Zea mays breeding in New Zealand:

Analysis of the probability of perpetuating transgenes

in breeding material

Allan K. Hardacre

May 2004

1 Executive summary

Maize is a large, valuable and important crop in New Zealand. In the US, where almost all the maize germplasm used in NZ is sourced, about 40% of the crops contain transgenic events. These are mostly for tolerance to the herbicides glyphosate (Roundup) and glufosinate (Liberty class), and for the production of a protein (Cry group from *Bacillus thuriengensis*), which stops insect feeding.

It may not be possible to prevent transgenes from reaching NZ in inbred or hybrid seed imported from the US, as testing can not detect every seed containing a transgenic event and low levels of transgenes are known to be present in many conventional hybrids.

The most likely sources of transgenic events in certified non-transgenic hybrids are:

 1) Pollen blown in from a transgenic crop during hybrid seed production.

 2) The accidental transfer of genes between transgenic and non transgenic versions of the same inbred.

3) The accidental transfer of gene blocks from a population containing transgenic event(s) in the early stages of a breeding program.

Transgenics can not and are not perpetuated in the maize production systems of NZ. This is not only because the probability of a GM plant outcrossing with an adjacent field of maize is extremely low, but also because maize is a hybrid crop for which the entire germplasm base is renewed each year.

At least three incursions of transgenic maize have been detected in NZ, and all have been at extremely low levels, nearing the technical limits of detection of

testing. For all of the events where the transgenic construct was identifiable, it had been approved in NZ and elsewhere as fit for human consumption.[1]

The polymerase chain reaction test itself can detect extremely small amounts of DNA (10 copies of the transgene). This accuracy cannot be routinely met, when all other sources of test error are considered

Unless New Zealand is prepared to consider either tolerance levels or the banning of all imports of *Zea mays* seed for sowing, costly incursion responses will continue to be required.

2 Introduction

The species *Zea mays*, generally known as maize[2], provides sweet corn, field corn for silage and grain, and popcorn to the world market. Maize has been a

[1] Standard 1.5.2 of the Food Standards Australia New Zealand Code:
http://www.foodstandards.gov.au/whatsinfood/gmfoods/gmcurrentapplication1030.cfm
[2] The term maize generally refers to all forms of *Zea mays*, but also specifically to field corn, the type traditionally grown throughout the centuries. Sweet corn and pop corn are relatively new types of Zea mays, developed in the 20th century.

significant crop in NZ since the 1900's. At this time, all production was from open pollinated races (OP) and seed was saved from one year to be grown the next. With the advent of hybrids in the 1920's and their widespread use in NZ in the 1940's, yields increased dramatically and the use of farmer saved OP seed was not economic. Because yields from crops grown from seed saved from the hybrids (OP seed) is inferior to genuine hybrid seed, OP hybrid seed is not sold by any of the seed companies in NZ.

It is not known if any growers save their own seed to use for forage production. However this seems extremely unlikely, as the yield decrease and therefore loss in economic return is greater than the cost of purchasing commercial hybrid seed.

Hybrids are created by crossing genetically homozygous inbred parent lines which when crossed, produce seed with marked hybrid vigor. It takes at least six cycles of systematic inbreeding to develop useful inbred lines from a genetically heterozygous parent. The identities and maintenance of commercial inbreds are closely guarded by the companies and protected by patents and plant variety rights. No company breeds maize in NZ by developing their own inbred lines, but there are several which license breeding lines from abroad, mainly from the US.

3 Maize Agronomy

3.1 General

In New Zealand, the species *Zea mays* is a valuable crop worth about $40 million as grain and $100 million as silage, depending upon annual cropping and economic conditions. Sweet corn is a unique genotype of *Zea mays* which contains one of two recessive mutations which cause the accumulation of sugars in the kernel prior to maturity. In 1996, sweet corn exports exceeded $34 million as fresh, frozen and processed products.   Popcorn is a very small market in NZ

and is a form of very hard seeded maize. All forms of *Zea mays* are completely inter-fertile. In NZ, all crops are hybrids produced from genetically homozygous inbred parent lines. This review will focus on maize for grain and silage, the type of maize involved in the recent GM incursion. Sweet corn was reviewed for a previous incursion (Appendix 1).

3.2 Crop development and the environment

The rate of crop development, and therefore the potential for pollen transfer from one crop to another, depends on the maturity of the crop, the environment in which it is grown, and sowing date.

 In NZ crops are planted once soil temperatures are above about 12°C. Below this temperature, germination is very slow and seedling survival is compromised. The seed is not dormant and therefore will not persist in the ground in an un-germinated and viable state for longer than the spring following the year of seed production. Plants are killed by frosts below about -2.5^{o}C and will not grow when day temperatures are below 15°C.

It is clear from Table 1 that large differences in climate (expressed as cumulative heat units) exist among growing regions and between season to season in NZ.

Table1. Maize cumulative heat units during growing season (15 October - 3 April) for three regions.

Region	Heat units	
	Season	
	2003-04	**30 Yr Avg**
Poverty Bay	1945	1900
Pukekohe	1735	1880
Manawatu	1620	1630

The 2003 maize growing season was warmer than average in the Poverty Bay region and cooler than average in the Pukekohe and Manawatu regions. In fact, in the Manawatu region, heat unit accumulation from February onwards has been amongst the slowest on record.

A comparison of the predicted dates for the 2003-04 growing season and the thirty year average for flowering (silk), physiological maturity (also called black layer or BL), and harvest at 23% moisture are shown in Table 2 for a late and an early maturing hybrid.

Table 2. Predicted dates to selected growth stages for maize crops sown with a late maturing or an early maturing hybrid on the 15 October for three maize growing regions of New Zealand.

Hybrid	Region	Season	Silk	BL	Harvest
Late	Poverty Bay	2003-04	8/1/04	21/3/04	16/4/04
107CRM[1]		30 Yr Avg	11/1	24/3	16/4
	Pukekohe	2003-04	16/1/04	7/4/04	14/5/04
		30 Yr Avg	14/1	28/3	30/4
	Manawatu	2003-04	24/1/04	21/4/04	13/6/04
		30 Yr Avg	23/1	17/4	10/6
Early	Poverty Bay	2003-04	2/1/04	11/3/04	2/4/04
94CRM		30 Yr Avg	4/1	15/3	5/4
	Pukekohe	2003-04	9/1/04	26/3/04	30/4/04
		30 Yr Avg	7/1	18/3	17/4
	Manawatu	2003-04	16/1/04	7/4/04	22/5/04
		30 Yr Avg	16/1	4/4	20/5

[1] Cumulative relative maturity as determined by seed companies.

For crops planted on the same date, the predicted date to the three selected growth stages varies significantly due to the rate at which the two crops mature. In particular, for the current season in the Poverty Bay region, mid pollen shed is estimated on the 8th of the January for the late hybrid and 6 days earlier for the early maturing hybrid. These differences increase to 7 days and 8 days respectively in the cooler Pukekohe and Manawatu regions.
As is shown later, the frequency of pollen transfer between adjacent crops is extremely low and for separations of >30m can be discounted.

3.3 Management

Typically, crops of about 100 day cumulative relative maturity (CRM) are sown in late October in the Manawatu which is close to the southern limit for grain cropping. These grain crops are harvested in late May or early June depending on the season. In warmer regions, crops of 115 day CRM can be sown in late September and harvested in late April.   Flowering usually occurs in January (Manawatu) and December (Gisborne), and physiological maturity (seed is mature) occurs in April (Manawatu) or February (Gisborne).

 Silage is harvested just prior to physiological maturity. Sweet corn is harvested about one month after flowering. During the sweet corn and popcorn harvests, the entire ear including cob and husk are removed from the field.

Grain is harvested by machine. Combine harvesters strip the ears from the plants and then remove the grain from the ears. All crop residue (stover) is returned to the field.

Crop management is minimal once detailed attention has been paid to land preparation, soil fertility and pH. Typically, weed control uses a single pre-emergence spray of Atrazine and Alachlor. Atrazine is very persistent in the soil, has been banned in some parts of the world, and was the major reason for the development of the Roundup Ready (RR) and Liberty tolerant (LibertyLink) maize varieties. In NZ, Atrazine is not banned although there are serious concerns regarding its continued presence in ground water. If Atrazine were banned, there may be compelling arguments for the use of the RR and LibertyLink maize in NZ, although there are alternatives including the Imidazolinone tolerant gene (not a transgenic) and other adequate herbicide systems.

The main insect pest of maize, army worm, is controlled biologically. Other insect pests such as black beetle and greasy cutworm are either very sporadic in attack and/or are controlled by current insecticide systems. No other caterpillar pests that could be controlled by the Bt conversion are important.

3.4 Uses

The majority of the $40 million 160,000-180,000 tonne maize grain crop (~70%) is used for poultry feed. About 40,000 tonnes, some of which is imported, is used in the starch industry. About 12,000 tonnes, again some of which is imported, is used for human food (cornflakes, extruded snacks and corn chips). The remainder is used in stock and animal feeds. It is the third most important crop in NZ behind barley (~$80 million) and wheat (~$80 million). The silage crop, estimated to be about 75% of the grain crop in area, is predominantly fed to dairy cattle, while a little is still probably used in beef cattle fattening operations.

4 Sources of seed

4.1 Overview

For all intents and purposes, virtually all maize seed for sowing in NZ comes from three general sources, within each of three companies maintaining breeding lines. Genetic Technologies (~ 65% of all *Zea mays* sold), Corson Grain (~30% of all *Zea mays* sold), and Pacific Seeds (~5% of all *Zea mays* sold) acquire seed by:

1) purchasing hybrid seed from external sources abroad,

 2) producing hybrid seed in NZ from imported inbred lines

3) producing hybrid seed in NZ from licensed inbred lines that are maintained in NZ.

A fourth company, Seed Solutions, occasionally produces hybrid seed under contract to Corson Grain and to Pacific Seeds. The remainder of their business is to produce seed for offshore companies. In this case, all the seed produced in NZ is either exported or destroyed.

Breeding operations are conducted in the Pukekohe region by Seed Solutions, and in the Poverty Bay region by Corson Grain, Genetic Technologies and Pacific Seeds. By far the greatest area of production for breeding is in the Poverty Bay region.

Inbreds are maintained in NZ by controlling pollination. In this process, the flowers are bagged to collect pollen and to prevent the uncontrolled pollination of the female flowers. Pollen from the donor is carefully poured into the stigmas of the female flower and the ears rebagged. In this system, the level of uncontrolled pollinations is very low, probably less than one in a million.

Where larger quantities of inbred seed are required, an inbred crop is grown so that the population is allowed to naturally self pollinate. Cross pollination from a source outside this population is kept to a minimum using spatial or temporal (time of maturity) isolation from possible donor crops.

Seed Solutions

Genetic Technologies
Corson Grain
Pacific Seeds
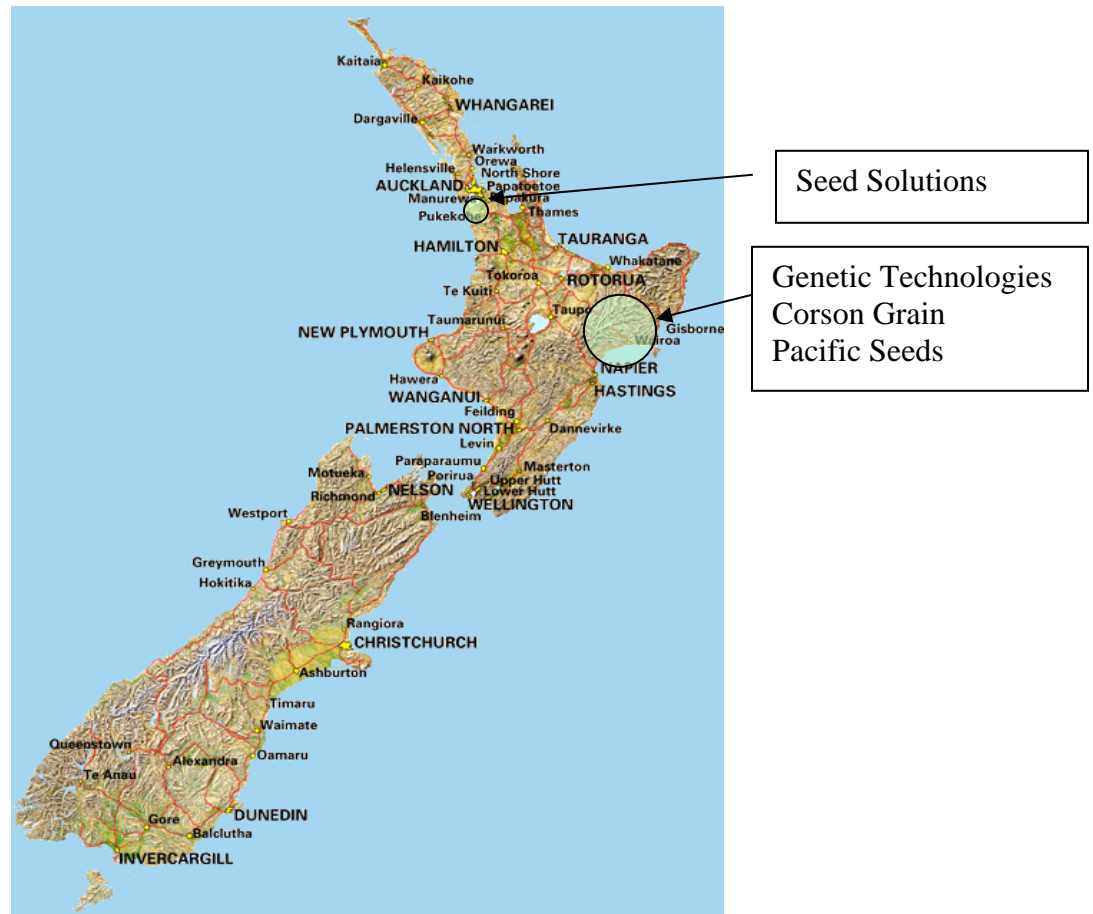
When inbred parent lines are planted in hybrid production nurseries, typically two designated male rows are inter-planted between a block of four designated female rows.

Any outcrosses which occurred during production of the inbred parent lines will show marked hybrid vigour. These hybrid "rogues" will be easily detected by their size and appearance amongst the inbred parent lines, and the process of removing them before flowering is a routine priority in hybrid seed production.

4.2 Imported hybrid seed

All imported hybrid seed is planted and harvested with no further regeneration of the population. It is usually planted the season following importation because germination rate decreases over time and long-term storage invites problems.

All plant material produced from the imported hybrids is used for consumption as forage, silage, human food, or animal feed, and none is used for plant breeding. This is the case that applies to the imported hybrid that has been recently shown to contain very low levels of the LibertyLink T25 transgenic event.

4.3 Hybrids made in NZ from imported inbreds

In the first year of hybrid production of a new variety grown in NZ, the inbreds have always been imported; in subsequent years, quantities of inbred seed necessary to satisfy the market may be produced in NZ (see Section 4.4). The inbreds are crossed together to make the hybrid, and all plant material produced from the hybrids is used for consumption as forage, silage, human food, or animal feed, and none is used for breeding.

4.4 Hybrids made in NZ from NZ grown inbreds

The inbreds imported in 4.3 above may be self pollinated in NZ and the resulting inbred seed saved and crossed in subsequent years to make the hybrid. All plant material produced from the hybrids is used for consumption as forage, silage, human food, or animal feed, and none is used for breeding. This technique is not used by Genetic Technologies.

5 Retention of transgenes in NZ maize

There is no clear pathway by which transgenic imports could be perpetuated throughout the NZ hybrid maize industry. Similarly, there is no clear pathway in NZ by which a transgene such as 'LibertyLink T25' could establish as a self-perpetuating entity in the NZ maize genome.

There is the possibility that a transgene could temporary persist within an inbred population.

5.1 Perpetuation of maize hybrids

Imported hybrid maize seed is required to be certified-free of transgenes prior to import into NZ. All hybrids are used without further breeding work.

All maize is annual and seed survival in fields left fallow following a maize crop is low. I estimate a maximum of 1 per hundred thousand kernels of the grain in the field will be left on the surface and will be available for germination either shortly after harvest or in the following season. The remainder are damaged, eaten by birds or animals, degraded naturally in the field or are otherwise unsuitable for growth.

It is important to note that precise agricultural practises are used for sowing maize seed and have been developed to ensure good seed 'strike' e.g. the seed in sown in rows 750 mm apart at a depth of 40-50 mm. Plants between the rows are usually cultivated and eliminated. Seed sown deeper than 100mm will not emerge and seed left on the surface does not germinate. If scattering seed, such as occurs post-harvest, resulted in good yield there would be no need for the expensive precision machinery required to establish the crop

The one in a hundred thousand kernels able to germinate post-harvest represents a maximum of 300 potential "volunteer" seedlings per ha (P), which at

the level of transgenic material in question (≤ 0.05%) will represent a maximum potential of less than one transgenic plant per 6 ha.

If, and this is usually the case, the stubble and stover is cultivated shortly after harvest, perhaps 1/6th of the 300 seed/seedlings will survive (S), for a maximum of 50 volunteer plants per hectare.

In cooler regions, frosts will kill all germinated seed during winter. If frosts do not occur, possibly half the seed/seedlings will survive winter (W) and perhaps one may survive birds, damp rot, and secondary cultivation (L) in the following spring to persist in a maize crop the following season. If a crop other than maize is grown the following season, or if the land is put into pasture following the maize crop, no maize plants will survive.

The following equation estimates the probability of volunteer transgenic plants:

$$P / (S \times W \times L) = 300 / (6 \times 2 \times 25) = 1$$

$$1 \times 0.0005 = 1 \text{ transgenic/2000ha}$$

In summary, at the levels of GM presence discovered in the imported hybrid in question (≤ 0.05%), the worst case scenario is that less than one transgenic volunteer plant will arise the following season for every 2000 ha of maize grown the previous season.

Refer to table 3 for a demonstration of the calculation

Table 3: Stepwise calculation of the survival of volunteer and transgenic volunteer maize plants through normal agricultural practise

Stage	Volunteers per 10 ha	Transgenic volunteers per 10 ha
At Harvest	3000	1.5
Post-harvest cultivation	500	0.25
Winter survival*	250	0.125
Spring cultivation	10	0.005

*Assumes no frost, no grazing

5.2 Perpetuation in inbred parent lines

Imported inbred seed. If a transgenic event is present in the inbred seed, it will be perpetuated in the hybrid at ½ the frequency at which it occurred in the inbred (note that if the construct is recessive it will not be expressed).

NZ produced inbred seed. If a transgenic event is present in the inbred seed, it will be perpetuated in the hybrid at ½ the frequency at which it occurred in the inbred (half of the genes from the GM parent, half from the other parent).

However, transgenic events will be perpetuated in the inbred during self pollination at the same frequency that they are present in the original inbred population (both sets of genes from same parent). Self pollination of the inbred in NZ will continue to maintain the transgenic construct in the inbred population. Since all inbred seed lines are tested for the presence of transgenic material when first imported, the maximum level of transgenic material persisting in inbred lines will be the false negative rate of the testing process.

A transgenic event in an imported hybrid will not be transferred to an inbred line produced in NZ, as any inbred x hybrid crosses will have hybrid vigour and

thence be "rogued" from the inbred nursery or from the hybrid production nursery (Section 4.1 above). Rogueing is a highly important process in these nurseries and is NOT neglected.

5.3 Transfer to other hybrid maize crops

Assuming that the flowering periods in two adjacent crops are synchronous, the probability of transgenic seeds arising in an adjacent receptive maize crop is a function of the density of GM plants in the donor crop and the amount of pollen transfer over the distance between donor and receptor crops. The frequency of transgenic plants along a row, assuming a plant spacing of 0.120m and a level of transgenic material of ≤0.05%, is about one transgenic donor plant per 240 meters of row.

Maize pollen is wind dispersed and although it has been proven to be blown large distances, the frequency of cross pollination from donor to receptor plants is surprisingly low.[1,3]

Ironically, in an experiment performed in the US to evaluate cross pollination from GM maize, the conventional hybrid check plot grown in isolation was found to have a low level (0.16%) of GM seed, making it difficult for the experimenters to determine whether the low levels of GM seed in their crossing plots were due to wind-carried pollen from their target source, or from inadvertent presence in the original seed.[1]

Maize plants shed pollen over a period of several days. Individual pollen grains can remain viable for up to two hours after shedding, although hand pollinations usually reveal a much shorter viability of up to twenty minutes.

For single donor plants in a crop, rates of pollination averaged about 1.1% at 1m and 0.14% at 3m, depending upon wind direction.[4] About 0.005% of the grain

---

[3] Paterniani and Stort. 1974. Effective maize pollen dispersal in the field. Euphytica 23: 129-134.

had been pollinated by the lone donor at 30m, dropping to 1/3 this frequency at 200m.[4]

Using these distance factors, the likelihood of a seed in an adjacent maize crop arising from transgenic pollen blown in from the crop in question has been calculated in Table 4 based on the following equation:

Probability of transgenic seed arising in crop plant at specific distance =

% GM plants in donor crop x % cross pollination at specific distance

Table 4. Probability of GM seed occurring in a receptor crop at specific distances from a donor crop having ≤0.05% GM presence.

Distance between donor and receptor	Percent cross pollination	Percent GM plants in donor crop	Probability of GM seed occurring in receptor crop at 1, 3, or 30 m distance from GM crop
1 m	1.1 %	≤0.05%	5.5×10^{-6} or ~1 : 180,000 seed
3 m	0.14%	≤0.05%	7×10^{-7} or 1 : 1.4 million seed
30 m	0.005%	≤0.05%	~25×10^{-9} or 1 : 40 million seed

5.4 Summary of gene transfer

Transgenic events in maize will not be transferred from hybrid to inbred. However, transgenic events could be transferred from a hybrid containing a

---

[4] Emberlin, J. 1999. A report on the dispersal of maize pollen. Soil Association website: www.soilassociation.org

transgenic construct to a new hybrid in a production nursery or to an adjacent hybrid crop. The frequency of this occurrence is very low.

6 Reliability of testing

The theoretical limits of detection and quantification of transgenes are very low. Currently the major testing laboratory used by MAF reports a limit of detection of 10 copies of a transgene and a limit of quantification of 0.05%. Other laboratories claim similar limits.

However, in the first "round robin" of proficiency tests for GM testing laboratories, the International Seed Testing Association (ISTA) found that when three maize samples, one free of transgenic material and two containing 1% of transgenic seeds, were submitted for evaluation, the error rate was about 30% with both false positives and false negatives being reported.

---

http://www.seedtest.org/en/content---1--1074.html

Proficiency Tests - Progress in 2003
The 2nd Proficiency Test on GMO Testing of *Zea mays* L.: 2003 was successfully finalised. 52 laboratories participated from 20 countries. 47 laboratories reported evaluable results. The results of the qualitative testing of this proficiency test showed that about 85% of the participants were able to identify all maize samples correctly **[This is an increase on the 70% reported for the first proficiency test (author Hardacres' note)].** In the 2nd proficiency test, in addition to the qualitative test, about 30% of the laboratories performed a quantitative test and about 30% performed a semi-quantitative test (sub-sampling test). Details of the test results can be found in *International Seed Testing,* no. 126, October 2003, p. 15-17. The results of both tests showed clear evidence that an information exchange between the laboratories participating in the proficiency tests and training were necessary. A first meeting to discuss the results was held in Bassersdorf, Switzerland, in December 2003.

The 3rd Proficiency Test on GMO Testing of *Zea mays* L.: The 3rd proficiency test was started in December 2003. Each laboratory received twelve samples with a sample size of 1500 maize seeds. The laboratory must detect the presence of GM seeds in the twelve samples for the obligatory qualitative test. Also, in this round, laboratories were encouraged to perform either a sub-sampling test or quantitative test. The results will be presented at the ISTA Ordinary Meeting, Budapest, Hungary in May 2004.
Further proficiency tests, e.g. decision of crop and test design, are discussed in the Proficiency Test WG.

In a review, Ahmed[5] reports an IUPAC (International Union of Pure and Applied Chemistry) collaborative validation study involving 29 laboratories in 13 countries, whereby samples containing 2% GM soy or maize were unequivocally identified by all laboratories, but the number of false negatives for 0.1% GM maize was 14.

In another review,[6] total error coefficients of variation (CV) (sampling error + PCR error) were considered to be around 25% for samples containing 1% of a GMO. For samples containing 0.1% of transgenic material, CV could reach values up to 200%.

Although proficiency rates in GM testing have been improving and are certain to continue improving, attempts to absolutely restrict transgenics in maize seed imports are impractical. Combined errors are large, ensuring that transgenic material will be imported into NZ particularly when the frequency of transgenics in the seedlot is low. It is, therefore, inevitable that intensive testing of seed imported into NZ and NZ food products will reveal the presence of transgenic material, even if that material has previously been declared free of transgenics.

[5] Ahmed, F.E. 2002. Detection of genetically modified organisms in foods. TRENDS in Biotechnology, 20 (5): 215-223.
[6] Giovannin, T. and L Concilio. 2002. PCR Detection of genetically modified organisms: A review. Starch/Starke 54: 321–327.