observed in transcript profiles in an Ericaceae shrub during cold acclimatation under field and cool room conditions (Dhanaraj et al. 2007).

Similarly, more than 95% of the selected sequences had similar expression levels in VT plants of MON810 and near-isogenic varieties (Student *t*, $P < 0.05$, Table 1). Only *pr9* was differentially expressed in Aristis Bt versus Aristis. It was 2.3-fold down-regulated in Aristis Bt V2 plants grown in vitro (Coll et al. 2008) but similarly expressed in GMO and comparable V2 plants grown in the field. Notably, no sequence was identified that was differentially regulated in the 2 pairs of MON810 and comparable varieties when plants were grown in a real environment. It should be noted that this study was restricted to 38 sequences, but they were selected to represent those differentially expressed in Aristis Bt versus Aristis and/or PR33P67 versus PR33P66 in vitro cultured V2 plants. Even though other differences between MON810 and comparable varieties grown in the field cannot be discarded, our results suggest high similarity between transgenic and conventional plants at the V2 and VT stages, and are in agreement with recent literature reporting that unintended variation between GM and comparable non-GM plants has very little impact other than the expected character (Baker et al. 2006; Catchpole et al. 2005).

For control purposes, all our GM samples were analyzed by real-time RT-PCR to compare the levels of expression of the transgene. The same three internal controls were used for normalization. The mRNA levels of *cryIA*(*b*) were the same among varieties (Aristis Bt and PR33P67, ANOVA significance level, 0.697) but were lower in V2 plants grown in vitro than under field conditions (ANOVA significance level, 0.000). This discounts differential expression patterns being directly attributable to higher transgene mRNA levels.

Natural variation across individual plants can mask any difference between samples from different varieties or different growth stage. In vitro cultured plants were used in our initial approach (Coll et al. 2008) to sensitively detect the possible regulation between transgenic and comparable varieties. The highly controlled experimental conditions were to minimise both variability among samples and the effects of factors other than the variety (such as environmental factors). Plants grown in the field were subjected to less homogeneous conditions. Residual variances of the expression values obtained (which cannot be attributed to the sequence or the variety) were to some extent lower for in vitro than in field samples (Aristis Bt and Aristis: 0.05, 0.12 and 0.15% for V2 in vitro, V2 and VT in field, respectively; PR33P67 and PR33P66: 0.02, 0.04 and 0.04% for V2 in vitro, V2 and VT in field, respectively), but these values are within an acceptable range.

In a control experiment we used triplicate samples of PR33P66 and PR33P67 V2 plants that were taken from 15 (instead of three) plants per sample. The calculated residual variance was 0.03%. As with the smaller samples, none of the 11 analysed sequences were differentially expressed between PR33P66 and PR33P67. Moreover, the results obtained from 3- and 15-plant samples were similar for all sequences and varieties tested (Student *t* pair wise comparison, $P < 0.05$; mean *P* value, 0.330 ± 0.229). Thus, the sample size or random variation among plants did not play a major role in the similar expression values obtained between GMO and comparable varieties grown in the field. This rather supported the conclusion that most differences observed in vitro were not statistically significant when plants were grown in field conditions.

Even though at the V2 stage maize plants essentially use their seed storage reserves, those cultured in vitro are subjected to different conditions compared to those grown in agricultural fields (e.g. light intensity, temperature and humidity fluctuations, substrate characteristics), and this result in physiological adaptations (for a review, see Afreen et al. 2000). In agreement with the known impact of environmental factors on the plant transcriptome (Fernandes et al. 2008), for all four varieties around 60% of analyzed sequences were expressed at different levels in V2 plants grown under in vitro compared to field

conditions (Student t pair wise comparison, $P < 0.05$, Supplemental material Table 1). V2 and VT field plants of the same variety had less than 40% of analyzed sequences up- or down-regulated. Importantly, these differential expression patterns were highly conserved in MON810 versus non-GM comparable varieties (around 80% analyzed sequences had the same regulation pattern) but were more divergent between non-GM or between MON810 varieties (around 60% analyzed sequences were similarly regulated). This further points towards the equivalence between MON810 and near-isogenic varieties, especially as compared to the similarity between conventional commercial varieties.

Conclusion

Previously (Coll et al. 2008), we reported the differential expression of $\sim$1.7 and $\sim$0.1% of transcripts in leaves of in vitro cultured plants of two MON810 versus near-isogenic variety pairs, AristisBt/Aristis and PR33P67/PR33P66. These varieties had been obtained by different seed companies through specific breeding programmes and were selected to represent the existing phenotypical diversity among commonly used varieties. As maize is of major agricultural interest, we further assessed the significance of these differential expression patterns in plants grown in a natural environment following common agricultural practices.

Our results show that most sequences selected as differentially expressed in plants grown in vitro were similarly expressed in MON810 and near-isogenic varieties cultured in the field. This suggests that unintended variation between MON810 and comparable varieties has little impact apart from the introduced character. In vitro methods have clear advantages with respect to sample uniformity, while field cultivation of GM plants is not only more difficult but is also subject to strict controls and prohibitions. But environmental conditions have an important effect on the plant growth and thus in its transcriptome at a given developmental stage. The expression patterns of 38 selected genes in V2 plants grown in vitro and V2 and VT plants cultivated in the field were highly conserved between MON810 and non-GM comparable varieties; and less conserved between conventional (or between GM) varieties: this is in agreement with recent literature reporting that differences between cultivars result in more variation than the comparison of GM versus non-GM. Our results highlight the importance of not only accurately assessing possible unexpected effects of GMO under highly controlled conditions, but also the need to study plants grown in a real agricultural environment. The two approaches should be considered as complementary to gain additional insights into potential unexpected effects of transgenes.

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References

Afreen F, Zobayed SMA, Kubota C, Kozai T (2000) Physiology of in vitro plantlets grown photoautotrophically. In: Kubota C, Chun C (eds) Transplant production in the 21st century. Kluwer Academic Publishers, The Netherlands, pp 238–246

Baker JM, Hawkins ND, Ward JL, Lovegrove A, Napier JA, Shewry PR, Beale MH (2006) A metabolomic study of substantial equivalence of field-grown genetically modified wheat. Plant Biotechnol J 4:381–392. doi:10.1111/j.1467-7652.2006.00197.x

Batista R, Saibo N, Lourenco T, Oliveira MM (2008) Microarray analyses reveal that plant mutagenesis may induce more transcriptomic changes than transgene insertion. Proc Natl Acad Sci USA 105:3640–3645. doi:10.1073/pnas.0707881105

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. Plant Biotechnol J 4:369–380. doi:10.1111/j.1467-7652.2006.00193.x

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc B 57:289–300

Bradford KJ, Van Deynze A, Gutterson N, Parrott W, Strauss SH (2005) Regulating transgenic crops sensibly: lessons from plant breeding, biotechnology and genomics. Nat Biotechnol 23:439–444. doi:10.1038/nbt1084

Catchpole GS, Beckmann M, Enot DP, Mondhe M, Zywicki B, Taylor J, Hardy N, Smith A, King RD, Kell DB, Fiehn O, Draper J (2005) Hierarchical metabolomics demonstrates substantial compositional similarity between genetically modified and conventional potato crops. Proc Natl Acad Sci USA 102:14458–14462. doi:10.1073/pnas.0503955102

Cellini F, Chesson A, 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. doi:10.1016/j.fct.2004.02.003

Chassy B, Egnin M, Gao Y, Glenn K, Kleter GA, Nestel P, Newell-McGloughlin M, Phipps RH, Shillito R (2008) Nutritional and safety assessments of foods and feeds nutritionally improved through biotechnology: case studies. Comp Rev Food Sci Food safety 7:65–74

Cheng KC, Beaulieu J, Iquira E, Belzile FJ, Fortin MG, Stromvik MV (2008) Effect of transgenes on global gene expression in soybean is within the natural range of variation of conventional cultivars. J Agric Food Chem 56:3057–3067. doi:10.1021/jf073505i

Coll A, Nadal A, Palaudelmàs M, Messeguer J, Melé E, Puigdomènech P, Pla M (2008) Lack of repeatable differential expression patterns between MON810 and comparable commercial varieties of maize. Plant Mol Biol 68:105–117. doi:10.1007/s11103-008-9355-z

Dhanaraj AL, Alkharouf NW, Beard HS, Chouikha IB, Matthews BF, Wei H, Arora R, Rowland LJ (2007) Major differences observed in transcript profiles of blueberry during cold acclimation under field and cold room conditions. Planta 225:735–751. doi:10.1007/s00425-006-0382-1

Dubouzet JG, Ishihara A, Matsuda F, Miyagawa H, Iwata H, Wakasa K (2007) Integrated metabolomic and transcriptomic analyses of high-tryptophan rice expressing a mutant anthranilate synthase alpha subunit. J Exp Bot 58:3309–3321. doi:10.1093/jxb/erm179

EFSA GMO Panel (2008) Safety and nutritional assessment of GM plants and derived food and feed: the role of animal feeding trials. Food Chem Toxicol 46(Supplement 1):S2–S70

Fernandes J, Morrow DJ, Casati P, Walbot V (2008) Distinctive transcriptome responses to adverse environmental conditions in *Zea mays* L. Plant Biotechnol J 6:782–798. doi:10.1111/j.1467-7652.2008.00360.x

Griffiths BS, Heckmann LH, Caul S, Thompson J, Scrimgeour C, Krogh PH (2007) Varietal effects of eight paired lines of transgenic Bt maize and near-isogenic non-Bt maize on soil microbial and nematode community structure. Plant Biotechnol J 5:60–68. doi:10.1111/j.1467-7652.2006.00215.x

Hernández M, Pla M, Esteve T, Prat S, Puigdomènech P, Ferrando A (2003) A specific real-time quantitative PCR detection system for event MON810 in maize YieldGard based on the 3′-transgene integration sequence. Transgenic Res 12:179–189. doi:10.1023/A:1022979624333

Hernández M, Esteve T, Pla M (2005) Real-time PCR based methods for quantitative detection of barley, rice, sunflower and wheat. J Agric Food Chem 53:7003–7009. doi:10.1021/jf050797j

Herrero M, Ibáñez E, Martín-Alvarez PJ, Cifuentes A (2007) Analysis of chiral amino acids in conventional and transgenic maize. Anal Chem 79:5071–5077. doi:10.1021/ac070454f

Ioset JR, Urbaniak B, Ndjoko-Ioset K, Wirth J, Martin F, Gruissem W, Hostettmann K, Sautter C (2007) Flavonoid profiling among wild type and related GM wheat varieties. Plant Mol Biol 65:645–654. doi:10.1007/s11103-007-9229-9

James C (2008) Global status of commercialized biotech/GM crops: 2008. ISAAA Briefs 39. ISAAA, Ithaca, NY

Kok EJ, Keijer J, Kleter GA, Kuiper HA (2008) Comparative safety assessment of plant-derived foods. Regul Toxicol Pharmacol 50:98–113. doi:10.1016/j.yrtph.2007.09.007

Kristensen C, Morant M, Olsen CE, Ekstrom CT, Galbraith DW, Moller BL, Bak S (2005) Metabolic engineering of dhurrin in transgenic *Arabidopsis* plants with marginal inadvertent effects on the metabolome and transcriptome. Proc Natl Acad Sci USA 102:1779–1784. doi:10.1073/pnas.0409233102

Lehesranta SJ, Davies HV, Shepherd LV, Nunan N, McNicol JW, Auriola S, Koistinen KM, Suomalainen S, Kokko HI, Karenlampi SO (2005) Comparison of tuber proteomes of potato varieties, landraces, and genetically modified lines. Plant Physiol 138:1690–1699. doi:10.1104/pp.105.060152

Manetti C, Bianchetti C, Casciani L, Castro C, Di Cocco ME, Miccheli A, Motto M, Conti F (2006) A metabonomic study of transgenic maize (*Zea mays*) seeds revealed variations in osmolytes and branched amino acids. J Exp Bot 57:2613–2625. doi:10.1093/jxb/erl025

Parrott W (2005) The nature of change: towards sensible regulation of transgenic crops based on lessons from plant breeding, biotecnology and genomics. In: Proceedings of the 17th North American Biothecnology Council, Nahville, Tenn., June 27–29 2005. Available from: http://nabc.cals.cornell.edu/pubs/nabc_17/parts/NABC17_Banquet_1.pdf. Accessed 4 Nov 2008

Poerschmann J, Gathmann A, Augustin J, Langer U, Gorecki T (2005) Molecular composition of leaves and stems of genetically modified bt and near-isogenic non-bt maize—characterization of lignin patterns. J Environ Qual 34:1508–1518. doi:10.2134/jeq2005.0070

Roles AJ, Conner JK (2008) Fitness effects of mutation accumulation in a natural outbred population of wild radish (*Raphanus raphanistrum*): comparison of field and greenhouse environments. Evolution Int J Org Evolution 62:1066–1075. doi:10.1111/j.1558-5646.2008.00354.x

Ruebelt MC, Lipp M, Reynolds TL, Schmuke JJ, Astwood JD, DellaPenna D, Engel KH, Jany KD (2006) Application of two-dimensional gel electrophoresis to interrogate alterations in the proteome of gentically modified crops, 3-Assessing unintended effects. J Agric Food Chem 54:2169–2177. doi:10.1021/jf052358q

Salvia J, López A, Capellades G, Betbesé JA, Serra J (2008) Varietats de blat de moro per a la campanya 2008. Dossier Tècnic 27:3–14

Shepherd LV, McNicol JW, Razzo R, Taylor MA, Davies HV (2006) Assessing the potential for unintended effects in genetically modified potatoes perturbed in metabolic and developmental processes. Targeted analysis of key nutrients and anti-nutrients. Transgenic Res 15:409–425. doi:10.1007/s11248-006-0012-5

Shewry PR, Baudo M, Lovegrove A, Powers S, Napier JA, Ward JL, Baker JM, Beale MH (2007) Are GM and conventionally bred cereals really different? Trends Food Sci Technol 18:201–209. doi:10.1016/j.tifs.2006.12.010