

The objective of this document is to give additional guidance for LMOs with stacked events generated through conventional crossing of single LMOs. As such it is meant to complement the road map on RA and RM and address special aspects of LMOs with stacked transgenes/traits resulting from conventional crossing of first-level transformation events. For the time being it will be restricted to plant LMOs

Introduction

Worldwide a growing number of (plant)LMOs with stacked transgenic traits are developed and cultivated. Thereby the number of stacked traits in a single LMO and the number of LMOs with two to multiple transgenic traits are growing. LMOs with stacked transgenic traits may be generated through a number of processes (see Appendix).

This document refers only to those LMOs resulting from conventional crossing of LMOs with one or more transgenic traits.

The road map is the base of the RA. This document is meant to complement the road map in those aspects which may need special consideration because of the stacking of (multiple) events.

Scope

The guidance presented in this outline focuses on stacked events (StaEv) resulting from conventional crossing of single transformation event (TraEv) parental lines (see definitions) which have been assessed previously in accordance with Annex III.

Additional points to consider

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

Rationale

The combination of transgenic traits via cross breeding may change the molecular characteristics of the inserted genes/gene fragments at the insertion site, and/or influence the detection and regulation of the expression of the transgenes. It is useful to confirm the presence and structure of the TraEvs in the StaEv LMO, and their inheritance, in order to appropriately assess possible adverse effects on the conservation and sustainable use of biological diversity in the likely potential receiving environment, and of potential adverse effects on human health¹.

1

Will be deleted in the final version. It is integrated here because the question was raised on the need of this point to consider and asked for scientific data to underline the recommendation:

It has recently been reported that a single nucleotide polymorphism (SNP) had been introduced into the 35S promoter sequence from Cauliflower Mosaic Virus of the transgenic cultivar 1507 maize during breeding process, resulting in a 16-fold lower sensitivity of a commonly used screening method (Morisset *et al.*, 2009). When the material used for the molecular characterisation is hemizygous in

2. Assessment of potential interactions between combined events and the resulting phenotypic effects

Rationale

The combination of two or more transgene events (TraEvs) in one LMO (ie. a StaEv LMO) may influence the expression level of each of the transgenes, and there may be interaction between the expressed products of the different transgenes. The genetic background may also influence the expression of the transgenes, as is the case for single-TraEv LMOs.

Therefore, in addition to information about the characteristics of the parental single-TraEv LMOs, specific information about the potential for interactions between the stacked proteins or modified traits in the StaEv LMO should be considered: for example, whether or not the different transgenes affect the same biochemical pathways or physiological processes, or are expected to or may have any additive, antagonistic and/or synergistic effects that may result in potential for new or increased adverse effects, relative to the parent LMOs.

3. Assessment of additive, cumulative, synergistic or antagonistic adverse effects of stacked transgenic traits on the conservation and sustainable use of biological diversity in the likely potential receiving environment, taking also into account potential adverse effects to human health.

Rationale

Assessment of additive, cumulative, synergistic or antagonistic adverse effects is based on the environmental risk assessment data for the StaEv LMO in comparison to the closely related non-modified recipient species and the parent LMOs in the likely receiving environment, taking into consideration the results of the genotypic and phenotypic assessments outlined above.

If potential new or increased adverse effects on the conservation and sustainable use of biological diversity or on human health are identified in relation to the StaEv LMO through the above analysis of possible interactions, additional supporting data on StaEv LMO may be required, such as:

- phenotypic characteristics, including the modified traits, compared to the parent LMOs and to relevant non-modified recipient organisms (plants);

concern to the genetically modified trait, the occurrence of SNPs and other nuclear changes at the insertion site or in the insert are even more likely during subsequent breeding processes.

Morriset, D., Demšar, T., Gruden, K., Vojvoda, J., Štebih, D. and Žel J. (2009) Detection of genetically modified organisms – closing the gaps, *Nature Biotechnology* 27 (8), 700-701

- compositional analysis, including the levels of toxins, allergens or anti-nutritional factors known to be present in the parent LMOs or non-modified recipients;
- additional information depending on the nature of the combined traits e.g. further toxicological analysis of the StaEv LMO may be required to address any synergistic effects arising from stacking of two or more insecticidal traits that result in a broadened target range or increased toxicity; and
- the levels of expression of any introduced proteins or modified traits, compared to the parent LMOs.

Indirect effects because of changed management procedures combined with the use of the transgenic stacked event LMO should be taken into consideration.

Intentional and unintentional StaEv LMO may have altered effects because of additive, cumulative, synergistic or antagonistic effects of the stacked traits. Changed impacts on non-target organisms or a change in the range of non-target organisms in the likely receiving environment have to be taken into account. Different StaEv LMOs of the same species may also trigger the possible occurrence of a number of varying unintentional StaEv LMOs, depending on the segregating patterns of the TraEvs in the parental StaEv LMOs. This potential should also be assessed.

Definitions

Stacked events (StaEv): contain the combination of the inserted genetic constructs of two or more single parental transformation events (TraEvs). These are typically physically unlinked and segregate independently. These may be generated by re-transformation of an existing LMO or by the consecutive crossing of two or more LMO plants with different TraEvs. Only those StaEv produced by crossing of LMOs are considered for the purpose of this guidance document.

A transformation event (TraEv) is the result of a single transformation with one transformation cassette. A transformation with a single gene transformation cassette or a multigene transformation cassette will equally result in one transformation event.

Unintentional stacked events are the result of outcrossing of stacked events into other LMOs or compatible relatives in the receiving environment. Depending on the segregation pattern of the stacked events this may result in different and new combinations of TraEvs.

Draft outline “stacked events”, January 2010

Supporting material

EFSA 2007: Guidance Document of the Scientific Panel on genetically modified organisms for the risk assessment of genetically modified plants containing stacked transformation events

Federal Environment Agency Austria 2007: Risk Assessment of “stacked events”

BEETLE-Report 2009, Federal Office of Consumer Protection and Food Safety Germany and EU-Commission. Long-term effects of genetically modified (GM) crops on health and the environment

Biosafety Assessment Tool (<https://bat.genok.org/bat/>)

Appendix

Stacked transgenic traits can be produced via different approaches. Besides the conventional cross hybridising of two LMOs multiple trait characters can be achieved by transformation with a multigene cassette, retransformation of a single trait transformation event with a second construct or simultaneous transformation with different transgene cassettes (cotransformation).

LMOs with multiple transgenic traits resulting from these processes should be assessed according to the road map especially taking into account interactions between the multiple transgenic traits as addressed in this additional guidance.