Regulatory Toxicology and Pharmacology xxx (2010) xxx–xxx

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

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

Gene expression profiling for food safety assessment: Examples in potato and maize

Jeroen P. van Dijk [a,*], Carlo Leifert [b], Eugenia Barros [c], Esther J. Kok [a]

[a] RIKILT Institute of Food Safety, P.O. Box 230, 6700 AE Wageningen, The Netherlands
[b] Nafferton Ecological FarmingGroup, Nafferton Farm, Newcastle University, Stocksfield, Northumberland NE43 7XD, United Kingdom
[c] Council for Scientific and Industrial Research (CSIR), Biosciences, Meiring Naude Road, Brummeria, Pretoria 0001, South Africa

ARTICLE INFO

Article history:
Available online xxxx

Keywords:
Transcriptomics
Microarrays
Genetically modified food
GMO
Food safety
Food
DNA
mRNA
Gene expression

ABSTRACT

Since the mid 1990s, microarray analysis has become one of the few tools that can analyze the entire contents of a cell regarding a specific information type. Especially since the development of whole genome microarrays the technique can be considered truly holistic. Most DNA based microarrays are used for the analysis of the total of messenger RNAs (transcriptome) and provide a snap-shot of what's going on in a cell population at the time of sampling. Within the last few years also full genome plant microarrays have become available for several crop species. With these it has been shown that several growing conditions can be separated based on their transcriptome pattern, such as location, year of harvest and agricultural input system, but also different cultivars of the same crop species, including genetically modified ones. A database comprising expression levels of the transcriptome in many different circumstances with a history of safe use would be a good comparator for evaluation of new agricultural practices or cultivars, genetically modified or otherwise obtained. New techniques as next generation sequencing may overcome issues on throughput time and cost, standard operation procedures and array design for individual crops.

1. Introduction

Transcriptomics has been the main tool to study broad, overall gene expression since its emergence in the second half of the 1990s. The technique offers a much larger scale of analysis than preceding techniques like Northern blotting, though essentially based on the same basic molecular biology principle: a single strand of DNA will hybridize to its complementary strand under the right chemical conditions. In the case of gene expression studies using microarrays, the transcriptome of a sample (the total messenger RNA (mRNA) pool) is generally converted to complementary DNA (cDNA) while being fluorescently labeled at the same time or in a subsequent step. This material is then allowed to hybridize to a collection of complementary probes, attached to a solid surface, at known positions. The intensity of the fluorescence at a particular position is a measure for the level of expression of the gene(s) hybridizing to the probe at that position.

In the early years of the technique cDNA microarrays dominated the scientific landscape. These usually consisted of a collection of PCR products spotted on a glass microscope slide. Probes were typically several hundreds of basepairs long and usually amplified from a cDNA library with universal primers. Typical sizes were microarrays with several thousand characteristics or spots on them, as they are commonly named after the spotting process used to deposit the DNA molecules on the slides. However, in recent years, more and more genomes of species, including plants, have been fully sequenced. This has allowed the design of microarrays consisting of single-stranded oligonucleotides (oligos) representing the entire mRNA population (or transcriptome) of an organism. The length and sequence characteristics of each oligo can be designed to be the same, so hybridization conditions are very similar for all spots. This has greatly enhanced the reproducibility of the microarray technique. Also, advanced manufacturing techniques have contributed to both a better reliability (i.e. spot quality) and more spots on an individual microarray. Initially, academic efforts dominated the microarray market, while today two companies are leading (Agilent and Affymetrix). However, some microarrays are produced by cooperation with one or more academic institutions. Affymetrix sells microarrays currently containing up to 6.5 million probe sets, while Agilent manages to synthesize 244,000 probe sets per microarray. Human and other mammalian microarrays were the first with full genome coverage, the two companies now offer complete or near complete genome arrays for several species, including plants, such as Arabidopsis, barley, cotton, maize, rice, tomato, wheat (both companies), Brassica, tobacco (only Agilent), citrus, Medicago, soybean, sugarcane, and grape (only Affymetrix). It should be noted that while called complete genome arrays, a better name would be complete transcriptome arrays, as they

* Corresponding author. Fax: +31 317417717.
E-mail addresses: jeroen.vandijk@wur.nl (Jeroen P. van Dijk), c.leifert@ncl.ac.uk (C. Leifert), ebarros@csir.co.za (E. Barros), esther.kok@wur.nl (E.J. Kok).

0273-2300/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.yrtph.2010.06.012

usually represent the coding regions of all genes expressed in the genome and no intergenic and intron regions. Plant genome microarrays are also available through academic efforts such as for maize, (the maize oligonucleotide array project www.maizearray.org), rice, (the rice oligonucleotide array project, www.ricearray.org), or tomato (http://ted.bti.cornell.edu/).

Apart from an obvious role in the study of gene function in plants, transcriptomics has been proposed to serve as an unbiased tool in food safety evaluation. The number of technological tools that are at the plant breeder's disposal to introduce new desired traits into the plant genome is increasing. In the past, this has only in rare cases led to safety issues in the newly bred plant varieties. Often cited adverse effects are white potatoes with high glycoalkaloid levels (Van Gelder et al., 1988) and the celery variety with high psoralen levels (Seligman et al., 1987). However, an assessment of the safety of new crop plant varieties is, not a routine part of approval procedures. With the current development of new plant varieties with more drastic changes in the plant's physiology, it can be questioned whether the assessment of the safety of new crop varieties should not become a more standard item in the approval procedure of these new varieties (Kok et al., 2008a,b).

The food and feed safety assessment of plant products derived from genetically modified organisms has been the subject of debate in a number of international expert meetings (FAO/WHO, 1996; OECD, 1996; Konig et al., 2004). One of the recommendations was to further investigate the use of 'omics' technologies as unbiased tools to assess any unintended changes in the plant's physiology (FAO/WHO, 1996; OECD, 1996). In the subsequent years, a number of national and European research projects have investigated the potential of transcriptomics, proteomics and metabolomics for the detection of undesirable side effects of genetically modifying plants (Cellini et al., 2004); http://www.entransfood.nl/RTDprojects/GMOCARE/GMOCARE.html; http://www.food.gov.uk/science/research/researchinfo/foodcomponentsresearch/novelfoodsresearch/g02programme/g02projectlist/g02001/).

Insight into the natural variation in gene expression has emerged as an important issue with respect to interpreting results from transcriptome experiments. With a holistic approach such as transcriptomics, some changes are likely to be discovered in all cases between wild type (WT) and new plant varieties, whether this new variety was created by traditional breeding or through genetic modification. Such changes would trigger unnecessary follow-up analyses if no data were present on the natural variation of specific genes in various conditions considered normal in agricultural practices. Natural variation of gene expression should be investigated in plants grown in for instance, different locations, climates, years of harvest, and under different farming practices, to make this overview as complete as possible. This would result in data on transcriptome variation under current growing conditions and serve as a benchmark. In all cases, observed differences will be a starting point for subsequent safety evaluation. Additionally, detected differences will need to be assessed for their food and feed safety implications and/or nutritional effects.

2. Examples

In recent years, a number of papers describing transcriptome analysis of GM plants have appeared. Baudo et al. (2006) used transcriptomics to analyze GM and conventional wheat varieties. They primarily investigated the bandwidth of natural variation and found the natural variation in gene expression patterns in conventionally bred wheat varieties to be much larger than the variation between GM lines and their non-transgenic control. Another study on the bandwidth of natural variation due to ripening stages was performed in tomatoes, evaluating the use of transcriptomics as part of safety assessment protocols (Kok et al., 2008a,b). Batista et al. (2008) compared an irradiated stable mutant rice line and a GM rice line with their respective parent lines, using transcriptomics. They found that transcriptome changes were more frequent in mutagenized plants, compared with GM plants. Similarly, Cheng et al. (2008) found differences in gene expression on the basis of whole genome soy microarrays to be more frequent and more pronounced between conventional lines than between GM and conventional lines. However, they also found changes in cysteine protease inhibitor expression levels as a potential unintended effect in GM soybeans, although they state that this could still fall within the natural variation if more conventional soy lines would have been included in the study.

Within the EU-funded SAFEFOODS project (www.safefoods.nl), ~omics techniques were explored for their use in food safety evaluation by comparing several WT and GM plants. Also, the natural variation was explored due to year of harvest, cultivar, agricultural input system, and location. One of the studies was a field trial from the Blight-MOP project (http://research.ncl.ac.uk/nefg/blightmop/index.php), studying the management of blight in EU organic potato farming. In the study analyzed with transcriptomics (van Dijk et al., 2009), two cultivars, Lady Balfour and Sante, that were exposed to late foliar blight, were grown under different organic regimes, including differences in fertilizer (manure or compost) and differences in blight protection (COMCAT, copper treatment or no treatment). An expected major effect was observed when comparing the two different cultivars (Fig. 1). The other factors did not show up in any of the principal components and showed limited differential gene expression in an analysis of variance (ANOVA), accompanied by high false discovery rates (FDRs). In contrast, many differentially expressed genes were found for the factor cultivar with ANOVA, with low FDRs. Additionally, differential gene expression of some members of the proteinase inhibitor (PI) family was confirmed with real time RT (reverse transcriptase) PCR, which is still the golden standard for gene expression

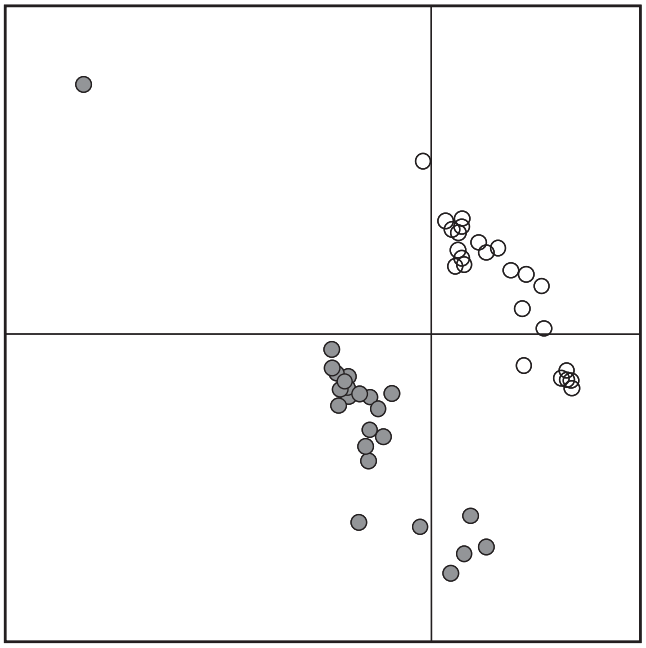

Fig. 1. Complete separation of the Lady Balfour and Sante cultivars in a principal component analysis of the gene expression data on the basis of the FSPM array. Representation of individual arrays in the combination of the first two components, gray circles represent arrays hybridized with Lady Balfour samples, open circles represent those with Sante samples. *X*-axis: 1st component, explaining 23% of the variation, *Y*-axis: 2nd component, explaining 20% of the variation (van Dijk et al., 2009). Reproduced with permission.

Please cite this article in press as: Dijk, J.P.v., et al. Gene expression profiling for food safety assessment: Examples in potato and maize. Regul. Toxicol. Pharmacol. (2010), doi:10.1016/j.yrtph.2010.06.012

quantification. The different cultivars showed a cultivar specific PI expression profile that might reflect the different genetic backgrounds with relation to blight resistance.

Another potato study analyzed within SAFEFOODS was from the QLIF (quality low input food) project (www.qlif.org), in which a potato cultivar was grown under either organic or conventional fertilizer conditions combined with organic or conventional crop protection systems. Transcriptomics revealed few but significant differences in gene expression for both factors. These results were obtained with two types of microarrays: the Food Safety-oriented Potato Microarray (FSPM, a 4 K cDNA array) as well as the Potato Oligo Chip Initiative (POCI) array (a 44 K oligo array, manuscript in preparation).

In the SAFEFOODS project maize studies were also included. Three maize genotypes were grown in different years and at different locations in South Africa. The genotypes were an insect-resistant (Bt) line, a herbicide-tolerant (Roundup ready) line, and a genetically close conventional variety. Samples were analyzed with transcriptomics, metabolomics, and proteomics. Overall, the variation explained by the location and year was much larger than the variation explained by the genotype (Fig. 2). Nevertheless, a potentially unintended effect due to the genetic modification was discovered (lower allergen m14 expression) in the Bt samples. While this difference was present in the dataset containing the three maize varieties grown in one location in different years, it was not confirmed in the dataset containing two maize varieties grown in 1 year but in three different locations. This could be due to a true difference which is only apparent under the circumstances in one location, or it could be a false positive discovery, as the false discovery rates (FDRs) were high in both analyses. However, this does not forestall the possibility of a statistically significant change in only one or a few genes while not causing a lot of variation in the whole dataset. In this case the next question is whether the observed differences may have any food or feed safety implications (Barros et al., 2010). Data from the South African field trial were combined with part of a German field trial for which metabolomics has been published (Rohlig et al., 2009). The 'German' transcriptomics data were obtained for one cultivar, different from the South African ones, grown in three different locations; in one of these locations five additional different cultivars were grown. The major source of variation was the difference between the cultivars grown in Germany and South Africa. The second major source of variation was the difference between the German cultivars, while differences in GM related gene expression were not observed in any of the principal components (unpublished data).

3. Benefits and challenges

Currently transcriptomics has a larger (near complete) coverage over the transcriptome, compared with proteomics and metabolomics over the proteome and metabolome, respectively. With the rapid increase of sequencing rate, genomic analysis will increasingly become a tool for safety analysis. Nevertheless, interpretation of the resulting data will require additional expression analysis in the years to come. The fact that whole transcriptome arrays are available for an increasing number of crop species, in combination with well-established protocols for gene expression analysis on the basis of these arrays, makes transcriptomics an informative way of analyzing novel plant varieties for unintended differences as a result of different breeding strategies.

Transcriptomics analysis leads to very large data sets, requiring dedicated analysis tools. Principal component analysis (PCA), hierarchical clustering (HC), and self organizing maps (SOMs) are examples of unsupervised, multivariate approaches for the analysis of such large datasets. These tools are designed for an assessment of trends in datasets, such as the groupings of samples. These methods take all variables into account simultaneously, so subtle changes in groups of variables may cause a grouping of samples, while none of the individual variables would have done so. These tools do not give a significance level, because there is no hypothesis testing involved. Also, it can be quite difficult to identify the variables driving an interesting grouping or separation of samples, especially if such an interesting grouping appears at a 7th or 8th component, explaining only a low percentage of the variation in the dataset. As a consequence, a *t*-test or ANOVA is frequently performed afterwards, to find individual genes driving the changes observed in the multivariate analysis. A drawback here is that such tests are univariate, so they analyze one variable at a time, disregarding dependencies in the dataset and potentially leading to high false discovery rates (FDR) due to multiplicity (type I error). While the FDR can be estimated, a more elegant solution lies in the analysis of biological pathways, to help identify significant differential gene expression. Several tools have been designed for pathway analysis, with a few specifically for plants; MapMan (Usadel et al., 2005; Rotter et al., 2007) and EasyGO (Zhou and Su, 2007). However, they do not yet provide a comprehensive picture of all genes acting in a single organism, with plants lagging behind mammalian species in the elucidation of gene functions. Ideally, pathway and network analysis will lead, in combination with other ~omics techniques, to a more complete understanding of cell biology, from gene to metabolite, often referred to as systems biology.

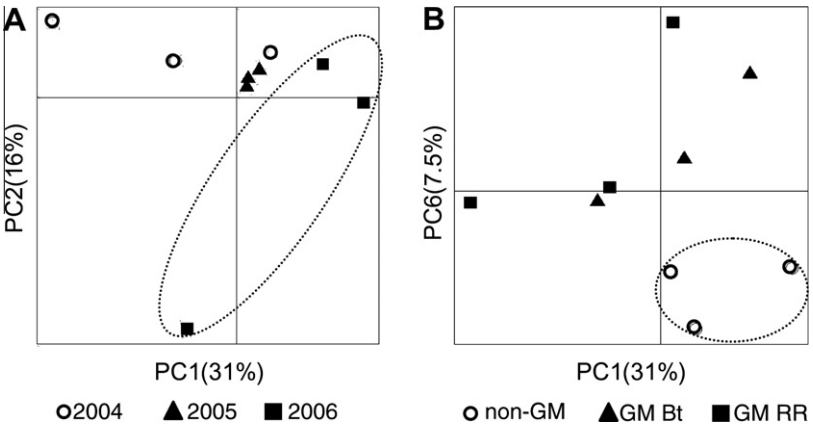

Fig. 2. PCA score plots for the samples grown at the Petit location. (A) Separation between the growing seasons: *X*-axis: component 1 (31%), *Y*-axis: component 2 (16%). (B) Separation between the conventional and GM lines: *X*-axis: component 1 (31%), *Y*-axis: component 6 (7.5%) (Barros et al., 2010). Reproduced with permission.

Please cite this article in press as: Dijk, J.P.v., et al. Gene expression profiling for food safety assessment: Examples in potato and maize. Regul. Toxicol. Pharmacol. (2010), doi:10.1016/j.yrtph.2010.06.012

Knowledge of biological interplay will not only be helpful for identification of relevant differences, it will also aid the interpretation of the differences found. In this respect, for safety evaluation purposes, performing studies on known unsafe or undesirable samples would be very informative. Examples are potatoes and tomatoes with high levels of glycoalkaloids, soy with elevated proteinase inhibitors, maize with elevated phytate levels, and cassava with elevated levels of cyanogens. Knowing the relation between gene expression and adverse levels of these kinds of antinutrients would indeed be helpful in the interpretation of differential gene expression found in the course of safety evaluation.

The construction of a database for transcriptomics data of selected varieties of several key species with a history of safe use would provide a very useful tool for safety assessment of novel plant varieties. This is analogous to the ILSI crop composition database (Ridley et al., 2004). Even without the complete knowledge of biological systems, such a database would be able to identify gene expression that clearly falls outside known natural variation observed in varieties considered to be safe. Such a database requires highly standardized methods and protocols. The use of a single platform for each crop species would be desirable. If multiple platforms are available for whole transcriptome analysis of a particular species, an adequate comparison should be performed between the different platforms for the same samples of the particular species. The sampling of the different crop varieties and derived edible (food/feed) products will also require strict standardization and should capture the broad variety of plant parts entering the market. This may lead to the sampling of several time-points post-harvest.

4. Future perspectives

Presently, gene expression analysis of novel crop varieties including GM varieties on the basis of whole transcriptome arrays can be performed in a reproducible way, resulting in informative data on the basis of limited numbers of experiments. In the years to come, other developments may again replace this current procedure, when the next generation sequencing techniques may prove to be even more efficient and informative. Several platforms are now available that generate up to 600 million short sequence reads per sample (Cloonan et al., 2008; Morozova et al., 2009; Ozsolak et al., 2009). This covers the whole transcriptome, up to several 100 times, depending on the species. The sequences are subsequently assembled into genes, after which the number of times a certain transcript is detected is counted, ergo: a digital expression profiling is obtained. Depending on platform and size of the genome investigated, a dynamic range of six orders of magnitude is possible for transcript quantification. Another benefit is that no a priori design of an array is needed, and as a consequence, previously unknown sequences will be detected. These can be novel fusion genes or translocations as well as introduced traits. Additionally, non-coding transcripts can be identified as well as alternative splicing. Finally, these sequencing techniques may provide a more uniform unit of expression: provided there is adequate coverage, the number of counts for gene *x*, relative to the total numbers of sequence reads, should be interchangeable between platforms. However, these developments are still in an early stage and the challenge will be to develop adequate procedures and tools for data analysis before any validation of the different protocols for food and feed safety evaluation can be considered.

5. Conclusions

Transcriptomics is currently the most comprehensive tool for the broad analysis of all processes in a cell. Microarray technology has provided the means for a true holistic view of plant cell expression profiles which can be useful for the evaluation of differences related to environmental factors as well as genetic background. Studies have shown that even within a multitude of data, minor significant changes can be discovered and confirmed. New sequencing strategies hold the promise for even higher accuracy and coverage of transcriptomics. A major challenge lies still in the biological interpretation of results. While transcriptomics may help with the analysis of novel foods, it is not yet a standard tool for risk assessors in food safety evaluation. The set-up of a well-controlled database with standardized (meta) data would be a good way to make maximal use of transcriptomics data for food safety evaluation. Furthermore, such a database would provide excellent data-mining opportunities for elucidation of biological pathways and networks, alone, or in combination with other ~omics technologies.

Conflict of interest statement

None declared.

Acknowledgment

The authors would like to acknowledge Dr. Louise VT Shepherd for coordination and distribution of samples in the SAFEFOODS project.

This work was financially supported by the EU under the FP5 RTD Project Blight-MOP (QLK5-CT-2000- 01065) and the FP6 Projects SAFEFOODS (Food-CT-2004-506446) and Quality Low Input Food (EU FP6 Contract No. CT-2004-506358) as well as by the Dutch Ministry of Agriculture, Nature and Food Quality.

References

Barros, E., Lezar, S., Anttonen, M.J., van Dijk, J.P., Rohlig, R.M., Kok, E.J., Engel, K.H., 2010. Comparison of two GM maize varieties with a near-isogenic non-GM variety using transcriptomics, proteomics and metabolomics. Plant Biotechnol. J. 8 (4), 436–451.

Batista, R., Saibo, N., Lourenco, T., Oliveira, M.M., 2008. Microarray analyses reveal that plant mutagenesis may induce more transcriptomic changes than transgene insertion. Proc. Natl. Acad. Sci. USA 105 (9), 3640–3645.

Baudo, M.M., Lyons, R., Powers, S., Pastori, G.M., Edwards, K.J., Holdsworth, M.J., Shewry, P.R., 2006. Transgenesis has less impact on the transcriptome of wheat grain than conventional breeding. Plant Biotechnol. J. 4 (4), 369–380.

Cellini, F., Chesson, A., Colquhoun, I., Constable, A., Davies, H.V., Engel, K.H., Gatehouse, A.M., Karenlampi, S., Kok, E.J., Leguay, J.J., Lehesranta, S., Noteborn, H.P., Pedersen, J., Smith, M., 2004. Unintended effects and their detection in genetically modified crops. Food Chem. Toxicol. 42 (7), 1089–1125.

Cheng, K.C., Beaulieu, J., Iquira, E., Belzile, F.J., Fortin, M.G., Stromvik, M.V., 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 (9), 3057–3067.

Cloonan, N., Forrest, A.R., Kolle, G., Gardiner, B.B., Faulkner, G.J., Brown, M.K., Taylor, D.F., Steptoe, A.L., Wani, S., Bethel, G., Robertson, A.J., Perkins, A.C., Bruce, S.J., Lee, C.C., Ranade, S.S., Peckham, H.E., Manning, J.M., McKernan, K.J., Grimmond, S.M., 2008. Stem cell transcriptome profiling via massive-scale mRNA sequencing. Nat. Methods 5 (7), 613–619.

FAO/WHO, 1996. Biotechnology and food safety. Report of a Joint FAO/WHO consultation, Rome, Italy. FAO Food and Nutrition Paper 61. Rome, Food and Agriculture Organisation of the United Nations.

Kok, E.J., Keijer, J., Kleter, G.A., Kuiper, H.A., 2008a. Comparative safety assessment of plant-derived foods. Regul. Toxicol. Pharmacol. 50 (1), 98–113.

Kok, E.J., Lehesranta, S.J., van Dijk, J.P., Helsdingen, J.R., Dijksma, W.T.P., Van Hoef, A.M.A., Koistinen, K.M., Karenlampi, S.O., Kuiper, H.A., Keijer, J., 2008b. Changes in gene and protein expression during tomato ripening – consequences for the safety assessment of new crop plant varieties. Food Sci. Technol. Int. 14 (6), 503–518.

Konig, A., Cockburn, A., Crevel, R.W., Debruyne, E., Grafstroem, R., Hammerling, U., Kimber, I., Knudsen, I., Kuiper, H.A., Peijnenburg, A.A., Penninks, A.H., Poulsen, M., Schauzu, M., Wal, J.M., 2004. Assessment of the safety of foods derived from genetically modified (GM) crops. Food Chem. Toxicol. 42 (7), 1047–1088.

Morozova, O., Hirst, M., Marra, M.A., 2009. Applications of new sequencing technologies for transcriptome analysis. Annu. Rev. Genomics Hum. Genet. 10, 135–151.

Please cite this article in press as: Dijk, J.P.v., et al. Gene expression profiling for food safety assessment: Examples in potato and maize. Regul. Toxicol. Pharmacol. (2010), doi:10.1016/j.yrtph.2010.06.012

OECD, 1996. Food Safety Evaluation. Paris, Organisation for Economic Cooperation and Development.

Ozsolak, F., Platt, A.R., Jones, D.R., Reifenberger, J.G., Sass, L.E., McInerney, P., Thompson, J.F., Bowers, J., Jarosz, M., Milos, P.M., 2009. Direct RNA sequencing. Nature 461 (7265), 814–818.

Ridley, W.P., Shillito, R.D., Coats, I., Steiner, H.Y., Shawgo, M., Phillips, A., Dussold, P., Kurtyka, L., 2004. Development of the international life sciences institute crop composition database. J. Food Compos. Anal. 17 (3–4), 423–438.

Rohlig, R.M., Eder, J., Engel, K.H., 2009. Metabolite profiling of maize grain: differentiation due to genetics and environment. Metabolomics 5 (4), 459–477.

Rotter, A., Usadel, B., Baebler, S., Stitt, M., Gruden, K., 2007. Adaptation of the MapMan ontology to biotic stress responses: application in solanaceous species. Plant Methods 3, 10.

Seligman, P.J., Mathias, C.G., O'Malley, M.A., Beier, R.C., Fehrs, L.J., Serrill, W.S., Halperin, W.E., 1987. Phytophotodermatitis from celery among grocery store workers. Arch. Dermatol. 123 (11), 1478–1482.

Usadel, B., Nagel, A., Thimm, O., Redestig, H., Blaesing, O.E., Palacios-Rojas, N., Selbig, J., Hannemann, J., Piques, M.C., Steinhauser, D., Scheible, W.R., Gibon, Y., Morcuende, R., Weicht, D., Meyer, S., Stitt, M., 2005. Extension of the visualization tool MapMan to allow statistical analysis of arrays, display of corresponding genes, and comparison with known responses. Plant Physiol. 138 (3), 1195–1204.

van Dijk, J.P., Cankar, K., Scheffer, S.J., Beenen, H.G., Shepherd, L.V., Stewart, D., Davies, H.V., Wilkockson, S.J., Leifert, C., Gruden, K., Kok, E.J., 2009. Transcriptome analysis of potato tubers – effects of different agricultural practices. J. Agric. Food. Chem. 57 (4), 1612–1623.

Van Gelder, W.M.J., Vinke, J.H., Scheffer, J.J.C., 1988. Steroidal glycoalkaloids in tubers and leaves of Solanum species used in potato breeding. Euphytica (Suppl.), 147–158.

Zhou, X., Su, Z., 2007. EasyGO: gene ontology-based annotation and functional enrichment analysis tool for agronomical species. BMC Genomics 8, 246.

Please cite this article in press as: Dijk, J.P.v., et al. Gene expression profiling for food safety assessment: Examples in potato and maize. Regul. Toxicol. Pharmacol. (2010), doi:10.1016/j.yrtph.2010.06.012