

Lack of repeatable differential expression patterns between MON810 and comparable commercial varieties of maize under in vitro and field conditions

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ABSTRACT

The introduction of genetically modified organisms (GMO) in many countries follows strict regulations to assure that only products that have been safety tested in relation to human health and the environment are marketed. Thus, GMOs must be authorized before use. By complementing more targeted approaches, profiling methods can assess possible unintended effects of transformation. We used microarrays to compare the transcriptome profiles of widely commercialized maize MON810 varieties and their non-GM near-isogenic counterparts (Coll et al. 2008). We further assessed the significance of these possible differential expression patterns in plants grown in the agricultural field (Coll et al. 2009). We present an overview of these results.

The expression profiles of MON810 seedlings are more similar to those of their corresponding near-isogenic varieties than are the profiles of other lines produced by conventional breeding. However, differential expression of ~ 1.7 and $\sim 0.1\%$ of transcripts was identified in two variety pairs (AristisBt/Aristis and PR33P67/PR33P66) that had similar *cryIA(b)* mRNA levels, demonstrating that commercial varieties of the same event have different similarity levels to their near-isogenic counterparts without the transgene (note that these 2 pairs also show phenotypic differences).

In the tissues, developmental stage and varieties analyzed, we could not identify any gene differentially expressed in all variety-pairs. However, a small set of sequences were differentially expressed in various pairs. Their relation to the transgenesis was not proven, although this is likely to be modulated by the genetic background of each variety.

Most sequences that were regulated in plants cultured in vitro, were not transcriptionally regulated in the same variety pairs when plants were grown in the field.

Key words: GMO (Genetically Modified Organism), MON810, maize, transcriptome, unintended effects, expression profile, field

Genetically modified (GM) crops are subjected to different legislation worldwide to cover aspects of consumer safety and protection. A number of publications (including work performed by the developing companies) show the equivalence of transformed and non-transformed lines of the same species [see reviews in (Cellini et al. 2004, Shewry et al. 2007)]. However, targeted approaches have detected some unpredicted differences between transgenic and conventional lines. Saxena and Stotzky (2001) described higher lignin levels in insect resistant transgenic maize than in conventional isogenic lines, and Poerschmann et al. (2005) also observed differences in lignin composition. As a consequence, the need for an in-depth study of any unexpected differences among GM and conventional lines by profiling techniques has been suggested by various authors (Cellini et al. 2004, Kok et al. 2008, Millstone et al. 1999). In this way, recent literature shows a narrow alteration of gene expression in GM and non-GM plants in several plant species such as *Arabidopsis thaliana*, potato, rice and wheat (Baudo et al. 2006, Dubouzet et al. 2007, El Ouakfaoui and Miki 2005, Gregersen et al. 2005, Kristensen et al. 2005).

As maize is the second most widespread GM crop, after soybean, with a global area of 37.9 million Ha in 2008 [ISAAA, (James 2008)] and the only GM crop cultured in the European Union (EU), in this paper we summarised a study of possible unintended variation between GM and non-GM maize. Specifically we present a transcriptome comparison between MON810 and their near-isogenic maize varieties cultured in vitro (Coll et al. 2008) as well as the significance of these possible unintended variation in plants cultivated in a natural environment following common agricultural practices (Coll et al. 2009).

The main objective of the study was to compare gene expression profiles of MON810 and comparable varieties which do not contain the transgene. We compared (by Affymetrix commercial microarrays) the transcriptome profiles of 2 MON810 and near-isogenic commercial variety pairs (Aristis Bt vs. Aristis and PR33P67 vs. PR33P66), using plants at the V2 developmental stage that were grown under in vitro conditions. The two conventional (Aristis vs. PR33P66) and the two MON810 (Aristis Bt vs. PR33P67) lines were compared.

As commercial varieties of maize MON810 have the same transgene in different genetic background, we used RT-qPCR to further compare the expression levels of a number of

selected transcripts in other MON810/near-isogenic pairs: Helen Bt/Helen, Beles Sur/Sancia and DKC6535/Tietar, overall representing different seed companies and breeding programs.

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. 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 grown in highly controlled conditions by analyzing comparable plants grown in the field.

The study was based on three biological replicates independently analysed in three microarrays for each variety (six microarrays per GM/near-isogenic pair). RT-qPCR analyses were carried out following the same design.

A total of 307 probes (equivalent to 282 genes) were differentially expressed in Aristis Bt and Aristis, which corresponded to approximately 1.7% of probes (or 2.1% genes) assayed (Figure 1). Only 25 probes (equivalent to 24 genes) were differentially expressed in PR33P67 and PR33P66 leaves, equivalent to around 0.14% of probes (or 0.18% genes) assayed (Figure 1). Aristis Bt/Aristis and PR33P67/PR33P66 had not only different numbers of differentially expressed sequences but the identity of these sequences was different. Just 14 sequences were regulated in both variety pairs ($p < 0.05$), representing about 5 % of the differentially regulated sequences in Aristis Bt vs. Aristis and almost 56 % of those in PR33P67 vs. PR33P66.

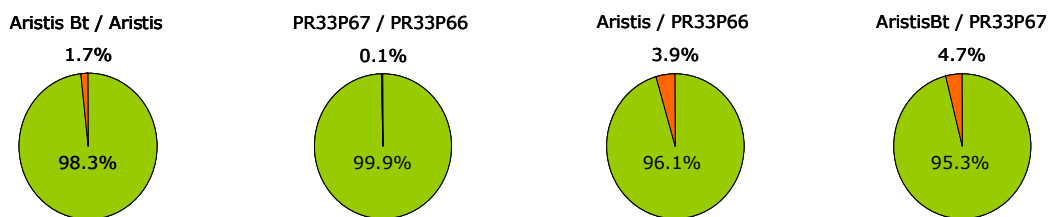


Figure 1. Pie charts representing differential gene expression in Aristis Bt/Aristis, PR33P67/PR33P66, Aristis/PR33P66 and Aristis Bt/PR33P67 based on the microarray analysis. Orange area represent the

percentage of differentially expressed sequences ($p < 0.05$, at least twofold differences) and green area represent the percentage of sequences with similar expression values.

These 14 sequences were subsequently analyzed in 3 other variety pairs. Pair wise comparison of each MON810 variety to its near-isogenic counterpart gave a complex pattern. Around 70% of these sequences were regulated in DKC6575/Tietar; only 10% were regulated in Beles Sur/Sancia; and none were differentially regulated in Helen Bt/Helen. All 5 MON810 varieties expressed similar levels of transgenic mRNA so discounting any differential expression pattern to be attributable to different *cryIA(b)* mRNA levels. These results further indicate that different variety pairs have different levels of similarity.

Comparison of the transcriptome profiles of Aristis and PR33P66 showed that they differed in around 4% of transcripts, likewise Aristis Bt vs. PR33P67 transgenic varieties differed in around 5% of the sequences. These results place the numbers of sequences differentially expressed in GM compared to near-isogenic varieties far below those with altered expression levels comparing conventional varieties (Figure 1).

As maize is of major agricultural interest, the significance of the differential expression patterns between MON810 and comparable varieties was further assessed in plants grown in natural environment following common agricultural practices. We analyzed by RT-qPCR the expression levels of 38 sequences that were differentially expressed in Aristis Bt vs. Aristis and/or PR33P67 vs. PR33P66 leaves of V2 plants grown in vitro. We monitored the expression of these sequences in leaves of MON810 and comparative non-GM plants at the same development stage (V2) and cultivated in a natural environment.

As shown in Figure 2, only 2 sequences were differentially expressed in Aristis Bt vs. Aristis cultured in the field, which corresponds to 10% of the analyzed sequences. None of the 11 sequences analyzed were differentially expressed in PR33P67 vs. PR33P66 in field conditions. None of the 14 sequences previously identified as being regulated in the two variety pairs grown in vitro were regulated in any of the two pairs grown in natural conditions. Transgene mRNA levels in V2 plants grown in vitro were lower than under field conditions.

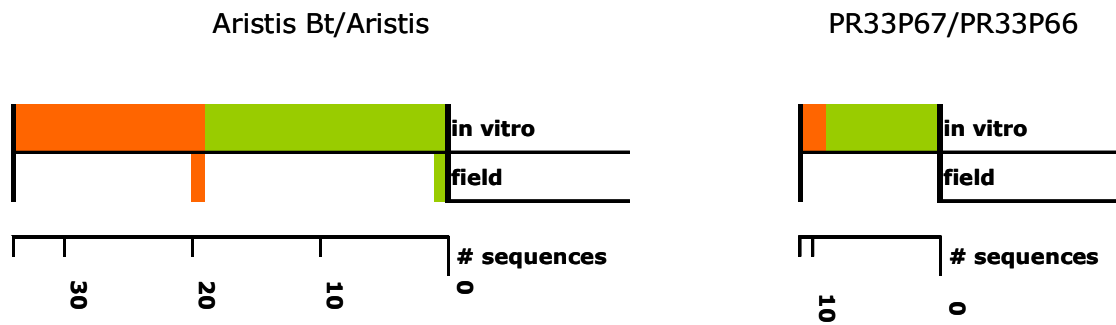


Figure 2. Schematic representation of differential gene expression in Aristis Bt/Aristis and PR33P67/PR33P66 under in vitro and field condition. Differentially expressed sequences ($p < 0.05$, at least, 2-fold difference) are represented in green (down-regulated in MON810 varieties) and orange (up-regulated in MON810 varieties). Sequences displaying similar expression values in GM and non-GM varieties (around 99% sequences analyzed) are not included.

According to recent publications, showing that unintended variation between transgenic and conventional lines did not considerably alter overall gene expression (Baudo et al. 2006, Baudo et al. 2009, Cheng et al. 2008, Dubouzet et al. 2007, El Ouakfaoui and Miki 2005, Gregersen et al. 2005, Metzdorff et al. 2006), our study revealed a low percentage of transcriptomic differences in leaves of Aristis Bt vs. Aristis and PR33P67 vs. PR33P66 grown under in vitro conditions. Although a narrow set of these sequences are differentially expressed in both variety pairs, any of them was consistently regulated in the other three MON810/near-isogenic pairs analysed by RT-qPCR. Similarly, 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); unexpected metabolic variations involving the primary nitrogen pathway were observed when comparing La73-Bt (MON810) and La73 (non-GM) (Manetti et al. 2006). Our results support that different GM and near-isogenic variety pairs have different levels of divergences.

Moreover, divergence levels between transgenic and comparable conventional maize lines were lower than those found between two different non-GM varieties obtained by conventional breeding. In fact, long-accepted plant breeding techniques cause transcriptome alterations which have been demonstrated that are greater than those caused by modern

DNA technology in wheat (Baudo et al. 2006), rice (Batista et al. 2008) and soybean ((Cheng et al. 2008).

To assess the biological relevance of differences between a new plant variety and its comparator, the bandwidth of natural variation of gene expression has to be taken into account (Van Dijk et al. 2009). To that end, we extended the analysis to 2 GM and non-GM variety pairs grown in the field. In agronomic fields, where plants were subjected to less homogeneous conditions, our results revealed that most differences observed in vitro were not significant in the field. This highlighted the importance to assess possible unintended effects of GMO, not only in vitro but also in real field conditions.

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