

## **Editorial: No Credible Scientific Evidence is Presented to Support Claims that Transgenic DNA was Introgressed into Traditional Maize Landraces in Oaxaca, Mexico**

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By Paul Christou (on behalf of the Editorial Board)

Because maize and its progenitor are wind-pollinated and capable of outcrossing, the eventual introgression of transgenes from commercial hybrids into landraces and wild relatives is likely should they be grown in close proximity. On 14th November 2001, a paper was published in the journal Nature which claimed for the first time to present evidence that transgene DNA had introgressed from commercially-released transgenic maize varieties into traditional landraces. It is not surprising that a scientific paper with such a strong claim in the title would be seized upon by the media and the public, including those who have been working with transgenic plants for many years. What is very surprising, however, is that a manuscript with so many fundamental flaws was published in a scientific journal that normally has very stringent criteria for accepting manuscripts for publication. Members of the Editorial Board of Transgenic Research, and a number of other scientists with many decades of experience in the area of transgenics, have provided comments that indeed demonstrate that the data presented in the published article are mere artifacts resulting from poor experimental design and practices. Consequently, this editorial focuses strictly on a purely scientific analysis of the data presented in the manuscript. We will not address implications or consequences if such an event had actually happened, as this is beyond the scope of this analysis. Our conclusion following detailed analysis of the results presented in this paper is that no credible scientific evidence is presented in the paper to support claims made by the authors that gene flow between transgenic maize and traditional maize landraces has taken place.

A careful analysis of the data presented in the paper strongly suggest the following:

- \* Sample contamination is the most likely explanation for the observed results.
- \* Rather than rely on questionable PCR results, plants that were alleged to contain introgressed DNA should have been grown out and subjected to more reliable confirming studies.
- \* The inverse PCR results are technically flawed.
- \* Cross pollination and introgression would not produce these results.

To avoid any confusion about what was being tested, it is important to clarify that this was maize, *Zea mays*, a landrace perhaps, but maize nonetheless, and not a progenitor species of maize. The experimental data Quist and Chapela cite as evidence for their conclusions is based solely on PCR and

inverse PCR (IPCR). All those working with these two techniques know very well that they are prone to artifacts and we have all learned in the course of many years to exercise caution in designing and interpreting experiments based on methodology involving PCR and IPCR. All evidence in the present study is based on PCR for various sequences (the CaMV35S promoter, nos terminator and Bt cryIAb). Among these, only the CaMV35S sequence was analysed by both PCR and IPCR. It was necessary to use nested PCR (two consecutive PCR reactions) to detect an obvious product. This is a particularly risky approach, since extremely low levels of contamination introduced during the handling of samples can be the cause of a positive result. The authors claim the Diconsa store sample gave a strong product in the first round of PCR but have cut out this track of the gel, without explanation. The Criollo samples represented a bulk of 150-400 grains. If the claimed presence of the transgene was due to pollination from commercial transgenic maize, one would not need PCR to prove it, given the level of 'contamination' in this relatively small number of kernels. An old-fashioned, but more reliable Southern blot would have sufficed, and would have provided more clear and unequivocal data. Indeed, the progeny of such pollinations would be very evident in terms of the dramatic change in the phenotype of the plant and cobs. Given that the authors relied on PCR, 40 PCR cycles would have been enough to amplify a single template molecule – a molecule which could have very easily been present because of contamination from a source other than plant DNA. The authors do not employ measures to eliminate any source of contamination, and therefore do not rule out the most likely explanation for the results they observed.

Further questions remain regarding the data actually presented. (1) Why did the Criollo maize samples A2, A3, B2 and B3 give weak signals while K1 gave a signal comparable to the Bt1 and RRI positive controls? Indeed, a close examination of Figure 1 shows that it does not support the statements made in the text, since the RR1 signal is strongest, and lanes for K1 and Bt1 have each been reconstructed. (2) Why are data from the historic control population never shown, when this is in fact the most appropriate control? The Mexican government results are stated without data or protocols and without citation. Given the uncertainties created by two consecutive PCRs and the likelihood of ground sample contamination, the authors should surely have grown out samples, even if more had to be collected, to prove beyond doubt that these were the result of cross-pollinations. None of this critical evidence is reported. If these really were cross-pollination events, it would be very easy to grow plants and test for kanamycin resistance, BT protein expression, or glyphosate resistance. The plants certainly would not grow true-to-type for the landrace, as mentioned above. The interpretation of the inverse PCR results in this article are flawed as well. The short fragments that in some cases abut unidentified DNA are interpreted by the authors to be the result of rearrangement of the sequences either during transformation or by recombination (during introgression?).

Transformation is not a likely explanation, since the sequences in these transformation events are well-accounted for, and stray CaMV promoters just are not present in these events. So, where did these CaMV sequences come from? Most likely not from transgenic corn, but again most probably from contamination

of the sample or some other technical artifact. The fact that the authors have not been able to show the presence of intact inserts, which are more likely to be present than fragments of unknown origin, casts further doubt that the results observed come from a transgenic plant source. Recombination is not a satisfactory explanation either, since multiple generations of crossing have been done with all these constructs, and they have been shown to be stable \_ or else they would not have made it through the regulatory system. It is highly improbable that these genes would experience a high degree of rearrangement upon crossing into a Criollo maize background. Finally, the authors show that not all CaMV positive samples were positive for the nos terminator. The absence of the nos terminator in some samples further casts doubt on the presence of intact, functional genes, which would have been expected if they were of transgenic plant origin. Furthermore, the lack of intact functional genes means that speculation about any effects would be scientifically unwarranted. Introgression through pollen is most likely to bring in the complete cassette (promoter-coding region-terminator) along with any flanking plasmid DNA that integrated into the original transgenic line. The IPCR products would, therefore, yield a sequence comprising partly of the gene and partly of the vector in almost all the cases. In two cases where the authors detected an adh1 gene sequence flanking the CaMV 35S promoter, IPCR reveals that the promoter is located within the adh1 gene sequence and not at one end as in the original construct. This calls for a most unlikely recombination event. There is no indication that the adh1 gene sequence was exactly that used in the construct. Adh is a complex gene family with a number of introns and exons. The authors did not investigate if this was an endogenous maize sequence, rather than the one normally used in trans-formation constructs. In fact, when the sequence they submitted (AF434757) is compared against the maize genome, it has 89% plus/ plus identity in 91 bases and 86% plus/ plus identity in 107 bases to the maize chro-mosome 9s bz locus. It also has 84% plus/ plus identity in 108 bases to the 22-kD alpha zein. It, of course, has an 86% plus/ minus identity in 89 bases to the adh1 gene. In all three cases approximately the same region of their submitted sequences is considered (ca 26\_ 130). The second 'adh1' gene sequence flanking the CaMV 35S promoter (AF434755) is very similar if not identical, and is homologous to the bz and zein sequences. It is not clear why the authors chose to report homology to the adh1 gene and not to the bz locus sequence or to alpha zein.

The inverse PCR result is said to show diverse adjacent sequence and the authors claim this implies insertion of the 35S sequence into multiple loci in the Criollo samples. In fact, this is not what would be expected from pollination from commercial transgenic maize. The likelihood of recombination very close the 35S promoter is infinitesimally small. The authors interpret their data as indicating that introgression events are relatively common. However, these results actually indicate technical failure in the authors' experiments. There are few transgenic events in commercial maize and all the inverse PCR results would be expected to conform to one or other of these.

While some degree of sequence rearrangement is expected, the sequences obtained by PCR are rather strange: none of the sequences contains an EcoRV site (which would be expected from the religation

event). Why are the amplified elements for A3 different/ longer than those for K1? That requires additional point mutations (to delete or create EcoRV sites) or further unexplained scrambling of sequences. The sequences that have been pulled out would generally be expected to contain vector sequence elements (for iCMV1 and iCMV2 amplicons) or transgene sequences (iCMV3 and iCMV4 amplicons) rather than zein, transposon and other unrelated sequences. Most strikingly, the sequences between the primer iCMV1 and the putative location of the EcoRV site are not well conserved at all! This implies that the original sequence between the 3' end of iCMV1 and the EcoRV site is lost/ altered in all cases, immediately after the primer.

It is most likely that the report by Quist and Chapela is a testimony to technical failure and artifacts which are common with PCR and IPCR. The PCR results are likely due to minute contamination of the ground sample powders. The inverse PCR results are problematic, internally inconsistent and not what is expected from cross-pollination by commercial transgenic maize. Most frustrating is the total failure of the authors to do the easy and incontrovertible experiment of growing out the suspected contaminated lines. Hybrids between Mexican landraces and trans-genic commercial maize would be very obvious. It is disappointing that the editors of Nature did not insist on a level of scientific evidence that should have been easily accessible if the interpretations were true. Consequently, no evidence is presented to justify any of the conclusions presented in the paper.

Reference Quist D and Chapela IH (2001) Transgenic DNA introgressed into traditional maize landraces in Oaxaca, Mexico. *Nature* 414: 541\_ 543. 3