Risk Assessment of Plants Containing Genetic Modification Events Combined by Crossing

Introduction

Transgenic traits can be combined by conventional plant breeding techniques into new commercial cultivars. This document considers the risk assessment for the commercialisation of plants containing genetic modification events combined by crossing, although the cases likely to be presented for risk assessment will vary according to the type of crop or plant species.

In maize most of commercial crop production is from hybrid seeds, with the exception of some open-pollinated types, for instance the traditional landraces of Mexico and Central America. In order to produce these hybrid seeds, breeders have their own collection of elite parental lines that are used for hybrid production. These lines are produced by crossing a line with itself a number of times (seven or eight at least) in order to be as near as possible to homozygosity or "purity". The hybrid produced will have many of the characters of the parental plants but will also show enhanced properties *e.g.* improved yield that is of interest to farmers. This occurs in the F1 hybrid due to the phenomenon known as heterosis, also referred to as "hybrid vigour".

In the production of genetically modified F1 hybrids, the transformation is carried out in parental lines that are suitable for transformation and regeneration but which may not necessarily be appropriate for commercial seed production. Once the transgenic plant is regenerated it is immediately crossed to one of the elite (higher performance) lines and backcrossing carried out several times to increase the proportion of the genome of the elite maize line as much as possible. The resulting transgenic line may then be used to transfer the event to any other elite line.

If the objective is to combine more than one event in an F1 hybrid, one possibility is to use two elite parental lines, each one with an event, or to cross two versions of the same line with each version having a different transgene. There are many potential variations to the example outlined and which might be used, for example, to combine three or more events.

In species other than maize this process might not be as complex because the use of hybrids is not as extensive. However, in species such as cotton the combination of events may also be produced either in the production of the F1 hybrid or maintained in a single variety. For example, the reproductive propagules planted may be seeds of F1 hybrids, or varieties or may be clonally propagated, vegetative materials such as potato tubers.

Rationale and scope of the document

Where single events have been approved for release into the marketplace, crops produced by crossing the approved GM event with a non-GM parent (a routine approach in plant breeding and using a range of non-GM parental material) are not required to undergo further risk assessment. However, where transgenic events are combined by the interbreeding of existing GM parents, a risk assessment is required as part of the approval process if such combinations are not already covered by the consent for the single events. The need for further assessment will depend, on a case-by-case basis, on the nature of the genetic modifications involved. It is recognized that the genetic background of parents used in the crosses will vary *e.g.* the selection of different elite lines for the

backcrossing process (EFSA's GMO Panel guidance document[1]).

With regard to the assessment of the plants containing genetic modification events combined by crossing the usual starting point is a risk assessment of the single events in parental materials. Clearly where the hybrids contain one or more events not previously evaluated (*e.g.* in parental material) these events require appropriate risk assessment taking into account the requirements of the GMO Panel guidance document.

Key elements of the evaluation process

If the parental GMO events have been assessed by the GMO Panel, the evaluation of the combined events produced from their crossing should concentrate on the following points:

1. Assessment of the intactness of the inserted loci and phenotypic stability

The requirement is to establish that each transgenic locus in the hybrid is the same as in the original independent transformation event. This information will also be important to confirm the identity of samples used in comparative studies, which would include compositional analysis and any trials involving animals. Intactness of loci and comparisons with insert structures in parental lines should be carried out on material which is representative of cultivars produced for commercial production *i.e.* which will enter the environment and the food/feed chain. To assess intactness of loci, applicants should use appropriate molecular approaches *e.g.* Southern blots and PCR analyses and ensure that probes and primers used cover the entire insert and flanking regions.

Stability can be assessed by confirming that the traits targeted by the genetic modification events (the phenotypes) remain unchanged. Changes in the expression of the trait/phenotype might indicate a potential stability issue with respect to the transgenes. For example, in the case of a herbicide-tolerant crop that the plants remain tolerant to the herbicide in question. Any significant change in the expression of the trait/phenotype targeted by the individual transgenes due to the presence of more than one event (*e.g.* events providing tolerance to biotic and abiotic stress) - in comparison to the expression levels of these transgenes in the parental events - should be taken into consideration during the safety assessment, including the environmental risk assessment (ERA). In such cases and where altered expression of the trait/phenotype is viewed as a potential safety issue (see example above) further assessment of the expression levels of the transgenes in plants segregating for the transgene traits is needed.

Applicants should also provide data to indicate the potential for biologically significant changes in the levels of target proteins when traits are combined by crossing. This may have consequences for the ERA.

2. Assessment of potential interactions between combined events

Applicants are requested to carry out a risk analysis on the potential for any interactions between the combined events which could impact on human or animal health and/or the environment.

A step-by-step approach in the risk assessment should be followed:

[1] http://www.efsa.eu.int/science/gmo/gmo_guidance/660_en.html

1 The risk assessment of the combined events should initially consider the characteristics and properties of each transgenic trait individually. This will also give information, where appropriate, on the segregants from the plants with the combined events.

2 Risk assessment should consider whether or not the occurrence of combined transgenes presents issues that were not addressed when considering the single events *e.g.* in parental lines. For example, the combination of genes may result in altered expression of both endogenous and/or novel traits in a plant.

a) Selection of appropriate comparators

The genetic backgrounds of the controls should be as close as possible to those used in producing the genetically modified plants containing the combined events. The applicant should provide information which validates the choice of controls used for the various parts of the risk assessment.

b) Comparative compositional analysis

Compositional analysis should be carried out alongside appropriate controls grown in the same location and in experiments designed to yield statistically meaningful data. Where the substantial equivalence of parental material containing genetically modified events has been fully tested in replicated field trials over at least 2 seasons, one years field trialling of events combined by crossing is acceptable where geographical localities are representative of the climatic conditions to which such crops will be exposed. Based on the outcomes of this assessment additional follow-up analysis of compositional characteristics over further growing seasons may be required if unexpected differences occur beyond the range of natural variation. On a case-by-case basis, this may trigger further assessment.

In terms of the nutrients, anti-nutrients and natural toxins to be analysed the OECD has published a series of consensus documents on the key components that should be considered in the comparative assessment of new crop varieties of particular species. Measurement of these components can be regarded as the minimum requirement for genetically modified events combined by crossing.

c) Assessments of toxicity, allergenicity and nutritional value

This would include, for example, an assessment of any potential for increased toxicity to humans and non-target organism or to modifications in nutritional value due to the combination of the events. This may arise from additive or synergistic effects of the gene products and may be particularly relevant where the combined expression of the newly introduced genes has unexpected effects on biochemical pathways. This will clearly require a case-by-case approach. The appropriate principles of risk assessment as described in the EFSA guidance document also apply to the assessment of genetic modification events combined by crossing.

d) Environmental risk assessment of plants with combined events

The environmental risk assessment (ERA) will take into account the evaluation of the single events and additional data from molecular characterisation and compositional analysis when determining potential interactions between genes or between gene products. The risk assessment of hybrids will then focus on the possible environmental effects as a result of these interactions. Any new interaction could result in changes to the physiology of the GM plant and related species and

potentially in modified ecological behaviour of these plants. Points to consider for interaction effects within the ERA are the same as those already listed in the EFSA guidance document, *e.g.*:

1. altered toxicity to target organisms and any consequential impact on the development of resistance in target organisms;
2. enhanced toxicity to non-target organisms (including changes in effects on the non- target range);
3. altered fitness of the GM plant or plants acquiring the transgene combination through gene flow;
4. enhanced capacity for gene flow and introgression;
5. altered effects on microbial diversity and activity in relation to biogeochemical cycles.

In addition to the step-by-step approach as described above the following issues need to be considered:

1 Within the framework of field-scale crop production, environmental exposure may be increased to the extent that there could be a significant biological impact which needs to be assessed (*e.g.* for a combination of insect resistance genes producing enhanced levels of toxins).

2 Monitoring plans**:** case specific monitoring should take into account the results of the ERA and any monitoring already established for approved single events. Consideration should be given to any added environmental exposure or effect due to the combination of the events identified in the ERA. General surveillance should be as for any other GM crop and take account of any general surveillance plans already established for the approved single events.

Definitions

Hybrid: The terms hybrid and F_1 hybrid can be defined and used in several ways.

(a) General - The F_1 generation of a cross between two genetically different plants, lines, cultivars, species, or genera.

(b) Specific - A cultivar in which all plants are the F_1 progeny from the crossing of two uniform inbred lines.

F_1's which do not fit in (b) are usually obtained in the process of breeding to obtain new combinations of germplasm, either for maintenance (*e.g.* by vegetative propagation) or for producing an F_2 generation. They may also occur naturally. Hybrids that fit into category (b) and which are produced for use as a commercial crop as the F_1 generation must be newly produced for each new seed crop.

Inbred Line: A line produced by continued inbreeding. In plant breeding an inbred line is a nearly homozygous line usually developed by continued self-fertilization, accompanied by selection.

Pure line: A strain homozygous at all loci, ordinarily obtained by successive self-fertilizations in plant breeding.

Isogenic lines: A group of individuals that possesses the same genotype, irrespective of their being homozygous or heterozygous.

Near-isogenic lines:	A group of lines that are genetically identical except at one or a few loci.
Recurrent parent:	The parent to which successive backcrosses are made in backcross breeding.
Backcross:	A cross of a hybrid to either of its parents. In genetic terms a cross between a heterozygote and a homozygous recessive. In wild inter-specific or intraspecific hybrid plants it is a cross with either of the parental types as a first step towards introgression of genes into populations of the parental type.
F_1:	The first generation of a cross.
F_2:	The second filial generation obtained by self-fertilization or crossing *inter se* of F_1 individuals
F_3:	Progeny obtained by self-fertilizing F_2 individuals.
S_1, S_2, S_3, . . .:	Symbols for designating first, second, third, *etc*., self-fertilised generations from the ancestral plant designated as S_0.
Heterosis	Heterosis, also known as "hybrid vigour", describes a situation where crossing two inbred lines yields progeny (F_1 hybrids) that are, for example, more healthy or vigorous than their parents or which fall outside the range of the parents with respect to other characteristics.
Locus:	The position that a given gene occupies in a chromosome.
Transgene locus:	The position occupied by the inserted (transgenic) construct in a chromosome.