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# Adventitious presence of transgenic events in the maize supply chain in Peru: A case study

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ABSTRACT

Cultivation and trade of transgenic or genetically modified organisms (GMO) and commodities has become widespread worldwide. In particular, production of transgenic crops has seen an accelerated growth along with a complex regulatory process. Current Peruvian legislation prohibits import of transgenic seeds and cultivation of transgenic crops in National territory but allows import of GMO-derived products and commodities. In addition, there is legislation that mandates the labeling of food products containing transgenic ingredients but the labeling threshold is still under discussion and the enforcement of this law is on hold. In this context, we evaluated adventitious presence of transgenic events in locally traded yellow maize using PCR- and immuno-based detection methods. Our results indicated that contamination during the distribution system of lots derived from non-transgenic maize was unavoidable and generally below 1.0% (w/w). Transgenic event MON810 was found in truck-loads of nationally grown maize. In general, frequencies of GMO-derived targets in whole-grain lots were 2.2% (GMO content $\geq$ 1%), 16.4% (GMO content $\leq$ 1%) and 81.3% (GMO content below our detection levels). When samples of de-germinated maize where evaluated, frequencies were 25.6% (GMO content $>$ 0.9%), 65.1% (GMO content $\leq$ 0.9%) and 9.3% (GMO content below our detection levels). We believe this information will aid policy makers in establishing a suitable threshold for trade and product labeling as well as to conduct further investigation on other crops and scenarios.



## 1. Introduction

Genetically modified organisms (GMO) are living organisms whose genetic material has been altered using recombinant DNA technology referred to as genetic engineering. During the last few decades plant breeders have taken advantage of genetic engineering to develop improved varieties that harbor desirable traits such as resistance to pathogens and herbicide tolerance, among others. These varieties are known as 'transgenic' and their global production has continuously grown during the last fifteen years (James, 2012).

Despite their advantage for dealing with day to day problems in food production, the cultivation and consumption of transgenic crops has become controversial due to health, environmental and socio-economic concerns (Romeis, Meissle, Brunner, Tschamper, & Winzeler, 2013). Although no scientific report has conclusively shown that food derived from transgenic crops is unhealthy for human consumption or the environment (Gilbert, 2013), public concern regarding their safety has triggered the establishment of policies that either prohibit or regulate transgenic-crops cultivation and trade (Gruere & Rao, 2007).

GMO legislation differs significantly among Countries, with various threshold levels for acceptance or rejection of food stocks being implemented worldwide for both products and processes (Gruere & Rao, 2007). Threshold levels are established to protect producers from unintentional or adventitious contamination of their products along the supply chain (Gruere & Rao, 2007; Zhang & Guo, 2011). In Peru, a ten-year moratorium for the production of GMOs in National territory was established in 2011 (Government-of-Peru, 2011), but import and trade of GMO commodities is allowed. Furthermore, Article 37 in Law No. 29571 mandates the labeling of food products that contain GMO-derived ingredients but the threshold level is still under discussion by National authorities (Government-of-Peru, 2010) and the enforcement of this law is on hold. Assessment of the likelihood of adventitious presence of

*Abbreviations:* GMO, genetically modified organism; PCR, polymerase chain reaction; qPCR, real-time PCR; dwb, dry weight basis; CaMV, cauliflower mosaic virus; CDS, coding sequence; CRM, certified reference material.

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transgenic events in the supply chain of commodities and intermediate products is thus necessary, to aid policy makers in the establishment of suitable thresholds for enforcement.

Maize (*Zea mays*) is a major commodity of which many transgenic varieties are commercially available (CERA, 2013). Peru imports around 60–70% of the yellow maize traded nationally, used mostly in the pork and poultry industries but also in the human diet as various processed products (OEEE, 2011). In 2010, Peru produced 1.2 million tones yellow maize with a net value of 344 million USD accounting for 2.6% of the agricultural GDP, and imported 1.9 million tones mostly from Argentina and the USA (OEEE, 2011). In addition, Peru is a major center of maize diversity (Rimachi, Alcantara, Aquino, & Ortiz, 2011), thus, discriminating transgenic events of this feedstock is relevant for domestic consumption and trade as well as for environmental control.

Various methods for GMO testing exist. The most widely used are the detection of recombinant DNA fragments by PCR and the detection of recombinant proteins by immunoassays (Zhang & Guo, 2011). Most of these methods have been validated and are available to the international community in specialized databases (Dong et al., 2008; Van Den Eede, 2010). In this study, we evaluate truck-loads of nationally grown maize from different suppliers and locations using real-time PCR and immunoassays to determine the scope of adventitious presence of transgenic events. Our findings from analyzing de-germinated and whole-grain lots of nationally grown maize are presented herein.

## 2. Materials and methods

### 2.1. Samples

Truck-loads of whole-grain maize and de-germinated maize kernels received at a Peruvian processing plant were targeted in the study. Whole-grain and de-germinated kernels consisted of yellow maize hybrids grown in various Peruvian fields and were presumably non-transgenic. Controls used in the study were (i) certified non-transgenic maize hybrid 'INIA 611' produced by the National Institute for Agricultural Innovation (Lima, Peru); (ii) certified reference materials 'ERM 413k-series', 'ERM 415- series', and 'ERM 412f' from the Institute of Reference Materials and Methods (IRMM, Geel, Belgium) corresponding to transgenic maize events MON810, NK603, and Bt11 respectively; and (iii) total DNA from a plant infected with the Cauliflower Mosaic Virus (CaMV) (Leibniz-Institut DSMZ GmbH, Braunschweig, Germany) to serve as donor-organism control for the 35S promoter (P35S).

### 2.2. Grain sampling

Bagged whole maize grains and de-germinated maize kernels were sampled following previously described guidelines (FAO, 1994; USDA, 1995). Each truckload contained about 30–35 tons of bagged grains (around 600–650 bags). A number of bags equal to the square root of the total number of bags in each truck were sampled following the pattern described in the ISO 950 standard (FAO, 1994); 200 g of grain were taken from each bag, pooled, divided in 4 equal portions and one of the quarters milled in a Corona grain mill. Moisture content was measured in a MAC 50/WH moisture analyzer (RADWAG, Radom, Poland). Milled samples were stored in double plastic bags at room temperature until analysis.

### 2.3. DNA extraction

4 g of Milled maize samples were poured into a grinding jar adapter (Qiagen, Venlo, The Netherlands), cooled with liquid nitrogen and pulverized in the Tissue Lyser II (Qiagen) operated for 1 min at 30 Hz, twice. 240 mg portions were used for DNA extraction in 2 replicates per test portion using the Axyprep Multisource Genomic DNA Miniprep Kit (Axygen Corning, NY, USA) following manufacturer's instructions. Genomic DNA was quantified using the Qubit Fluorometer 2.0 (Invitrogen). The quality of extracted DNA from each matrix was verified by agarose gel electrophoresis (Sambrook & Russell, 2001). Presence of PCR inhibitors in the DNA preparation was tested according to previously described guidelines (Žel et al., 2012), evaluating the $C_q$ difference in the qPCR amplification of a control DNA mixed 1:9 with ultrapure water or the DNA preparation. DNA samples were stored at −20 °C until analysis.

### 2.4. Quantitative real-time PCR (qPCR)

qPCR reactions were run in the 7500 Fast real-time PCR System (Applied Biosystems, CA, USA) using Fast Reaction Tubes and MicroAmp Optical Caps (Applied Biosystems), Power SYBR Green Master Mix 2X (Applied Biosystems), 0.5 μM for each primer (Eurofins MWG Operon, AL, USA), and 50 ng DNA in 25 μL reactions. Two PCR reactions per DNA sample (4 reactions per test portion) for each primer set were conducted. An initial screening for targets P35S, T-NOS, and in some cases MON810, NK603 and the phosphinothricin n-acetyltransferase '*pat*' gene (Table 1) was conducted. Quantification was done for the P35S fragment only using absolute quantification with independent standard curves for the taxon-specific gene 'maize starch synthase II' (*SSIIb*) and the Cauliflower

**Table 1**
PCR primers used in the study.

| Name | Sequence | Target name | AF (bp)[b] | Ref. |
|---|---|---|---|---|
| p35S 1-5 | 5′-ATT GAT GTG ATA TCT CCA CTG ACG T-3′ | P35S | 101 | (Kuribara et al., 2002) |
| p35S 1-3 | 5′-CCT CTC CAA ATG AAA TGA ACT TCC T-3′ | | | |
| tNOS 2-5 | 5′-GTC TTG CGA TGA TTA TCA TAT AAT TTC TG-3′ | T-NOS | 151 | (Kuribara et al., 2002) |
| tNOS 2-3 | 5′-CGC TAT ATT TTG TTT TCT ATC GCG T-3′ | | | |
| M810 2-5 | 5′-GAT GCC TTC TCC CTA GTG TTG A-3′ | M810 | 113 | (Kuribara et al., 2002) |
| M810 2-3 | 5′-GGA TGC ACT CGT TGA TGT TTG-3′ | | | |
| NK603 01-5 | 5′-TAT CTT GCT CGA TGC CTT CTC C-3′ | NK603 | 143 | (Dong et al., 2008) |
| NK603 01-3 | 5′-ACA CCA TTG CAG ATT CTG CTA ACT-3′ | | | |
| KVM-5 | 5′-TTG AGG GTG TTG TGG CTG GTA-3′ | *pat*[a] | 68 | (Weighardt et al., 2004) |
| KVM-6 | 5′-TGT CCA ATC GTA AGC GTT CCT-3′ | | | |
| SSIIb-5 | 5′-CTC CCA ATC CTT TGA CAT CTG C-3′ | SSIIb | 151 | (Mano et al., 2009) |
| SSIIb-3 | 5′-TCG ATT TCT CTC TTG GTG ACA GG-3′ | | | |
| CaMVF | 5′-GGC CAT TAC GCC AAC GAA T-3′ | CaMV | 89 | (Cankar et al., 2005) |
| CaMVR | 5′-ATG GGC TGG AGA CCC AAT TTT-3′ | | | |

[a] Phosphinothricin N-acetyltransferase (pat) gene from *Streptomyces viridochromogenes*.
[b] Amplified fragment length (base pairs).