

GM Quantification In Various Maize Products For Managing Coexistence Between GM And Non-GM Production

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Abstract

GM levels in maize harvests are usually quantified by real-time PCR on the grains. In Northwestern Europe, maize is mostly harvested as whole plant forage. Any transgenic DNA in the grains due to outcrossing can be expected to be “diluted” by the non-transgenic DNA from the plant’s vegetative parts. The effects of this “dilution” on the final outcome of GM quantification will depend on the amount and quality of DNA extracted from the different plant parts. Therefore, we tested mixtures of grains and vegetative parts to assess the effect of vegetative parts on the outcome of the real-time PCR.

Introduction

For the benefit of coexistence of GM and non-GM cultivation of maize in The Netherlands, trials testing isolation distances from GM fields for their effect on transgene outcrossing levels in non-GM fields were performed in The Netherlands during 2006 and 2007 (Van de Wiel et al. 2009). In these trial, a standard method for assessing transgene levels in maize samples from non-GM receptor fields was used, i.e. real-time PCR quantification (qRT-PCR) of the transgenic event in DNA extracted from the grains. The results of the quantification are expressed as a percentage representing the ratio of the number of transgenes to the number of haploid genome equivalents (HGE), based on a calibration curve from a reference ground grain sample (produced by IRMM, Geel, Belgium). However, the greater part of maize production in Northwestern Europe is harvested and used as whole plant forage. With GM quantification using DNA from this product, any transgenic DNA present in the grains due to outcrossing is expected to be “diluted” by the non-transgenic DNA from the vegetative parts of the plant. The effects of this “dilution” on the final outcome of transgene quantification will depend on the amount and quality of DNA that can be extracted from the different plant parts. Therefore, we tested artificially made mixtures of grains and vegetative parts (both GM and non-GM) to assess the effect of vegetative parts on the outcome of the qRT-PCR.

Methods

Three types of plant materials were arranged during the 2007 Dutch coexistence trials (Van de Wiel et al. 2007) located in Lelystad and Schaarsbergen, using the GM (MON810) hybrid DKc3421YG and its ‘near-isogenic’ non-GM counterpart DKc3420. The materials consisted of plant samples of (1) GM plants pollinated by GM plants, (2) non-GM plants pollinated by non-GM plants and (3) non-GM plants pollinated by GM plants. Two random plant samples of ten plants each were taken per pollination type, and separated in vegetative parts and ears. Each fraction was then dried at 40°C, the vegetative parts after chopping. The ears were subsequently threshed. Separate plant fractions, grains, cobs and the rest of the vegetative parts, were ground to obtain proper source materials for DNA extraction. DNA was extracted from ca. 100 mg of powdered sample using a standard CTAB/Qiagen DNAeasy Plant minikit protocol. After extraction, the yield of DNA was determined with help of a Nanodrop[®] spectrophotometer. In addition, two UV wave length ratios, 260/280nm and 260/230nm, resp., were determined spectrophotometrically to evaluate the DNA purity; the ratios indicate the degree of impurity due to the presence of proteins and polysaccharides, respectively. Levels of the transgene in the grains were measured using a validated qRT-PCR method for the MON810 event (<http://gmo-crl.jrc.it>).

Results

DNA extractions from grains were superior in quality to those from vegetative parts and in extracts from grains, it was estimated that almost ten times more copies of the maize HMG gene that is used as representative for the number of genome copies (HGE), were detectable than in corresponding amounts of vegetative part DNA. QRT-PCR on mixtures of grain and vegetative part DNA also indicated that the vegetative parts contributed relatively few detectable maize genome copies to the mixtures, while having a negative effect on DNA quality. A series of grain-vegetative part mixtures with expected GM ratios in the range from 0-3%, which is in the vicinity of the 0.9% EU threshold for adventitious presence of GM in non-GM products, were composed by mixing grain and vegetative parts in 1:1 weight ratio, and from these DNA was extracted for qRT-PCR. All tests showed that the contribution of the vegetative component to the variation in GM ratio of the mixture was very small. This is in line with previous observations in samples from field trials by Weber et al. (2007), although in their study no direct comparison was made between grain and forage from the same field. The difficulty to extract good quality DNA from the vegetative parts was a likely reason for the poor detection rate of transgene from grain-vegetative mixture DNA.

Conclusions

It is possible to assess GM ratios in whole-plant forages but it will be less accurate and more laborious than a similar assessment solely based on grain material. Regardless of the method chosen, the ratio obtained will mainly reflect the ratio in the grain fraction, as a “diluting” effect of non-GM DNA from the vegetative part of the plant appeared to be almost absent. Assessing GM ratios proved all the more difficult in silage and digestate samples, as these underwent post-harvest processing involving microorganisms that severely affect the amount and quality of extractable maize DNA. The final choice of the sampling methodology, e.g. for monitoring GM levels of maize products, will also depend on practical or logistic conditions at the field or farm/storage.

References

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