GMO FREE EUROPEAN REGIONS NETWORK

PROBLEMS RELATED TO THE PRODUCTION OF SEED BELONGING TO GENETICALLY MODIFIED CROPS

1. Introduction

The incorporation of genes into the gene pool of one population from one or more other populations is a definition of Gene Flow (GF). The practical importance of the analysis of the consequences of crop to wild and crop to crop GF increased with the introduction in agriculture of the Genetically Modified Crops (GMCs). Therefore a distinction is made among:

- introgression of a transgene from cultivated to wild relatives (WRs);
- casual or technically unavoidable presence of a transgene in conventional or organic crops for food or feed;
- casual or technically unavoidable presence of a transgene in conventional or organic seed crops.

The risk of GF from a GMC to WRs is connected to the extent of GF itself and to the possible selective advantage given by the transgene in natural conditions. It is possible to identify transgenes able to give positive, negative or neutral fitness to individual plants (fitness is the survival value and the reproductive capability of an individual, compared to that of competitor individuals of the same or other species within a population or an environment). As a consequence, in the analysis of the environmental impact of a transgene, both the presence of WRs and their potential invasiveness and the impact of the transgene itself must be taken into consideration. The possibility of fertile crosses between cultivated plants and WRs is at the base of GF to the wild; in Europe in some cases it can occur (canola or sugar beet) while in other cases the absence of WRs makes GF impossible (corn and soybean). In the latter cases the only, purely hypothetical, possibility of GF to the wild is connected to horizontal transfer via micro-organisms.

GF from GMCs to conventional or organic crops for food or feed only concerns the casual or technically unavoidable presence of a transgene in the commercial product used for food or feed and not the consequence of the spreading of a transgene in the wild. The practical problem is to define the distances from GMCs to conventional or organic crops able to avoid the risk of overcoming the limit of unintended presence of a transgene defined by law (at the moment 0,9% for conventional crops). For what the organic production is concerned, from a technical point of view it should be useful to define an official limit so that it could be possible to develop the technical procedures needed to assess it. In case of a widespread presence of GMCs, the assessment of very low levels of unintentional presence of a transgene in an organic production could be difficult and expensive to reach.

Regarding the maximum level of the casual or technically unavoidable presence of a transgene in seed lots to be used in conventional or organic agriculture there isn't a definition from the EU. Indeed, the official limits of transgene contamination for seed production have not been defined for the EU. The definition on this matter is lacking and the same is true for official EU laboratory procedures to determine the level of contamination in seed lots. Nothing in this area is published by JRC and ENGL while it would be deeply needed, due to the technical difficulties to approach the problem (see the paragraph devoted to unintentional GMO contamination in seed crops and its detection).

A simple system certifying and traceability for the seed sector could be based on those that already exist in the individual member states of the EU. In Great Britain a code was defined for managing GM crops , that was verified by experimental protocols based on the identification of the critical points when accidental contamination an take place during the course of production.

Generally speaking, it appears that the control of unintentional transgene contamination has to be much more stringent for seed crops than for food or feed crops and a clear regulation has to be implemented at EU level.

2. Unintentional GMO contamination in seed crops and its detection

Contamination of seed lots and other plant propagation materials with seed of GM varieties can be due to several causes, whose relative importance differs among crops.

It is known that the European Commission is evaluating the minimum tolerance thresholds to the accidental or technically inevitable presence of GMO in seed. First of all, the Commission must define the concept of " technically inevitable" and, if the value of the minimum contamination threshold is established, it will be the minimum detectable by the analytical methods for crop.

The EU recommendation 2004/787/CE deals with the technical methods for sampling and analyses for the presence of contaminating GM material. Reference is made to the rules set by International Seed Testing Association (ISTA), to the ISO rules for sampling, to the methods provided by the Community Reference Laboratory for GM Food and Feed of ISPRA, Italy, and by the European Network of GMO Laboratories (ENGL).

To detect GMO contamination in seed lots the key factors are sampling and analytical methods. Both are crucial because sampling error and analytical error are additive.

a. Sampling procedures

It is very important that specific sampling procedure are adopted that are designed for GMO detection.

Definitions and information on sampling in the context of GMO detection are provided by Kay (Kay S, Comparison of sampling approaches for grain lots. JRC Draft document 2001).

Therefore, sampling should receive much attention. It depends on the threshold limit chosen for acceptance of the presence of GMO: the lower the limits the greater the demand on the sampling plan and its cost. For instance, in quantitative analyses, to reliably (95% confidence and a sampling error of less than 20%) detect a 1% contamination in a homogeneous seed lot a sample of 3,500 grains is needed, but in the realistic case of a heterogeneous seed lot 10,000 grains should be used. To reliably detect a 0.1 % contamination under the hypothesis of a realistic heterogeneity of the seed lot, Lischer indicates a sample of approximately 20 kg of soybean kernels and 30 kg of maize kernels (Lischer, P., Sampling procedures to determine the proportion of genetically modified organisms in raw materials. Part 2: Sampling from batches of grain. Mitt. Lebensm. Hyg., 2001, 92: 305-311). Sample size depends on seed size and the level of contamination to be detected and increases rapidly as the latter decreases. The definition of a threshold should take into consideration the economic feasibility of its assessment.

A research project aimed at developing sampling approaches specifically designed for GMO detection and quantification is underway (http://biotech.jrc.it/sampling.htm).

The sampling methods designed for seed lots are not applicable for sampling crops in the field. It appears very difficult to effectively and economically sample plants in the field when the detection of a low level of contamination is desired. For example, sampling 3,000 plants in a maize field would presents technical difficulties and high cost. Likely, the error rate of this type of analyses will be very high. The technical difficulty and the high cost of sampling correlated with very low threshold levels, suggest that checks should not be carried out on seed crops in the field but rather on seed lots.

b. Methods of analysis

The Community Reference Laboratory of Ispra provides a list of methods validated by trials conducted by the ENGL (http://biotech.jrc.it/methodsdatabase.htm). All these methods are based on the Real Time PCR technique and are event-specific, that is, designed specifically for each GMO on the market. To date, validated methods are available for only a few events and validation is underway for others. No specific validated method for seed analysis is reported.

If detection, not quantification is required, standard, qualitative PCR can be a much effective and cheap alternative to Real Time PCR. It should be either a nested PCR, or a normal PCR

complemented by further analyses like digestion of the amplification product with diagnostic restriction enzymes or sequencing of the amplification product.

The sensitivity of PCR is very high. The limit of detection (LOD) of a target (GMO-specific) DNA sequence is the minimum amount of copies that can be detected by qualitative PCR; this is much lower than the limit of quantification (LOQ) that applies to quantitative PCR. Theoretically, one copy of a DNA sequence present in the DNA sample subjected to the analysis is detectable, so it is the genome size of the plant species tested and the amount of DNA used for a PCR reaction to set limits. For example, up to 36,697 copies of the haploid *Zea mays* genome are present in a typical 100 nanograms (ng) DNA analytical sample, given the 1C value (weight of one copy of the haploid genome) of 2.725 picograms (pg). It follows that a single copy of the haploid *Z. mays* genome in a 100 ng DNA sample is 0.0027%. Levels of GMO DNA below this threshold cannot be detected reliably (Kay and Van Den Eede 2001).

Using pure DNA, 0.05% GMO could be detected by the official German PCR method, whereas using certified reference materials (maize and soybean flower) the limit of detection was 0.1%.

The prevalent trend is not to give official recognition to all of the possible analytic methodologies, but rather to determine accredited laboratories by means of the organisation of official ring tests. To this and the following should be carried out:

- determination of an authority responsible for updating survey methods;
- to guarantee to the accredited laboratories in the all countries the access to the information to the every single notification of OGM;
- to organise the ring test to guarantee a better equality in analyses.

3. Effect of agronomic practise on gene flow from transgenic to conventional and organic crops.

The correct agronomic practices are recommended in EU legislation to be adopted to avoid contamination of crops intended for seed production. It should be need to introduce the definition of the critical points in production chain where accidental contamination may take place.

From an agronomic perspective, the introduction of transgenic crops raises three main reasons of concern: (1) genetic flow from GM crops to conventional crops in nearby fields, through pollination; (2) genetic flow from GM crops to wild relatives (hybridization) and, subsequently, to conventional crops; (3) diffusion of volunteer transgenic plants, i.e. transgenic plants which emerge within conventional crops, from seeds or propagules set by GM crops grown on the same fields in previous years. Other reasons of agronomic concern are mainly related to herbicide resistant GM crops, which may encourage a less efficient use of herbicides, contributing to the spread of resistant weeds and to herbicide residue accumulation on plant tissues and on the environment.

Besides, the adoption of infesting flora-control methods should allow the removal of the "*volunteer GM crop*" from the fields which are cultivated with conventional or biological crops. Bibliographic data should clarify which DISERBANTE utilise, and what intervention methods should be used.

For the "*Wild relatives"* , instead, due to the practical impossibility to remove them with certainty from the borders of the fields or nearby locations , which could be contaminated, and because of the potential irreversibility of the phenomenon in case of allogame GMO, in regards to the precaution principle, forbidding the coexistence in environments in which this risk will occur will be necessary.

Several agronomic practices may help to reduce the above problems. A first factor is a careful selection of fields, to ensure a good spatial separation between transgenic and conventional crops and to avoid cross pollination. Bibliographic data should clarify what distances may be appropriate, according to mating system and pollen dispersal capacity of each GM crop species of interest.

Weed control measures are as well important to eliminate volunteer GM plants from conventional fields, as well as wild relatives of GM crops from field borders or nearby locations. Literature data should clear up the most appropriate techniques (active ingredient, application date and dosage) to perform an effective weed control.

Crop rotation appears to be a very important factor to avoid the problem of volunteer plants. Indeed, volunteer GM plants are particularly dangerous if a conventional crop follows strictly a GM crop of the same species. Therefore, the selection of an appropriate rotation cycle appears to be fundamental. Literature data may clarify seed viability and dormancy patterns of the different GM species, to be used to set up rational rotation cycles.

Rotation cycle can be strictly related to tillage system: it has been shown that deep ploughing may control seeds characterised by short longevity and no secondary dormancy. On the contrary, no tillage systems may control seeds characterised by high longevity and secondary dormancy, due to *in situ* mortality and predation.

Other agro-technological aspects to be considered are false-seed bed preparation, which may contribute to early eliminate volunteer plants from conventional crops, and an accurate cleaning of harvesting machinery and stocking equipments used for GM crops.

4. Horizontal transfer

The hypothesis of a genetic transfer from Genetically Modified Plants (GMP) to environmental microbes, or to non-GMP through microbes, has been considered at the theoretical and experimental level with a series of studies focusing on specific phenomena included in the whole process. The absence of a systematic experimental work throughout the EU allows only a

critical reading and interpretation of scientific literature available in order to evaluate the possible risks inherent to transgenic seed production.

The mechanism causing the transfer of genetic material from GMP to other organisms is basically the Horizontal Gene Transfer (HGT), which overcomes sexual reproduction and can overcome the barriers represented by the segregation of the organisms in different species. In this context, HGT can occur according to at least three different models, of which the first is a simple HGT from GMP to microbes, producing Genetically Modified Micro-organisms (GMM). The other two models imply a HGT from the MGM to other microbes or to non-GMP.

A critical evaluation of these three models shows that indeed there is some probability that a HGT from plants to microbes can occur, whereas no , at the moment, experimental evidence has been provided to confirm the other two hypotheses. Moreover, it is important to consider that the diffusion of a transgene from GMM to other microbes appears to be a minor problem in Western countries where the use, and sometimes the abuse, of antibiotics caused the spread of antibiotic-resistant bacteria in several habitats. This observation is not instrumental to underestimate the problem, but rather to draw the attention on a actual risk, which seems to raise less concern than a theoretically possible drawback of GMP production.

These preliminary observations suggest to focus the attention on the possibility of a simple HGT from GMP to microbes. The scientific literature available indicates that some bacterial species belonging to the genera *Bacillus*, *Pseudomonas* and *Acinetobacter* can be transformed with exogenous DNA thanks to some form of natural competence. Unfortunately, most of the valuable works presenting these data had to be carried out in the laboratory and therefore in conditions not necessarily similar to those found in the real agricultural environment where such HGTs could occur. One of the main drawbacks is the fact that most of the preliminary data, necessary to devise the experiments, are lacking or are so heterogeneous that drawing general conclusion seems to be difficult. All together, these pieces of literature indicate that the frequency of transformation is relatively low. Another crucial point is the relative fitness of the GMMs after the transformation. This aspect has been even less studied than the possibilities of HGT in natural environments, although it has been considered the real bottleneck of the whole process in recent papers. As a matter of fact, if GMMs have similar or even lower fitness than the untransformed members of the same species, the HGT is likely to represent an interesting phenomenon for the biological speculation but not a real practical risk. Moreover, the fact that several successful transformation used plasmids as vectors and did not progress to any type of integration in the genome of the microbe is suggestive that the stability of the transgene cannot be considered high.

Taking all these information together we can draw a preliminary conclusion saying that HGT from GMP to microbes is possible, but the permanence and spread-out in the environment of the GMM is unlikely. As long as more data will not be available and systematic research will not be carried out throughout the EU territory, the problems raising from the production of GMP could pragmatically be solved simply by confining transgenic production in specific areas where conventional plants cannot be cultivated for a period which should be prudentially evaluated on the basis of the transgenic DNA permanence in the soil, currently estimated in four years at the most.

5. Economical aspects

From the economic point of view, a major problem of co-existence is the economic feasibility of a multi-track marketing and handling system and fixation or less some tolerance thresholds. The tolerance level is critically important, especially for crops that are particularly susceptible to gene flow. The economic implications of the co-existence of GM and non GM crops consist in two main problems:

1. the cost of segregation, to avoid or minimise the adventitious presence of traces of GMOs in conventional seeds;
2. the economic consequences of exceeding the tolerance level.

To avoid the increase of the isolation costs for the cultivations from seed identified zones closed where the coexistence is no admitted go . At the same time to avoid too much raised of sampling and analytical costs for the quantitative determination of GMO in the seed and make the controls easier fixed tolerance thresholds do not go.

Other issues are involved in the economic analysis of the co-existence of GM and non GM crops. Recent studies came to the conclusion that a major cost in non GMO segregation and identity preservation does not come from cleaning farm machinery, from cleaning handling equipment, or other on-farm measures, but rather from the “reshuffling” of the handling system. Existing storage facilities are too few, too large, inefficiently located. However, the adjustment of the handling infrastructure to a new economic equilibrium is seen as a long and gradual processes, due to the large fixed costs of building more, smaller and efficiently located handling facilities.

6. Conclusions

Ensuring the coexistence between transgenic, conventional and organic agriculture has a particular importance, especially when this concept is applied to seed production. In seed production, the risk is that if we tolerate even very low accidental contamination thresholds, we can anyway have high contamination levels in conventional and organic crops

For this, as far as seed production is concerned, not to assume tolerance thresholds is strategic; if unavoidable, their value should be next to zero.

Economical costs due to non-contamination certification guarantee (transports, stocks, processing…) should be undertaken by who sells the products.