

The Behaviour of Transgenic Cereals in Brewing

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Introduction

Public awareness - but not necessarily public understanding - of GM technology has increased dramatically over the past 6 - 9 months. Unfortunately, this increase of interest has been accompanied by an equally increased distrust of the new technology and a growing reluctance to accept GM foods. There are some geographical differences. One year ago, opposition to GM technology was largely confined to Europe - and to northern Europe at that. Since then, however, there are signs that concern is growing elsewhere, in Asia, here in Australia and even in the parts of the third world which are generally considered to have the most to gain from GM technology.

The legislative background

The legislation governing GM technology in the European Community is amongst the most comprehensive and restrictive in the world. Yet it still is not sufficient to satisfy consumer concerns, and more regulations are being drafted. A brief overview of the EU legislative position will be given here in order to explain the background which prompted the brewing studies at BRi.

The basic piece of legislation is the so-called "Deliberate Release Directive", 90/220/EEC. This defines what is meant by GMOs and lays down the requirements, in terms of biological information about the organism itself and a comprehensive assessment of any potential risks, before a new GMO can be released on to the market. The risk assessment must cover both risks to the environment and, where appropriate, the suitability of the product to be used as food. It relates only to actual organisms - animals, plants or microorganisms, which can reproduce. It does not cover foods made from such organisms. This tier of legislation requiring safety assessment of GMOs is common to most countries, although the details may differ.

Where the EU does differ substantially from other countries - most notably from the USA, is in its requirements for labelling of foods made from GMOs. This tier of legislation has been imposed directly as a result of consumer unrest. Quite reasonably, legislation was passed in 1997 (Regulation 258/97) to ensure that consumers were informed if a novel food (which definition includes foods made from GM organisms), although accepted as safe, differed from the traditional version of the same food in a way which could have implications for certain sections of the population. What was envisaged here was, for example, the insertion of animal genes into plants, of which vegetarians would wish to be aware. Likewise the insertion of genes from a species known to cause serious allergenic reactions, such as peanuts. But apart from such cases, if the food was deemed to be "substantially equivalent" to traditional counterparts, then labelling would not be required.

It very soon became evident that consumers did not regard foods containing "foreign" DNA as equivalent to traditional foods. Not only that, but the two commonest GM foodstuffs, maize

and soya, were not covered by 258/97 since they were already on the market and therefore no longer novel. A second Regulation (1139/98) followed swiftly. This extended the labelling requirements to GM maize and GM soya and defined “substantial difference” as containing detectable transgenic DNA or foreign protein. Allowances were made for a negative list to be drawn up foodstuffs shown to be free of DNA or protein and also for a minimum threshold to be defined, below which labelling would not be triggered. To date neither of these have been agreed.

Brewing Trials at BRI

It was against this legislative background that the programme of investigation at BRI was initiated. The aim was to brew using substantial proportions of GM maize grits and GM maize starch in order to determine whether the transgenic DNA could survive processing and still be detectable in the beer, thus triggering labelling. It was decided to concentrate on DNA since methods for detecting specific proteins in processed foods are very much less sensitive than those for DNA. The findings would not only be applicable for beers prepared using GM maize adjuncts, but would also be relevant if and when GM malting barleys became commercially available.

Materials and methods

Maize grits and maize starch were prepared in pilot scale processing plant from the GM maize line MON 809, marketed by Pioneer Hi-Bred International. This line has been modified to express the *cryIA(b)* protein from *Bacillus thuringiensis* subsp. *Kurstaki*, which confers resistance against the European Corn Borer and is commonly known as Bt protein. However, codon usage in the construct has been modified from that in the bacterial gene. As with most of these GM maizes, a promoter from Cauliflower Mosaic Virus (CaMV 35S) is used. Genes for kanamycin resistance (*nptII*) and a selectable marker protein, EPSPS (5-enolpyruvylshikimate-3-phosphate synthetase) (which confers glyphosate resistance, but is poorly expressed in MON 809) are also present.

Detection of DNA

Methods used for extraction of DNA from maize grits and starch and detection by PCR (Polymerase Chain Reaction) were based on those validated by the Joint Research Centre (JCR), Italy. DNA is extracted and purified using chloroform and isopropanol. The JCR exercise only involved solid maize products prepared from Novartis Bt maize, thus extraction and concentration techniques for liquid samples such as wort and beer were developed at BRI. 100ml of beer / wort was freeze-dried overnight. The powder (approx. 4g) was resuspended in CTAB extraction buffer (20g/l CTAB; 1.4M NaCl; 100mM Tris.Cl, pH8.0; 20mM EDTA) and incubated at 65C for an hour. Chloroform:isoamyl alcohol (20 ml) was then added, mixed and centrifuged. The aqueous layer was removed and the DNA pelleted by ethanol precipitation before being further purified through a Promega Wizard DNA Clean-up kit. PCR was then performed using an aliquot of this purified DNA.

A plant universal primer (for a multicopy chloroplast gene) was used in order to confirm that DNA of suitable quality could be extracted and detected by the techniques used. Additional primers against maize invertase and the CaMV 35S promoter were also used. Semi-quantification of positive results was achieved by comparison with reference standards containing 0, 0.1, 0.5 and 2.0% Novartis Bt 176 GM maize. The level of detection was 0.1%. Sub-samples of the beers were also sent to an independent laboratory for cross-checking.

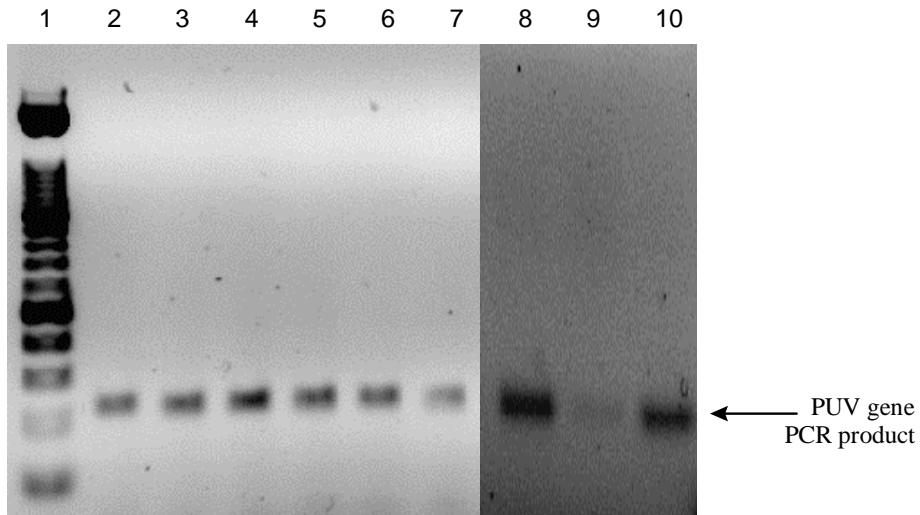


Figure 1. PCR Detection of Plant Universal DNA.

Lanes; 1. 100bp ladder. 2-5. Reference standard maize DNA (containing 0, 0.1, 0.5 and 2% GM maize respectively). 6. GM maize grits DNA. 7. GM maize starch DNA. 8. DNA purified from beer produced with GM maize grits. 9. DNA purified from beer produced with GM maize starch. 10. DNA purified from beer produced with control maize grits and starch.

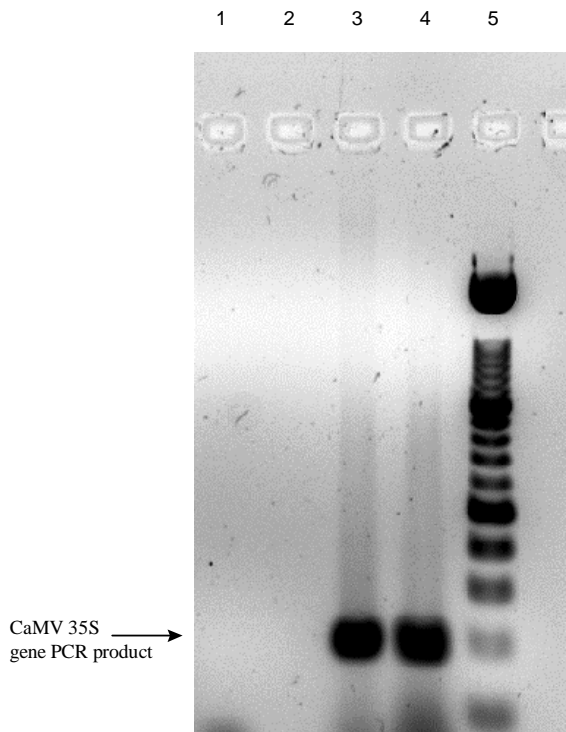


Figure 2. PCR Detection of CaMV 35S

Promoter.

Lanes; 1. DNA extraction blank. 2. PCR control blank. 3. GM maize grits DNA. 4. GM maize starch DNA. 5. 100bp ladder.

Brewing

Brews were carried out in the BRI 100 litre pilot brewery. Two lager beers were prepared, using 15 kg of lager malt and 6.5 kg of GM maize starch and GM maize grits respectively. In each case the maize adjunct was cooked for 10 min at 70°C followed by a 30 min boil before adding to the malt mash in the mash conversion vessel, where it was held for a further 60 min at 65°C. After lautering the mixture was boiled for 60 minutes and the trub removed in a whirlpool. The cooled worts were fermented at approximately 10°C for 7 days using a standard BRI lager yeast. After conditioning for 7 days the beers were bottled, pasteurised and stored at 0°C.

Results

Good quality DNA could be extracted and amplified from both the maize grits and maize starch, as evidenced by the strong positive bands for the universal plant DNA (Figure 1) and the maize invertase gene. Both products also gave strong positive results for the CaMV 35S gene (Figure 2). Amplifiable universal plant DNA could be extracted from the worts and beers prepared with GM maize products and also from control beers prepared with traditional maize (Figure 1). However, all beers gave negative results with the CaMV 35S primer, indicating that there was no transgenic DNA present above a concentration of 0.1%. The independent laboratory was also unable to detect transgenic DNA in the beers.

Table 1. DNA in raw materials, beers and process samples.

Sample	Plant Universal DNA	Maize DNA (Invertase)	Transgenic Maize DNA (CaMV 35S)
Control maize Grits starch wort spent grains beer	Positive Positive Positive ND Positive	Positive Positive ND ND ND	Negative Negative ND ND Negative
MON 809 maize grits brew Grits wort spent grains beer	Positive Positive ND Positive	Positive ND ND ND	Positive ND ND Negative
MON 809 maize starch brew Starch wort spent grains beer	Positive Positive ND Positive	Positive ND ND ND	Positive ND ND Negative

(ND = not analysed)

Discussion

The results reported here indicate that if sufficiently sensitive methods are used, amplifiable, although partially degraded, DNA can be detected in beer. Since the universal plant genes are common to all plants, such DNA may be derived from the malt, from any cereal adjuncts used, or from the hops, and will always have present at low levels in beer. However, no transgenic DNA has been detected in beers, even when they have been prepared with substantial quantities of adjuncts derived from 100% GM maize. Thus under the present European novel foods legislation such beers do not trigger labelling.

However, in the current climate, consumer demand may prove stronger than the legislation. A sizeable proportion of the population in Europe, when questioned, insist that they wish to know whether or not a food has been prepared from a GM crop, or the derivatives of that crop, regardless of whether “foreign” DNA or protein can be detected. Most of the large retail food chains in the UK have reacted to this pressure, initially by promising that own label products will be GM free or labelled accordingly. Supermarkets such as Sainsbury’s which, with their policy of full voluntary labelling have been active - and indeed successful - in launching GM tomato paste, are now withdrawing such products. It is of course very difficult to police a GM free policy since not only is there no agreement as to what GM free should mean, but for many derivatives such as refined oils or lecithin any DNA has been completely destroyed by the processing. Thus claims of GM free are reliant on paper audits of supplier guarantees and purchasing records. Whether this approach can be supported in the long term remains to be seen.