Opinion

Cell PRESS

Plants with stacked genetically modified events: to assess or not to assess?

Esther Kok[1], Jan Pedersen[2], Roberta Onori[3], Slawomir Sowa[4], Marianna Schauzu[5], Adinda De Schrijver[6], and Teemu H. Teeri[7]

[1] RIKILT Wageningen UR, Akkermaalsbos 2, 6708 WB, Wageningen, The Netherlands
[2] National Food Institute, Technical University of Denmark, Mørkhøj Bygade 19, 2860 Søborg, Denmark
[3] Istituto Superiore di Sanità, Viale Regina Elena, 299, 00161 – Rome, Italy
[4] Plant Breeding and Acclimatization Institute National Research Institute, Radzikow 05-870 Blonie, Poland
[5] Federal Institute for Risk Assessment, Max-Dohrn-Strasse 8–10, 10589 Berlin, Germany
[6] Scientific Institute of Public Health, J. Wytsmanstraat 14, 1050 Brussels, Belgium
[7] Department of Agricultural Sciences, Latokartanonkaari 7, FI-00014 University of Helsinki, Helsinki, Finland

The principles for the safety assessment of genetically modified (GM) organisms (GMOs) are harmonised worldwide to a large extent. There are, however, still differences between the European GMO regulations and the GMO regulations as they have been formulated in other parts of the world. One of these differences relates to the so-called 'stacked GM events', that is, GMOs, plants so far, where new traits are combined by conventional crossing of different GM plants. This paper advocates rethinking the current food/feed safety assessment of stacked GM events in Europe based on an analysis of different aspects that currently form the rationale for the safety assessment of stacked GM events.

Background on the policy for stacked GM events

Global harmonisation has been reached to a large extent on the principles for the safety assessment of GMOs, based on well-accepted advisory reports that have been published by, for instance, the Food and Agriculture Organization/World Health Organization (FAO/WHO) and the Organisation for Economic Cooperation and Development (OECD) [1–3], and incorporated into GMO regulation. Differences can, however, still be observed between the European GMO regulations and those in other parts of the world. One of these differences relates to the so-called stacked GM events, that is, plants where new traits are combined by conventional crossing of different (GM) plants.

In the EU, stacked GM events are assessed as new GMOs, although not all aspects of the safety assessment for single GM events are deemed to be as relevant for stacked GM events [4]. As stated in a report from the European Commission Directorate General for Health and Consumers [5], 'This procedure is not defined as such in Regulation (EC) No 1829/2003, which covers applications for putting "a GMO" on the market. It derives from the assumed rationale that a stack of two GMOs is simply another distinct GMO, a "new" entity requiring as such a full application, . . .' This rationale seems to some extent in contradiction with the definition of a GMO as defined in EU Directive 2001/18/EC: '. . .an organism. . . in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination'. All stacked GM events have so far been obtained by conventional crosses of plants with single GM events. In other parts of the world the Codex guideline is mostly used, which does not mention stacked GM events as new GM plant varieties [6].

As a result of this EU decision also to evaluate stacked GM events, applicants need to provide many of the same data for stacked GM events as for new single GM events. In the Commission Implementation Regulation (EU) No 503/2013, which came into force in June 2013 (EC, 2013), it is stated that stacked GM events need to be assessed in relation to: (i) stability of the inserts; (ii) expression of the introduced genes and their gene products; and (iii) potential synergistic or antagonistic effects. This also pertains to any subcombinations, in the case of a multiple stacked GM event, of the stacked GM event that has not yet been authorised separately.

The food/feed safety assessment of stacked GM events was introduced after the *de facto* moratorium on the authorisation of new GM varieties was lifted in 2004 and when the first stacked GM events moved towards the European market [7]. This assessment was then subsequently substantiated in the European Food Safety Authority (EFSA) guidance documents on the safety assessment of food and feed derived from GM plants [4,8]. The revision with respect to the stacked GM events in the guidance document as published in 2011 was based on another guidance document issued by EFSA [9], which specifically focused on the stacked

Corresponding author: Kok, E. (esther.kok@wur.nl).
Keywords: stacked GM event; safety assessment; genetically modified organism.

0167-7799/$ – see front matter
 http://dx.doi.org/10.1016/j.tibtech.2013.12.001

Table 1. Stacked events for which EFSA have issued their scientific opinion

Dossier reference	EFSA Scientific Opinion adopted	Stacked event
EFSA-GMO-BE-2010-81	6-09-2012	MS8 × RF3 rapeseed
EFSA-GMO-NL-2009-73	26-01-2012	MON 87701 × MON 89788 soybean
EFSA-GMO-NL-2009-65	8-09-2010	1507 × MON 89034 × NK603 maize
EFSA.GMO-CZ-2008-62	8-09-2010	MON 89034 × 1507 × MON 88017 × 59122 maize
EFSA-GMO-UK-2008-56	29-04-2010	Bt11 × GA21 × MIR604 maize
EFSA-GMO-UK-2007-50	29-04-2010	Bt11 × MIR604 maize
EFSA-GMO-UK-2007-49	15-09-2009	Bt11 × GA21 maize
EFSA-GMO-UK-2007-48	29-04-2010	MIR604 × GA21 maize
EFSA-GMO-NL-2007-39	10-03-2010	MON 89034 × MON 88017 maize
EFSA-GMO-NL-2007-38	9-09-2009	MON 89034 × NK603 maize
EFSA-GMO-CZ-2006-33	2-07-2009	MON 88017 × MON 810 maize
EFSA-GMO-UK-2005-21	3-04-2009	59122 × 1507 × NK603 maize
EFSA-GMO-UK-2005-20	19-11-2008	59122 × NK603 maize
EFSA-GMO-NL-2005-16	26-05-2010	281-24-236 × 3006-210-23 cotton
EFSA-GMO-NL-2005-15	21-04-2009	1507 x 59122 maize
EFSA-GMO-UK-2005-09	8-03-2012	MON 1445 x MON 531 cotton
EFSA-GMO-BE-2004-07	6-07-2005	MON 810 x MON 863 x NK603 maize
EFSA-GMO-UK-2004-06	6-07-2005	MON 863 x NK603 maize
EFSA-GMO-UK-2004-05	28-03-2006	1507 x NK603 maize
EFSA/GMO/DE/2004/03	8-06-2005	MON 810 x MON 863 maize
EFSA-GMO-UK-2004-01	13-10-2005	NK603 x MON 810 maize
EFSA-GMO-RX- (renewal)	08-03-2012	MON 1445 x MON 531 cotton

GM events. EFSA did not deem it necessary to require similar comprehensive datasets for the stacked GM events compared to the single GM events.

At that time, arguments were also put forward as to why assessment of stacked GM events was not necessary from a scientific point of view (UK Advisory Committee on Releases to the Environment; ACRE) [10]. These arguments against assessing stacked GM events were not strongly expressed, perhaps because food/feed safety assessment of new single GM events was still in its infancy and a cautious approach was adopted. In the past decade EFSA has assessed more than 20 different stacked GM events (Table 1). In all cases the conclusion was that the crossing of the single GM events did not result in interactions that cause compositional, agronomic, or phenotypic changes that would raise safety concerns. Now, after over 20 years of experience with the safety assessment of GM events, it is time to rethink the current food/feed safety assessment of stacked GM events in Europe.

Safety assessment of stacked GM events

Safety assessment for plants with single GM events

Within the EU, EFSA performs science-based safety assessments of each single GM event for which an application is filed by the applicant for authorisation of introduction into the European market. This assessment includes a molecular characterisation of the genetic construct that was inserted in the GM plant variety, including the flanking sequences; a toxicological assessment of the new gene products (including potential allergenic properties); an agronomic, phenotypic, and compositional comparison of the GM plant variety with its comparator(s); and a nutritional assessment of the whole GMO-derived food/feed [4,5,8]. The comparative compositional analysis of the GM plant variety and its comparators includes relevant micro- and macronutrients, anti-nutrients, and natural toxins. The OECD has issued consensus documents on all major crops providing overviews of these key nutrients, and key anti-nutrients per crop, as well as recommendations on which components should be included in the comparison [11,12]. On the basis of these basic data, it is evaluated whether additional toxicological or nutritional studies are required to form an opinion on the safety of the new GM plant variety. All member states have access to the full dossier and their views and comments are replied to by EFSA and, where relevant, incorporated in the final opinion of EFSA.

The EFSA assessments of single GM events provide insight into the effect of the genetic modification on the physiology of the respective plant variety. These assessments are deemed sufficient to allow subsequent crossing of the assessed GM plant variety with any conventional plant variety to obtain new cultivars without a need to assess each of these cultivars for their food or feed safety. Crosses allow for many new interactions of genes, gene products, and plant components. In the EFSA evaluation, foreseeable interactions between the newly introduced trait and traits as known from commercial varieties have been part of the assessment.

Safety aspects of plants with stacked GM events

When looking at the different aspects that, according to the Implementation Regulation (EU) No 503/2013, need special attention in cases of plants with stacked GM events, it is clear that singling out crosses of single GM events compared to crosses of GM plant varieties with conventional plant varieties is no longer justified.

Genetic stability. The first aspect is the stability of the insert. Including this aspect in the safety assessment of stacked GM events reflects the concern that stability of the insert would be at greater risk when crossing GM plant

varieties compared to the genetic stability in crosses of conventional varieties or when crossing a GM plant variety with a conventional plant variety. Such concerns are unfounded. In all three types of plant crosses there may be changes at the genetic level, but there is no evidence from the safety assessment evaluations (Table 1) that the genetic stability is different in the case of stacked GM events.

Gene expression. The second aspect is the expression of introduced genes. This requirement assumes that gene expression may change more often and/or to a larger extent in the case of a stacked GM event compared to any other type of cross. It is well established that the genomic background of a plant can influence the expression levels of genes, including newly introduced genes in the case of GM plant varieties. Similarly, it is well known that following an approval of a single GM event, subsequent traditional breeding with the GM line may lead to expression levels of inserted genes that may differ considerably from the expression levels of the initially authorised GM event [13]. However, there is no scientific basis to the assumption that the changes in expression levels in a stacked GM event are in any way different to those in any other type of cross. This includes the frequency by which these changes might occur.

Furthermore, it is well known that mutations of a plant genome occur naturally, as the result of transposable genetic elements, errors in the normal duplication of DNA, or induced by external factors such as UV radiation, chemicals, or viral or bacterial infection. Such mutations can also alter the expression of genes, including those inserted in GMOs. Also, environmental conditions (e.g., soil or climate conditions) will greatly influence expression levels of many plant metabolic routes [14,15]. Therefore, it will implicitly be assumed in the safety assessment of any single GM event that gene expression levels might fluctuate considerably during breeding procedures, seed production, and cultivation, which may take place after approval has been given. This implicit inclusion of the variability in expression of introduced genes in the evaluation will be obvious in some cases, for example, in GM plant varieties in which allergens are no longer expressed, it is crucial that this trait will be a stable characteristic; also in the case of subsequent crosses with any other (conventional) varieties. But also in the assessment of any other single GM events, it will implicitly be assumed that expression levels might fluctuate considerably during breeding procedures, as well as, after market approval, subsequent seed production and cultivation.

Potential synergistic or antagonistic effects. The last aspect relates to the potential synergistic or antagonistic effects that may occur in plants with stacked GM events, that is, the interaction between the two, or more, respective genetic modifications and the newly expressed gene products or altered components that result from these modifications. Interaction between GM plant components can take place at two different levels. The first level is at the level of GM plant components, where the interaction is caused by the presence at the same time of two or more newly expressed gene products, for instance, proteins, or of two or more altered components in the food or feed. A possible safety issue could be a combined effect or interaction beyond that which can be expected from the consumption of the individual gene products or components. This potential risk is, however, not limited to the stacked GM event, but is also feasible when the underlying single GM events have already been approved and are consumed simultaneously. Given that foreseen interactions with food/feed on the market are expected to be part of the safety assessment done by EFSA, the assessment of interactions at food/feed level for stacked GM events can be considered as a duplication of the assessment of the single GM event, with no additional value in terms of food and feed safety. Moreover, the same phenomenon of interaction between gene products and/or plant components is not unique for GM plants and may occur in any new mixture in food or feed derived from two conventional plant varieties.

The second level where interaction can take place is at the level of the living cells of the stacked GM event plant. Here, the interaction is due to some common action in the cell, for example, when the new gene products of GM plants are interfering with or acting on the same pathway and thereby change the level of cell components or even give rise to new components. This interaction is one of many new interactions within plant cells that may occur with any new cross. Therefore, this type of hazard is in no way unique for stacked GM events. As a consequence, there is no reason to assess separately and specifically this specific interaction for stacked GM events when these interactions can be and have been implicitly part of the safety assessment of the underlying single events. Interaction of newly expressed gene products with a pathway in stacked GM events is part of the limited set of interactions that to some extent can be foreseen and assessed beforehand, when the individual GM events are assessed for market introduction. For instance, we can foresee interactions on pathways in the case of the high oleic acid GM soy variety that may act on the oleic acid pathway within a conventional variety. Similarly, foreseeable potential interactions with other known pathways, including pathways that have been introduced by genetic modification, could form part of the assessment of a GM single event.

There is thus no reason why the interactions of individual GM plant varieties in general would require extra attention. From experience so far from many different applications, there have been no indications that would justify this separate attention for crosses of GM plant varieties with relation to new interactions in the resulting GM plant variety.

Concluding remarks

For food and feed safety assessment by default there should thus be no further assessment required for plants with stacked GM events. There have been no indications that would justify this separate attention for crosses of GM plant varieties with relation to insert stability, expression of the new genes, and new interactions in the resulting GM plant variety. However, this does not exclude the possibility to consider case-by-case assessment if there are any

specific science-based reasons to require specific further information or data on the stacked GM event. This would also be fully in line with the science-based case-by-case approach as has always been advocated by FAO/WHO [1], OECD [3], EFSA [4,8], and others [16–18]. It would therefore be prudent to have a notification procedure for new stacked GM event varieties so that the requirement for a food/feed or environmental safety assessment can be determined on a case-by-case basis.

The focus of this paper is on food and feed safety aspects of plants with stacked GM events, but we expect the same arguments will apply to the environmental safety assessment, although the hazards are different in that case.

When looking at the full spectrum of new plant varieties moving to the market, there is no sound scientific argument to require full dossiers for stacked GM event varieties that comprise single events that have already been elaborately assessed. This view is reinforced by the fact that in non-GM plant varieties that are considered conventional, changes are introduced that may lead to large, often not very well understood, alterations in the genetics and/or physiology of the plant.

There currently is a considerable difference worldwide between the data that need to be provided by applicants that wish to market a new GM variety compared to the marketing of a new plant variety that does not fall into the category of GMOs. From a scientific point of view this difference is not justifiable, especially in the light of the accumulated experience during many years of safety assessment of GMOs. With the advent of new breeding techniques, such as the different targeted approaches on the basis of zinc finger nucleases and oligonucleotide-directed mutagenesis (ODM), the potential of inducing significant and specific changes in plant physiology is not limited anymore to genetic modification as defined under the European GMO regulations. Safety assessment procedures and regulations should reflect these insights and adjust to new developments in plant breeding.

At present the discrepancy is most obvious for the safety assessment of stacked GM events where the underlying single GM plant varieties have already been adequately assessed. To bring the assessment of new GM plant varieties in line with current scientific insights, it would therefore be a good start to have a notification procedure in place for new plants with stacked GM events to guarantee that specific data on the stacked GM event will only be required when there is a scientific rationale for this.

Whether there are plant varieties that are currently regarded as conventional but for which a food/feed safety assessment would be justifiable is another issue. Discussing this issue could be a next step in the global harmonisation of the assessment of new (GM) plant varieties and at the same time serve to fine-tune the assessment in light of the latest scientific insights.

Acknowledgements

The authors would like to thank Dr. Patrick O'Mahony, Maryvon Noordam, ERT, and Dr. Josep Casacuberta for critical reading of the manuscript.

References

1 Food and Agriculture Organization/World Health Organization (1996) *Biotechnology and Food Safety. Report of a Joint FAO/WHO Consultation, Rome, Italy, FAO Food and Nutrition Paper 61*, FAO/WHO
2 Food and Agriculture Organization/World Health Organization (2000) *Report of a Joint FAO/WHO Expert Consultation on Foods Derived from Biotechnology – Safety Aspects of Genetically Modified Foods of Plant Origin*, FAO/WHO
3 Organisation for Economic Cooperation and Development (1996) *Food Safety Evaluation*, OECD
4 European Food Safety Authority (2011) EFSA Panel on Genetically Modified Organisms (GMO); Scientific Opinion on Guidance for Risk Assessment of Food and Feed from Genetically Modified Plants. *EFSA J.* 9, 2150
5 European Commission (2013) Commission Implementation Regulation (EU) 503/2013 on Applications for Authorisation of Genetically Modified Food and Feed in Accordance with Regulation (EC) 1829/2003. *Official J. Eur. Union* L157/1
6 Codex Alimentarius Commission (2009) *Foods Derived from Modern Biotechnology*. (2nd ed.), FAO/WHO
7 De Schrijver, A. *et al.* (2007) Risk assessment of GM stacked events obtained from crosses between GM events. *Trends Food Sci. Technol.* 18, 101–109
8 European Food Safety Authority (2006) Guidance document of the scientific panel on genetically modified organisms for the risk assessment of genetically modified plants and derived food and feed. *EFSA J.* 99, 1–100
9 European Food Safety Authority (2007) EFSA Panel on Genetically Modified Organisms (GMO): Guidance Document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants containing stacked transformation events. *EFSA J.* 512, 1–5
10 Advisory Committee on Releases to the Environment (ACRE) (2004) Scientific safety assessment of GM hybrids. http://webarchive.nationalarchives.gov.uk/20050216120000/http://defra.gov.uk/environment/acre/pubs/pdf/efsa-maizehybrids-030804.pdf
11 Organisation for Economic Cooperation and Development (2006) *An Introduction to the Food/Feed Safety Consensus Documents of the Task Force. Series on the Safety of Novel Foods and Feeds* (14), OECD
12 Organisation for Economic Cooperation and Development (2012) *Revised Consensus Document on Compositional Considerations for New Varieties of Soybean [Glycine max (L.) Merr.]: Key Food and Feed Nutrients, Antinutrients, Toxicants and Allergens,* Series on the Safety of Novel Foods and Feeds No. 25, ENV/JM/MONO 24, OECD
13 Cellini, F. *et al.* (2004) Unintended effects and their detection in genetically modified crops. *Food Chem. Toxicol.* 42, 1089–1125
14 Chen, W. *et al.* (2002) Expression profile matrix of *Arabidopsis* transcription factor genes suggests their putative functions in response to environmental stresses. *Plant Cell* 14, 559–574
15 Ni, F-T. *et al.* (2009) Gene expression and regulation of higher plants under soil water stress. *Curr. Genomics* 10, 269–280
16 Kuiper, H.A. *et al.* (2001) Assessment of the food safety issues related to genetically modified foods. *Plant J.* 27, 503–528
17 König, A. *et al.* (2004) Assessment of the safety of foods derived from genetically modified (GM) crops. *Food Chem. Toxicol.* 42, 1047–1088
18 Kok, E.J. *et al.* (2008) Comparative safety assessment of plant-derived foods. *Regul. Toxicol. Pharmacol.* 50, 98–113