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**ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND
THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY**

**Report of the OECD Workshop on Environmental Risk Assessment of products derived from New Plant
Breeding Techniques (February 2014)**

**Series on Harmonisation of Regulatory Oversight in Biotechnology
No. 61**

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No. 61

Report of the OECD Workshop on
Environmental Risk Assessment of Products Derived from
New Plant Breeding Techniques
held on 10 February 2014

Environment Directorate
Organisation for Economic Co-operation and Development
Paris 2016

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- No. 47, Guide for Preparation of Biology Consensus Documents (2008)
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- No. 49, Consensus Document on the Biology of *Picea mariana* [Mill.] B.S.P. (Black spruce) (2010)
- No. 50, Guidance Document on Horizontal Gene Transfer between Bacteria (2010)
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- No. 55, Low Level Presence of Transgenic Plants in Seed and Grain Commodities: Environmental Risk/Safety Assessment, and Availability and Use of Information (2013)
- No. 56, Consensus Document on the Biology of Sugarcane (*Saccharum* spp.) (2013)
- No. 57, Consensus Document on the Biology of Cassava (*Manihot esculenta* Crantz) (2014)
- No. 58, Consensus Document on the Biology of Eucalyptus spp. (2014)
- No. 59, Consensus Document on the Biology of Common bean (*Phaseolus vulgaris* L.) (2015)
- No. 60, Consensus Document on the Biology of Cowpea (*Vigna unguiculata* (L.) Walp.) (2015)

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FOREWORD

The major output of the Working Group on the Harmonisation of Regulatory Oversight in Biotechnology over the years has been its Consensus Documents. They contain information for use during the regulatory risk/safety assessment of a particular organism. In the area of plant environmental biosafety, these are being published on information on the biology of certain plant species, selected traits that may be introduced into plant species, and biosafety issues arising from certain general types of modifications made to plants.

The scope of this document is different from that of the Consensus Documents. It constitutes the report of the OECD Workshop on Environmental Risk Assessment of Products derived from New Plant Breeding Techniques, held on 10 February 2014.

This document was prepared by OECD Secretariat in co-operation with the Bureau of the Working Group on the Harmonisation of Regulatory Oversight in Biotechnology, and has been revised based on the input from member countries and stakeholders.

This document is published under the responsibility of the Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology.

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INTRODUCTION

1. ‘New Plant Breeding Techniques’ (NPBT) have been and remain the subject of discussion by regulators, risk assessors, researchers and plant developers over recent years. The Working Group on the Harmonisation of Regulatory Oversight for Biotechnology (WG-HROB) develops guidance to support the environmental risk/safety assessment (ER/SA) of transgenic organisms, including plants. Aware of the significant discussion regarding NPBT, the WG-HROB proposed to hold a workshop to explore whether NPBT raise new issues for ER/SA. The workshop provided a forum for discussion of NPBT and sharing experiences of the ER/SA of NPBT. The information from the workshop will help inform the future work program of the WG-HROB.

2. *The Workshop on Environmental Risk Assessment of Products Derived from New Plant Breeding Techniques* was held at the OECD in Paris, France on 10 February 2014. The workshop specifically discussed the ER/SA of plants¹ developed through some of these techniques. This report describes the main outcomes of this workshop.

Key Message from the workshop:

Experience to date indicates that current guidance and tools for Environmental Risk/Safety Assessment of transgenic plants are applicable to plants developed using New Plant Breeding Techniques, where Environmental Risk/Safety Assessment of such plants may be required.

¹ The term “products” was used in the title of the workshop since ER/SA is not done on techniques but on organisms or products developed using NPBT. However, taking into consideration that the ER/SA of plants was the target of the workshop, the term “plants” is used in this report.

SECTION I. SHARING INFORMATION

1.1 NPBT

3. Recent scientific progress has enabled the development of a new generation of techniques being applied to plant breeding which are often referred to as *New Plant Breeding Techniques* (NPBT). Some of these techniques are different from classical transgenic approaches in their way of introducing traits to a plant, while some of these techniques are refinements of traditional techniques and insert genetic material that is derived from sexual compatible species. Many NPBT resemble conventional breeding methods, and some of the NPBT result in plants that differ only through a few base pairs and are practically indistinguishable from varieties bred through conventional breeding methods. Many/most countries have regulatory frameworks which require pre-market approval, including ER/SA, of plants developed through modern biotechnology. Over recent years there has been increasing discussion and debate by policy makers, regulators, industry and scientists as to whether or not regulatory oversight is required for plants developed by some or all of these techniques.

4. NPBT is a collective descriptive term that has been applied to a range of techniques. The workshop and the focus of the questionnaire was on those techniques identified by Lusser et al. (2011) as NPBT (Table 1).

Table 1. NPBT after Lusser et al. (2011)

Agro-infiltration	Genetic material, so-called T-DNA, is inserted in a plant to express transiently by vector such as <i>A. tumefaciens</i> .
Cisgenesis/Intragenesis	Genes derived from cross-compatible species are inserted into a plant genome.
Grafting on GM rootstock on wild-type Scion	GM rootstock is grafted to non-GM scion without possessing transgenic elements in the leaves or fruits.
Oligonucleotide Directed Mutagenesis (ODM)	Specific mutation is introduced in a defined place in a plant genome by introducing synthetic oligonucleotides as a target to homologous genes.
Reverse Breeding	Homozygous parental plant is generated from selected heterozygous plant by the suppression of meiotic recombination by RNA interference.
RNA-directed DNA methylation (RdDM)	Methylation of promoter region is induced by the introduction of RNA fragments, which results in silencing of the downstream gene.
Site Directed Nucleases (SDN)	Targeted mutagenesis of genes or targeted insertions/deletions of genetic material are achieved by some protein complexes.

1.2 Workshop

5. As agreed at its 27th meeting, the WG-HROB organized a workshop to address NPBT. The main purposes of this workshop were: (i) to improve the general understanding of these techniques and products derived through them; and (ii) to share experiences of, and perspectives on, the ER/SA of products derived

through NPBT. The content of the workshop was informed by a questionnaire circulated among the participating countries in the WG-HROB in September 2013.

6. The workshop took place on 10 February 2014 at the OECD Headquarters in Paris, France. In total, 135 participants from 35 countries, including official participating country delegations to the WG-HROB and individuals from a range of organisations, attended the event. In preparation to the workshop, the OECD Secretariat developed two documents: (i) a background paper that provided an overview of the different NPBT and information on relevant publications [ENV/JM/BIO(2014)4]; and (ii) a summary of the responses by delegations to the WG-HROB questionnaire on their experience with NPBT [ENV/JM/BIO(2014)5]. Both documents are reproduced in Annexes II and III to this report.

1.3 Outcomes of Questionnaire and Presentations

7. A questionnaire was circulated among the participating delegations of the WG-HROB in September 2013, to obtain insight into the experiences of and perspectives on ER/SA of plants developed through NPBT. It also provided an opportunity to obtain information on current development of plants with these techniques. In total, 21 delegations submitted a response to the OECD Secretariat. The blank questionnaire, and a summary of the replies received, are both reproduced in Annex III to this report.

8. During the workshop, experiences of and perspectives on plants derived with NPBT were presented by several delegations of the WG-HROB representatives (the Netherlands, Japan, the United States, South Africa, Australia, Argentina and the Business and Industry Advisory Committee (BIAC) to the OECD), as well as from the European Commission Joint Research Centre (JRC), the Wageningen University Plant Breeding Research centre, and the European Food Safety Authority (EFSA). The agenda is included in Annex I to this report. Perspectives from Canada were also provided. The remarks in paragraphs 9-14 below summarize the main conclusions of the questionnaire and presentations.

9. There were two evident themes from the questionnaire responses: (i) NPBT is a broad category, not an homogeneous set of techniques and does not have a set definition; (ii) triggers for regulation, and therefore legal requirements for ER/SA by regulatory agencies, generally do not explicitly mention NPBT. Some delegations mentioned that they consider the term NPBT to be irrelevant from a regulatory perspective, since regulatory oversight in their jurisdiction is not triggered by the technique used to develop new plant varieties. For example, in Canada, regulation is triggered by the novelty of the product.

10. Some countries reported knowledge of plants being developed by NPBT. The techniques that were mentioned most frequently include Oligonucleotide Directed Mutagenesis (ODM), cisgenesis and applications of Zinc Finger Nuclease (ZFN). The traits mentioned most frequently were pathogen resistance (e.g. late blight, fire blight or scab resistance) or herbicide tolerance. The crops mentioned as most frequently being developed by the application of NPBT were food crops (e.g. apple, potato and maize), although most of them were still in the research phase and not yet approaching commercial use.

11. Some countries mentioned that they were informed of the perspective of the public and private sectors on plants developed using NPBT, many of which advocate following a science-based evaluation in which new characteristics of plants, i.e. phenotype, should determine whether an ER/SA is required and not whether the plant is obtained by a NPBT. If this approach were followed, some plants obtained by NPBT might be exempted from regulation and ER/SA procedures, since many NPBT produce plants that resemble conventional breeding methods that have an established history of safe use (e.g. chemical mutation breeding) or natural crossing techniques. Indeed, plants that are developed by these techniques are often indistinguishable from conventional bred varieties. It was also noted that such an approach would be dependent on the requirements of each country's regulatory framework. As many speakers provided a brief overview of the regulatory context in which ER/SA took place, it became clear that notable

differences exist in the criteria that trigger regulatory oversight among jurisdictions. Lowering the regulatory burden could stimulate innovation since it enables small and medium enterprises (SMEs) to develop plants with NPBT.

12. Most countries indicated that, to date, they did not have practical experience in performing an ER/SA for plants developed through techniques considered to be NPBT. However, some countries have established expert committees for providing advice and recommendations on the use of NPBT. A few countries indicated that they have performed an ER/SA for plants developed by NPBT because the techniques and/or plants are subject to regulation through existing regulatory frameworks. In these cases no new safety issues were identified and according to these countries current guidance tools for ER/SA were considered adequate.

13. Another consideration regarding NPBT, namely the one of “null segregants” was raised. Null segregants are plants in which recombinant genetic material is present during the breeding process, but not in the end-product (plants intended for commercial cultivation). Scientific consensus on the presence of inserted genetic material could resolve the issue whether an ER/SA of null segregants derived from NPBT is required.

14. Several other issues were raised and considered relevant for countries. A first issue was the potential difficulties in detecting the difference in sequences between the plants derived from some NPBT from ones derived through conventional breeding methods; the regulatory/legal status of the end-product (i.e. resultant plants); and the use of these techniques in animals. Secondly, the question was posed as to how certain jurisdictions could anticipate NPBT that might emerge in the future, if the need for regulatory oversight in these jurisdictions is dependent on the technique used.

SECTION II. DISCUSSION

15. For the final part of the programme, a discussion was held on the topics identified during the workshop. The remarks below aim to provide an overview of the issues raised by the different participants and delegations during this discussion. These statements are thus intended to give an impression of some of the considerations that came out of the discussion and *cannot be seen as conclusions adopted by the WG-HROB*.

2.1 The definition of NPBT

16. It was reiterated that the term ‘*new plant breeding techniques*’ is used to describe a diverse range of techniques, that there is no definitive list of NPBT, and that what techniques have been included in discussions to date are somewhat arbitrary and varied. It was also observed that some of the techniques are a refinement of traditional techniques and have existed for over two decades. Furthermore, some of the techniques are not restricted to plants but can also be applied to other organisms. According to some participants, the term NPBT might be confusing for the general public because it suggests that NPBT represents one fixed ‘new’ category of plant breeding techniques or that the techniques identified as falling into the category “NPBT” have the same characteristics.

17. Some delegations noted that within their regulatory frameworks the term “NPBT” is not relevant for determining whether a plant is subject to regulation because the requirement for regulatory oversight was either not determined by the technique used in plant development or the term “NPBT” or any of the techniques that might fall under the term are not a definitional trigger in legislation.

2.2 Approach to and experiences of ER/SA of plants developed through NPBT

18. During the presentations and the discussion it became clear that the current principles of ER/SA were considered applicable when considering plants produced by NPBT. The amount of data required might differ among jurisdictions, but the technical and scientific approaches are, to a large extent, equivalent. Many participants mentioned that the phenotype should be the focus in ER/SA of a plant developed using NPBT.

19. Another question brought up during the discussion was whether, given the similarities with conventional breeding, plants produced using some or all NPBT (if regulated) might be subject to streamlined ER/SA and lesser data requirements (in comparison to transgenic plants for example). Some delegates mentioned that this might be a possibility on a case-by-case basis, but that such an approach would be governed by the particular regulatory and policy framework.

2.3 Detection and identification of plants produced by NPBT

20. Regulatory frameworks for products of modern biotechnology heavily rely on detection since the presence of inserted genetic material is the trigger. It was noted that actual genotypic changes introduced by some NPBT techniques may be indistinguishable from the same changes achieved through conventional breeding approaches. Similarly, the resultant plant’s trait or phenotype from such changes would be indistinguishable from those developed traditionally. Detection and identification of plants

developed by NPBT would be problematic, in contrast to the ability to detect and identify particular transgenic plants using molecular techniques.

21. Some participants observed that the lack of specific detection of plants produced by NPBT might have implications for risk management, monitoring or compliance actions should they be required.

22. One participant mentioned that detection possibilities are currently improving, methods for genomic sequencing are increasingly becoming quicker and cheaper and that such methods could enable the detection of small genetic changes. However it was also noted that differentiating whether changes were introduced by NPBT or by conventional techniques would still be problematic.

2.4 Other issues regarding NPBT

23. A number of other issues related to but distinct from the question of whether current guidance for ER/SA is applicable to plants developed through NPBT were raised and discussed by workshop participants: 1) whether particular NPBT are subject to regulation and require ER/SA under regulatory frameworks and definitions; 2) whether NPBT or plants produced using them should be subject to regulation; and 3) whether there is a scientific or risk basis for regulating noting that many NPBT can produce similar or indistinguishable outcomes to conventional breeding.

24. It was noted that the development of new techniques represents a continuous challenge for regulatory frameworks with regulatory triggers based on definitions of techniques. It was also noted that definitions vary between regulatory frameworks and that there is uncertainty about whether plants produced by NPBT will be subject to regulation under some frameworks but not others. Some participants observed that this represented a challenge for researchers and industry in the development of new plant varieties, in the context of regulatory approvals and trade.

25. It was mentioned that the main goal of the WG-HROB is to ensure that the types of elements used in ER/SA, as well as the methods to collect such information, are as similar as possible amongst countries. This is achieved by facilitating the harmonisation of tools of ER/SA (e.g. consensus documents) and by providing a platform where countries can share their experiences and practices. In contrast, the scope and application of regulatory frameworks for products of modern biotechnology are determined by individual jurisdictions. Participants noted that the discussions around NPBT in various fora include an intersection of several issues, including policy, legal and scientific considerations. Thus when discussing NPBT within in the WG-HROB, the distinction between guidance for undertaking ER/SA and policy considerations for the scope of regulatory frameworks should be kept in mind.

SECTION III. CONCLUDING REMARKS

26. The discussion and the different presentations brought up many different perspectives, experiences and considerations. However, consensus existed among participants that to date, current guidance and tools for ER/SA of transgenic plants are applicable to plants developed using NPBT, where ER/SA of such plants may be required. The workshop participants also acknowledged that the application of NPBT in plant development will remain topical because it raises a range of other issues including policy, legal and trade, and that these were beyond the scope of the workshop.

REFERENCES

Lusser, M. et al. (2011), *New plant breeding techniques. State-of-the-art and prospects for commercial development*, JRC (European Commission's Joint Research Centre).

ANNEX I. AGENDA OF THE WORKSHOP

The draft agenda for the workshop [ENV/JM/BIO/A(2014)2] was circulated on 23 February 2014.
The final agenda is reproduced below.

**OECD WORKSHOP ON ENVIRONMENTAL RISK ASSESSMENT (ERA)
OF PRODUCTS DERIVED FROM NEW PLANT BREEDING
TECHNIQUES (NPBT)**

Agenda

10 February 2014, OECD Paris, FRANCE

Opening and introduction			
1.	09:00	<u>Welcome & activities of Working Group</u>	- Sally McCammon, Chair of the WG-HROB - Peter Kearns, OECD
2.	09:20	<u>Workshop on ERA of products derived from NPBT</u> <ul style="list-style-type: none"> • The outline and the scope of the workshop • The relevance for the WG to consider NPBT • The relevant results of the questionnaire 	- Sally McCammon - Peter Kearns
3.	09:50	<u>New Plant Breeding Techniques</u> <ul style="list-style-type: none"> • An overview of the different NPBT 	- Maria Lusser, Joint Research Centre, European Commission
	10:20	Coffee Break	
Current research and developments – Adinda De Schrijver, Belgium, Chair			
4.	10:50	<u>On-going research and development</u> <ul style="list-style-type: none"> • Example(s) of on-going R&D project(s) concerning NPBT • An outlook on future research of NPBT 	- Jan Schaart, Wageningen University and Research Centre
5.	11:20	<u>Corporate activities</u> <ul style="list-style-type: none"> • Drivers for companies to develop products with NPBT • Example(s) of product(s) developed with NPBT • Perspective of industry on ERA and NPBT • An outlook on commercialisation of products developed with NPBT 	- Gary Rudgers, BIAC, Dow AgroSciences
	11:50	Lunch	

ERA of products derived from NPBT (in various countries/regions)			
- Sol Ortiz Garcia, Mexico, Chair			
6.	13:20	<u>Perspectives from different regulators/risk assessors</u> <ul style="list-style-type: none"> • Perspective on ERA of products derived from NPBT when compared to: (i) ERA of transgenic crops and (ii) procedures for environmental release of conventional bred crops • Practical experiences with ERA of NPBT • Outlook and recommendations on ERA of NPBT 	- Boet Glandorf, The Netherlands
7.	13:40	<u>Idem 6.</u>	-- Dean Oelofse, South Africa
8.	14:00	<u>Idem 6.</u>	- Hiroshi Kamada, Japan
9.	14:20	<u>Idem 6.</u>	- Sally McCammon, United States
10.	15:00	<u>Idem 6.</u>	- Heidi Mitchell, Australia
	15:20	Coffee Break	
11.	15:50	<u>Idem 6.</u>	- Andrea Gennaro, EFSA
12.	16:10	<u>Idem 6.</u>	- Patricia Gadaleta, Argentina
12.	16:30	<u>Idem 6.</u>	- Phil Macdonald, Canada
Discussion and closure			
13.	16:50	<u>Panel-led discussion on the workshop</u>	- Peter Kearns, moderator
14.	17:30	<u>Closure and wrapping up</u>	- Sally McCammon
	18:00	Cocktail	

ANNEX II. BACKGROUND PAPER

In preparation to the workshop, a background paper on new plant breeding techniques [ENV/JM/BIO(2014)4] was circulated on 30 January 2014. It was made available to all attendees at the workshop, and is reproduced below.

27. In recent years scientific advances have enabled the development of more sophisticated plant breeding techniques (NPBT). New traits can be introduced in a plant species more precisely and often without the introduction of foreign genetic material into the genome. This paper discusses seven techniques generally considered as NPBT: (I) Zinc Finger Nucleases; (II) Oligonucleotide Directed Mutagenesis; (III) Cisgenesis and Intragenesis; (IV) Reverse Breeding; (V) RNA-directed DNA methylation; (VI) Grafting on GM-rootstock; and (VII) Agro-infiltration. For these techniques the underlying biological mechanisms will be explained and an overview will be provided of relevant publications, including: (I) a selection of relevant research papers; (II) a selection of relevant reviews; (III) publications on commercial product development; and (IV) known publications on risk assessment. This document aims to support the workshop on environmental risk assessment (ERA) of NBPT that has been organized by the OECD Working Group on Harmonisation of Regulatory Oversight on Biotechnology.

Introduction

28. During, the last decades, scientific advances have continuously improved existing plant breeding practices. As knowledge on molecular biology and genetics has increased, plant breeding has become ever more sophisticated. From 1996, when the first transgenic crops were introduced in the US, the surface of cultivated transgenic crops across the globe has increased up to 170 million hectares in 2012 (Clive, 2012).

29. Recently, a new generation of plant breeding techniques has emerged that enabled developers to achieve highly targeted genetic modifications in a plant often without the introduction of foreign genetic material. Furthermore these new techniques sometimes result in crops that are genetically indistinguishable from their conventional counterpart.

30. To discuss environmental risk assessment (ERA) of these new plant breeding techniques, the OECD Working Group on Harmonisation of Regulatory Oversight in Biotechnology agreed to organise a Workshop on New Plant Breeding Techniques on 10 February 2014 in Paris. This background paper discusses a non-exhaustive selection of the seven techniques that are the focus of the workshop and commonly considered as new plant breeding techniques (Lusser et al., 2011; COGEM, 2006; Schaart and Visser, 2009). It was prepared as a supportive document for the workshop that provides definitions of the techniques and an overview of relevant publications on research and corporate activities.

Site Directed Nucleases (SDN)

Applications of SDNs

31. The term Site Directed Nucleases (SDN) refers to different types of protein complexes that are able to achieve targeted mutagenesis of genes or targeted insertions/deletions of genetic material. All SDN complexes have a nuclease domain that can induce a site-specific double strand break (DSB) in the DNA, in which both complementary strands of DNA are broken. When native cellular mechanisms repair this break, mutations can be induced (deletion, additions or mutations) or genes can be inserted. Relying on this mechanism, three different applications of SDN exist:

- In a SDN-1 approach the SDN complex binds to the DNA sequence targeted for mutation and generates a site-specific DSB. Gene repair mechanisms of the cell intervene to repair the break using non-homologous end joining and may generate site-specific mutations that can consist of changes in a few base pairs, deletions or insertions. In this way gene functioning can be altered or disturbed (Lusser et al., 2011).
- In a SDN-2 approach SDN genes are delivered to cells along with a repair template, comprising of a DNA sequence homologous to the targeted area with the exception of a point mutation, a small deletion or small addition. The SDN binds to a specific DNA sequence and generates a site-specific DSB. Gene repair mechanisms of the plant cell intervene to repair the break using homology directed repair and by doing so creating specific mutations through homologous recombination with the repair template. In this way a specific point mutation can be induced in the genome (Lusser et al., 2011).
- In a SDN-3 approach SDN genes are delivered to cells along with a stretch of DNA (e.g. a gene of interest). The SDN binds to a specific DNA sequence and generates a site-specific DSB. The ends of the inserted DNA stretch are homologous to the sites flanking the DSB and will be inserted between both ends of the DSB. In this way genes can be introduced in a genome via a site-specific approach (Lusser et al., 2011).

Different types of SDNs

32. Three different techniques that rely on SDN will be discussed: Zinc Finger Nucleases (ZFN), Transcription-activator Like Effector Nucleases (TALENs) and Mega Nucleases (MN),

- ZFN are artificial protein complexes that consist of a nuclease domain coupled to a zinc finger domain. A zinc finger is a protein motif that interacts specifically with three base pairs of DNA. Different zinc fingers exist that each can target a specific triplet of nucleotides, by fusing multiple zinc fingers together a specific sequence can be targeted. The nuclease that is coupled to the zinc finger complex can subsequently induce a DSB on the targeted site in the genome (Puchta and Fauser, 2013; Podevin et al., 2013).
- Via a mechanism similar to ZFN, TALENs can induce site-specific mutations in a genome. TALENs are artificial protein complexes in which a nuclease is coupled to a Transcription Activator-Like (TAL) effector domain. The TAL effector is a protein complex derived from plant pathogenic bacteria that is able to recognize and bind specific genetic sequences. It is comprised of multiple repeats of approximately 34 amino acids, that each can recognize one nucleotide in a genetic sequence. The residues on positions 12 and 13 of each repeat are variable and determine which nucleotide is preferred by the repeat. Via this mechanism TAL effectors can be engineered to target specific genetic sequences and induce mutations via the attached nuclease domain (Podevin et al., 2013; Puchta and Fauser, 2013; Bogdanove and Voytas, 2011)

- MNs are naturally occurring monomeric nucleases that can recognize DNA sequences up to 40 nucleotides and induce a DSB. The long recognition site makes MNs specific, since the targeted sequence is not likely to occur anywhere else in the genome. The limited amount of natural available MNs restricts the use of MNs for genome editing. However, currently existing MNs have been redesigned resulting in an expanded repertoire of new tailor-made MNs (Puchta and Fauser, 2013; Podevin et al., 2013).

PUBLICATIONS

Selection of research publications

- Shukla et al. (2009) used a ZFN-3 approach to simultaneously disrupt the IPK1 gene in maize and introduce herbicide tolerance. This was achieved by inserting, an exogenous gene cassette conferring herbicide tolerance inside the IPK1 gene. The disrupting of the IPK1 resulted in lower the levels of phytate (an anti-nutrient indigestible for most mammals).
- Townsend et al. (2009) used a ZFN-1 approach to develop herbicide tolerant tobacco by disrupting the acetolactate synthase (ALS) gene.
- Wright et al. (2005) report the targeted repair of a disrupted reporter gene via a ZFN-2 approach through homologous recombination with a repair template.
- Cai et al. (2009) report the insertion of gene conferring herbicide tolerance via a ZFN-3 approach.
- Li et al. (2012) report the development of rice variety resistant to blight via a TALEN mediated mutation.
- Gao et al. (2010) report achieving a successful mutation in maize with MNs.

Selection of reviews

- Reviews have been published by Durai, Weintal, Porteus, Kumar, Saika, Curtin, Tzfika, Puchta and Podevin (Durai et al., 2005; Weintal et al., 2010; Porteus, 2009, Kumar, Allen and Thompson, 2006; Saika and Toki, 2009; Curtin, Voytas and Stupar, 2012; Tzfira, 2012; Puchta and Fauser, 2013; Podevin et al., 2013).

Product development for commercialisation

- Based on ZFN-3 technology, Dow AgroSciences have developed EXZACT™ Precision Technology. With this technology a variety of herbicide tolerant maize was developed (Dow AgroSciences, 2013; Shukla et al., 2009).

Regulations and risk assessment

- The European Food Safety Authority (EFSA) published a scientific opinion on ZFN-3 (EFSA, 2012a).

Oligonucleotide Directed Mutagenesis (ODM)

33. Oligonucleotide Directed Mutagenesis (ODM) is an approach by which specific mutations can be introduced in a defined place in a plant genome. Synthetic oligonucleotides are introduced in the cell which is homologous to the targeted gene except for the nucleotides that are targeted for mutation. Once the oligonucleotide hybridizes to the targeted gene, it creates a mismatch in base pairing. The cells native repair mechanism corrects this mismatch, giving rise to small specific mutations. The oligonucleotides can be composed of DNA, RNA or both RNA/DNA (Lusser et al., 2011).

PUBLICATIONS

Selection of research publications

- Beetham et al. (1999) developed herbicide tolerant tobacco by inducing site-specific mutations in the ALS gene with full-DNA and DNA/RNA-hybrid oligonucleotides.
- Okuzaki and Toriyama (2004) developed herbicide tolerant rice by inducing site-specific mutations in the rice' ALS gene with DNA/RNA-hybrid oligonucleotides.
- Zhu et al. (1998) developed herbicide tolerant maize by inducing a site-specific point mutation in the acetohydroxyacid synthase (AHAS) gene through DNA/RNA-hybrid oligonucleotides.

Selection of reviews

- A review has been published by Oh and May (2001) and Hohn and Puchta (1999).

Product development for commercialisation

- The American company Cibus has developed the Rapid Trait Development System™ (RTDS™) based on oligonucleotide directed mutagenesis. Currently HT canola (expected 2013) and HT flax (expected 2015) are in development (Cibus, 2013).
- The Dutch company Keygene developed Keybase®, a technology that uses ODM for achieving targeted point mutations (Keygene, 2013).

Regulations and risk assessment

- Breyer et al published a commentary addressing regulatory ambiguities of plant breeding approaches using oligonucleotides in the context of EU GM legislation [30].
- The British Advisory Committee of Releases to the Environment (ACRE) published an opinion about HT Canola produced by Cibus' RTDS™ (Breyer et al., 2009).
- The Dutch COGEM published an opinion on the use of oligonucleotides in plant breeding (COGEM, 2010).

Cisgenesis/Intragenesis

34. In both cisgenesis and intragenesis genes are inserted into a plant genome that is derived from cross-compatible species. In cisgenesis the transferred gene is flanked by its native promoter and terminator. No marker genes or vector-backbone sequence are present in a cisgenic plant, except for so-called T-DNA borders that are co-inserted when *Agrobacterium tumefaciens* is used for transformation. In intragenesis, the transferred gene can be inserted in combination with promoters and/or terminators that are derived from other cross-compatible species than the coding sequence of the gene. No marker genes or vector-backbone sequence are present in an intragenic plant (Lusser et al., 2011; Holme, Wendt and Holm, 2013).

PUBLICATIONS

Selection of research publications

- Vanblaere et al. (2011a; b) published several papers on the development of a cisgenic apple variety with scab resistance.
- Rommens et al. (2008, 2006) used an intragenic construct to develop potatoes that contain lower amount of the toxic acrylamide, suffer less from enzymatic browning and are less prone to pressure bruising during storage.
- Haverkort et al. (2009) report on developing a cisgenic potato with resistance against the fungus *Phytophthora infestans*. This fungus causes late blight, a disease that causes major damage to potato production.
- Holme et al. (2012) report work on a cisgenic barley variety with increased phytase activity. This enzyme can release phosphate from phytate, a compound that is not digested by most mammals. In a conventional barley plant low phytase levels are present, resulting in low phosphate absorption when used as animal feed.

Selection of reviews

- Reviews have been published by Holme (intragenesis and cisgenesis) and Rommens (intragenesis) (Holme, Wendt and Holm, 2013; Rommens, 2001).

Product development for commercialisation

- The American company Simplot has developed an intragenic potato with reduced acrylamide levels (Simplot, 2013).
- Researchers in Wageningen are developing a cisgenic potato resistant against the fungus *Phytophthora infestans* (WUR, 2013).
- The JRC maintains a database with notifications on deliberate environmental release of GMOs for experimental purposes. This database mentioned field trials for a scab resistant apple and a barley variety with increased phytase levels.

Regulations and risk assessment

- Some researchers/developers working on cisgenesis suggest the exemption of cisgenesis from the GM regulatory framework since it does not exceed natural genetic boundaries between species (Schouten, Krens and Jacobsen, 2006).
- EFSA (2012b) published a scientific opinion about the risk assessment of plants developed by cisgenesis and intragenesis.

Reverse Breeding

35. Reverse breeding is an approach that allows the generation of a homozygous parental plant from selected heterozygous plants by the suppression of meiotic recombination by RNA interference of genes involved in meiosis. Subsequently, the obtained homozygous lines are hybridised, in order to reconstitute the original genetic composition of the selected heterozygous plants (Lusser et al., 2011; Dirks et al., 2009).

PUBLICATIONS

Selection of research publications

- Wijnker et al. (2012) report the proof-of-concept of reverse breeding in *Arabidopsis thaliana*.

Selection of reviews

- Reviews have been published by Wijnker and Jong (2008) and Dirks et al. (2009).

Product development for commercialisation

- No products are known to have been developed.

Regulations and risk assessment

- No publications concerning risk assessment of reverse breeding are yet known to have been published.

RNA-directed DNA methylation (RdDM)

36. RNA-directed DNA methylation (RdDM) is a technique that relies on transcriptional gene silencing guided by RNA fragments. The mechanism involves the introduction of a transgene into a cell that can be transcribed into double stranded RNA (dsRNA). This dsRNA is cleaved into short interfering RNAs (siRNAs) that share homology with the promoter region of the targeted gene. The siRNAs then induce methylation of the promoter region, resulting to silencing of the downstream gene. This results in the silencing of the gene without changing its genetic sequence (epigenetics). The changed methylation pattern will be transmitted to progeny even if the transgene that induced the methylation is deleted. A major drawback is that after several generations the effect is shown to fade out (Lusser et al., 2011; Aufsatz et al., 2002).

PUBLICATIONS

Selection of research publications

- Okano, Miki and Shimamoto (2008) report that in rice siRNAs do induce methylation of the promoter region of the targeted gene but that this did not result gene silencing.
- Aufsatz et al conducted fundamental research on RNA-directed DNA methylation in Arabidopsis (Aufsatz et al., 2002).
- Cigan, Unger-Wallace and Haug-Collet (2005) succeeded in obtaining male sterile maize plants by silencing a fertility gene through methylation of its promoter.

Selection of reviews

- Reviews are published by Matzke et al. (2007, 2004), Chan, Henderson and Jacobsen (2005) and Zhang and Zhu (2012).

Product development for commercialisation

- No products are known to have been developed yet.

Regulations and risk assessment

- No publications concerning risk assessment of RdDM are yet known to have been published.

Grafting on GM-rootstock on wild-type Scion

37. Grafting is a common used agricultural technique that provides opportunities for enhancing crops by combining beneficial traits of rootstocks and scion of different varieties. By grafting transgenic rootstocks to non-transgenic scions, the crop may benefit from traits conferred by transgenes in the rootstock without possessing transgenic elements in the leaves or fruits. Furthermore it is considered an advantage that once a GM rootstock is developed it can be used for grafting onto different scions (Lusser et al., 2011).

PUBLICATIONS

Selection of research publications

Most research is conducted in fruit crops and focusses on the mobility of transgenic elements between the rootstock and the scion.

- Smolka et al. (2010) report on research conducted on transgrafted apple trees. The inserted transgene is proved to improve rooting when overexpressed. Transgrafted trees show declined growth and flowering, while fruit quality was not affected. No transgenic DNA or mRNA was detected in the scion.
- Haroldsen, Chi-Ham and Bennett (2012) report research on walnut and tomato crops in which the rootstock is genetically engineered. The inserted transgenic construct contains two marker genes and a gene that expresses siRNAs that confer resistance to Crown Gall disease. Subsequent analysis revealed that no transgenic proteins, mRNA or DNA could be detected in the scion. However, transgenic short interfering RNAs were detected in the kernels of the walnut.
- Nagel (2010) report on the development of transgrafted plum tree in which the rootstock is genetically engineered to confer resistance against *Phytophthora cimamoni* and *Meloidogyne incognita*. Analysis of both the rootstock and the scion revealed that the mRNAs and proteins encoded by the transgene are present in the rootstock, but not in the scion [57].

Selection of reviews

- A publication by Haroldsen et al. (2012) reviews studies addressing the mobility of DNA, RNA and proteins between the GM rootstock and the wild-type scion.

Product development for commercialisation

- A walnut crop with resistance to Crown Gall disease, a plant disease caused by *A. tumefaciens* causing tumour formation in certain plants, is close to commercialisation (Escobar et al., 2002).
- A grape vine with moderate resistance to Pierce disease, a bacterial infection affecting Grape plants (Agüero et al., 2005).
- The JRC database mentioned several field trials that have been conducted with transgenic rootstocks: fungal resistant orange variety, apple and pear varieties with enhance rooting characteristics and a grapevine variety with viral resistance (<http://gmoinfo.jrc.ec.europa.eu/Default.aspx>).

Regulations and risk assessment

- Lemgo et al. (2013) published a review in which they discuss biosafety considerations of RNAi-mediated virus resistance in fruit crops. Within this context also transgenic rootstocks grafted to non-transgenic scions are discussed.

Agro-infiltration

38. Agro-infiltration provides a quick approach for transient high level gene expression in plants. *A. tumefaciens* is able to insert genetic material (so-called T-DNA) to the nucleus of a plant cell, hence enabling foreign genetic sequences to be expressed. In agro-infiltration plant tissues are infiltrated with a liquid suspension that through *A. tumefaciens* introduces high quantities of foreign genetic material into the plants' genome. This enables rapid, high level expression of the targeted protein in the infected tissue. Expression of the foreign gene is limited to the infected tissue and will not be stably inherited to by progeny. Different subclasses of agro-infiltration exist:

- In an approach known as *agroinfiltration sensu stricto* a non-germline tissue (typically a leaf tissue) is infiltrated with a liquid suspension of *Agrobacterium*. The transferred construct is non-replicative in order to obtain localised expression in the infiltrated tissue, ensuring that the transgene will not be inherited to progeny (Lusser et al., 2011).
- In an approach known as *agroinfection* a non-germline tissue is infiltrated within a replicative virus vector that spreads through the entire plant (Lusser et al., 2011).
- In an approach known as *floral dip* a germline tissue is infiltrated by *Agrobacterium* carrying a DNA construct leading to a stable transformation of some of the embryos (Lusser et al., 2011).

PUBLICATIONS

Selection of research publications

Agro-infiltration is often used as a diagnostic approach in which transient expressed genes are tested for evoking immune responses. Furthermore genes with unknown functions can be expressed under a strong promoter to analyse their function.

- Gomez et al. (2013) use agro-infiltration to obtain *Nicotiana Benthamiana* plants that transiently express the VP2 protein of the Infectious Bursal Disease Virus (IBDV). The VP2 is used as a plant-derived vaccine and is shown to evoke an immune response in chickens.
- Bhaskar et al. (2009) developed an *Agrobacterium*-mediated tool for rapid functional gene assays in potato.

Summary

39. Over recent years, a new generation plant breeding techniques emerged as a result of increased knowledge on plant genomes and molecular biology. This paper described seven of these techniques generally considered as new plant breeding techniques.

40. Some of these techniques induce site-specific genome alterations at defined sites in the genome (SDN and ODM), whereas others introduce beneficial traits through epigenetic modifications (RdDM) or modifications with genes derived from cross-compatible species (cisgenesis and intragenesis). Also techniques have emerged in which transgenes are only expressed in certain plant tissues (grafting and agro-infiltration).

- **SDN** are protein complexes that can induce breaks in the genetic sequence on defined locations in the genome. Different types of SDNs exist (e.g. ZFN, TALENs and MNs) that can be used in different approaches (e.g. SDN-1, SDN-2 or SDN-3). Dow Agrosiences used ZFN to develop a maize variety with lowered levels of phytate (an anti-nutrient) and herbicide tolerance (HT).
- **ODM** is a technique in which small, specific mutations are induced through synthetic oligonucleotides. ODM is currently in an early phase of commercialisation, scientific advances have been adopted by companies like Keygene and Cibus. A HT canola variety developed through ODM is approaching market introduction.
- With **cisgenesis and intragenesis** only genes are transferred into a plant genome that are derived from cross-compatible species, so the approach does not exceed natural genetic boundaries. Current products developed involve potatoes, apples, barley, melons and alfalfa. Traits introduced are mainly fungal resistance or altered compositional characteristics.
- **RdDM** is a technique that enables epigenetic silencing of a targeted gene in a plant genome. This is achieved via a complex mechanism in which DNA-methylation induces chromosomal rearrangements in the promoter region of the targeted gene making it inaccessible for enzymes involved in transcription. As far as current publications reveal, RdDM is still in research phase and no commercial products are developed yet. A current major challenge in RdDM is to achieve stable gene silencing over multiple generations.
- **Reverse Breeding** is a technique in which RNA interference is used to suppress meiotic recombination so homozygous parental plants can be obtained from selected heterozygous individuals with beneficial traits. Reverse breeding is currently still in an early phase of development: only a few papers have been published so-far.
- **Grafting on transgenic rootstocks** is mainly done with fruit crops and woody species (plum, grape, tomato, walnut). The rationale behind this approach is that the transformed rootstock can confer enhanced genetic characteristic (e.g. resistance traits) without the presence of transgenic DNA in the fruit or nut. Current developments known to be approaching commercialisation are a walnut variety resistant to Grown Gall disease and a grape vine with moderate resistance to Pierce disease.
- **Agro-infiltration** enables rapid, transient high level expression of transgenes in defined plant tissues through transformation by *A. tumefaciens*. Agro-infiltration is reported mainly as approach for producing pharmaceutical proteins and as system to test genes with unknown function.

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ANNEX III. QUESTIONNAIRE AND SUMMARY OF RESPONSES

A. QUESTIONNAIRE

The blank questionnaire circulated to delegations of the WG on 25 September 2013 in preparation to the workshop, is reproduced below.

Questionnaire on environmental risk/safety assessment of plants developed with New Plant Breeding Techniques (NPBT)

COUNTRY:

Contact details:

Preamble

Science is continuously developing new techniques for advancing plant breeding. It is important to begin to understand whether and how countries are contemplating regulation and environmental risk/safety assessment (ER/SA) of the ever-evolving continuum of emerging plant products and the biotechnologies used to develop them. Certain these new techniques have been identified by some as New Plant Breeding Techniques (NPBT, Lusser *et al*, 2012)². Examples include:

- Agro-infiltration
- Cisgenesis/intragenesis
- Grafting on GM rootstock
- Oligonucleotide directed mutagenesis (ODM)
- Reverse breeding
- Site-directed nucleases (e.g. zinc finger nucleases)
- RNA-dependent DNA methylation

However, we want to understand what types of plants and techniques are currently being discussed in countries **whether or not they are included in these examples.**

Workshop

At the 27th meeting of the Working Group on the Harmonisation of Regulatory Oversight in Biotechnology it was agreed to have a workshop on NPBT, 10th February, 2014, in association with the upcoming 28th meeting of the Working Group. To prepare for the workshop it was agreed to circulate a questionnaire to the delegates in order to provide input for discussions. This questionnaire was developed to obtain an understanding of the types of plants under development, the phenotypic changes being introduced and the new technologies deployed to develop them.

² Lusser *et al.* (2012), "Deployment of New Biotechnologies in Plant Breeding", *Nature Biotechnology*, Vol. 30, pp. 231-239.

The upcoming workshop will likely be composed of several aspects including: 1) an overview of the science behind the application of some techniques, 2) presentations by specific countries and 3) discussion. In addition, the workshop may set the stage for future project proposal(s) to be developed. **The goal of this questionnaire is to characterise the perspective from which countries identify, address and assess new plants and NPBTs being used to develop them, particularly from an ER/SA perspective.** Ultimately, the responses can serve to guide the scope of work for future OECD discussions and projects in this area.

We would like to receive responses to the questionnaire **by 30th November 2013** in order that we might use the information relayed to best advantage in preparations for the workshop and during the workshop itself, as well as in the subsequent meeting of the Working Group. We realize that this does not allow a lot of time for preparation of a response, so be assured that if a project is developed subsequently in which it would be advantageous to include country responses, it will be possible to add additional information later.

Question I

Does your country consider NPBT? Which techniques does your country consider as NPBT?

Question II

Is your country seeing any plants developed with NPBT in the private or public sector (industry and/or academia)?

If yes,

(a) please describe this plant and the phenotypic change(s) introduced.

(b) please describe the NPBT involved in the development of the plant.

(c) when do you anticipate that a developer will apply for commercial release for this plant?

Question III

Does your country have any practical experience in performing an environmental risk/safety assessment on plants developed with NPBT?

If yes,

- (a) which technique(s) were involved?*
- (b) did you encounter any new environmental risk/safety assessment issues?*
- (c) did your country issue any specific guidance or recommendations on environmental risk/safety assessment?*
- (d) have any environmental risk/safety assessments for plants developed with NPBT been made available to the public?*
- (e) please provide all relevant reports, guidance documents and links, if possible.*

If no,

- (a) do you expect plants developed using NPBT will give rise to new issues in environmental risk/safety assessment and, if so, what are those issues?*

Question IV

Have the public or private sector (academia and/or industry) provided their perspective regarding environmental risk/safety assessment of plants developed with NPBT?

- (a) If yes, please describe them.*

Question V

Are there other questions on NPBTs do you consider to be of importance in your country?

Question VI

What do you consider to be important objectives and outcomes for the OECD workshop (10th February 2014)? Are there NPBT that are of particular interest to your country?

A. QUESTIONNAIRE

B. SUMMARY OF RESPONSES

The OECD Secretariat prepared a summary of the replies received from delegations to the Questionnaire on environmental risk assessment of products derived from new plant breeding techniques [ENV/JM/BIO(2014)5], circulated on 30 January 2014. This document is reproduced below.

In preparation for the workshop, a questionnaire was circulated among the delegations of the Working Group to obtain more insight in their perspectives on and experiences with environmental risk assessment (ERA) of products developed with NPBT. This document provides a summary of the received responses. The following twenty-one delegations submitted a response to the OECD Secretariat:

Argentina, Australia, Austria, Bangladesh, Belgium, Canada, Czech Republic, Germany, Finland, Ireland, Japan, Mexico, Netherlands, Norway, Switzerland, South Africa, Turkey, United Kingdom, United States, European Commission, and BIAC.

I. Does your country consider NPBT? Which techniques does your country consider as NPBT?

1. Most countries do discuss NPBT and consider techniques described in *Lusser, 2012* as NPBT. Furthermore, other techniques mentioned (amongst others) are: accelerated breeding, RNAi, CRISPr/Cas, TALENs, transplastomics.
2. The US and Canada do not use the term NPBT in a regulatory framework to date, since in their regulatory oversight the product rather than the technique plays a central role.

II. Is country seeing any plants developed with NPBT in the private or public sector?

1. Cisgenic apples with scab resistance (Switzerland, the Netherlands).
2. Fireblight resistant apples through accelerated breeding (Switzerland).
3. Citrus trees with transgenic rootstocks (Argentina).
4. Male-sterility technology in Maize (Argentina).
5. Cisgenic potatoes resistant to late blight (the Netherlands, Belgium, Ireland).
6. Different food crops developed via ODM (the Netherlands).
7. Trees with altered lignin composition developed via RNAi (Belgium).
8. Accelerated breeding using Apple latent spherical virus-based vectors (Japan).
9. Herbicide tolerant oilseed rape developed through ODM (UK).
10. Cereal varieties developed through site-directed nucleases (UK, Ireland).
11. Cisgenic maize lines that are drought and cold resistant (Mexico).
12. Cisgenic papaya resistant for a specific fungus (Mexico).

13. Several applications of transplastomics and agroinfiltration for pharmaceutical purposes (Mexico).
14. Herbicide tolerant flax via ODM (Canada).
15. In the US various queries have been submitted at the regulatory agencies as to whether the concerned product is considered as a regulated article. In several of these inquiries NPBT are discussed (e.g. cisgenesis, ZFN), but also other techniques are under consideration (e.g. meganucleases and null-segregant plants).
16. Intragenic ryegrass with improved forage qualities (Australia).

III. Does your country have any practical experience in performing an ERA on plants developed from NPBT?

1. Most countries do not have practical experience in performing an ERA in plants developed from NPBT.
2. Many countries do have scientific committees studying these techniques.
3. Ireland did an ERA on cisgenic potatoes with blight resistance. No new issues were identified.
4. The Netherlands have practical experience in doing an ERA on plants/trees derived from cisgenesis and intragenesis. Furthermore, there is discussion with third parties about Reverse breeding and ODM. No new issues were identified.
5. Australia performed an ERA for the controlled release of intragenic ryegrass with improved forage qualities. No new issues were identified.
6. Belgium has experience with ERA on plants/trees developed with RNAi or cisgenesis. The ERA was performed using existing guidelines for conventional GMOs. No new issues were identified.
7. EFSA published a scientific opinion on the use of cisgenesis and intragenesis in plant breeding.
8. EFSA published a scientific opinion on SDN-3: site-directed nucleases that facilitate the targeted integration of a gene.

IV. Have the public or private sector provided their perspective regarding ERA of plant developed with NPBT?

1. Some countries refer to position papers of the plant breeding sector and/or seed associations.
2. Some countries mention the request from industry for a science-based risk/safety assessment in a product-based approach rather than a process trigger.

V. Are there other questions on NPBT that you consider important for your country?

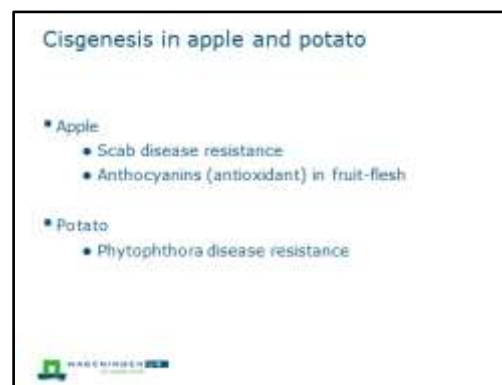
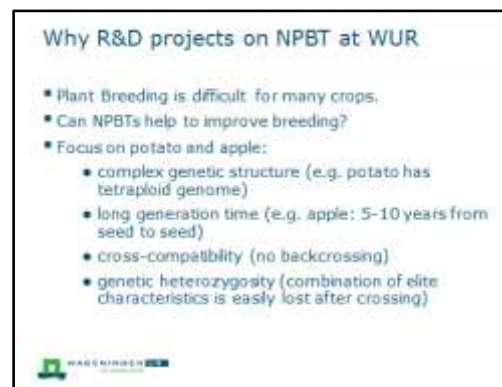
1. Legal/regulatory status of NPBT and possible differences among jurisdictions that might give rise to trade disruptions.
2. Are sufficient detection possibilities available for distinguishing plant developed by NPBT from conventional bred plants?
3. When the regulatory focus remains on the technique rather than the product, how would it be possible to anticipate new breeding techniques that might be developed in the future?
4. Animals developed with new gene techniques.

VI. What do you consider important objectives for the workshop?

1. Sharing practical experiences of countries in performing an ERA of plants developed through NPBT.
2. Different perspectives of different countries on the approach towards risk assessment of product derived from NPBT.
3. General scientific introduction on techniques.
4. What is considered NPBT in different countries?
5. Inventory of plants developed with NPBT and their status in the development pipeline.

VII. What do you consider important outcomes of workshop?

1. Increased understanding of the techniques and the plants being produced using these techniques, including understanding what are considered NPBT in different countries.
2. Increased understanding of the experience with ERA and approaches to ERA of crops developed using NPBT including the challenges, any new safety concerns or issues and rationales for doing or not doing an ERA.
3. Potential for the development of guidance on ERA and NPBT; including the application of existing principles.

ANNEX IV. SLIDES PRESENTATIONS DELIVERED AT THE WORKSHOP³**1. Wageningen UR Plant Breeding**

³ The slides presented during the workshop were not prepared with the intention that they be published. As a result, some of them are not included in the document or revised from the original.

Cisgenesis

- Introduction of extra gene(s) to existing, high quality cultivars
- Challenges:
 - Appropriate cisgenes must be available
 - It should be able to transform species and specific cultivar of interest



Availability of cisgenes, eg resistance (R-) genes

- Cisgenes:
 - Identification of genes in donor species (genetics)
 - Localization and cloning of genes (genetics, genomics)
 - Testing function of genes (transgenesis)
- Challenging task
- Availability of whole genome sequence can speed up identification of essential genes



Availability of transformation system

- Transformation of apple is difficult, laborious task
- Successful transformation of apple requires direct selection step:
 - kanamycin resistance gene + removal
 - visual selection (specific case)
- Transformation of potato is easy, routine task
- Transformation without direct selection possible:
 - screening using PCR amplification technique



Scab in apple

- Apple scab is major disease in apple
- Common culture: 20-30 sprays/season



Scab-resistance genes from wild apple species

- For apple scab resistance 3 resistance genes identified
 - *Vf*, cloned from *Malus floribunda*, already used in cisgenesis
 - *Vr2*, cloned from *M. pumila*, tested in transgenic plants, ready for cisgenesis
 - *V25*, identified, we are still busy with cloning (started in 2011)



Classical introgression of *Vf*-gene in apple

- Different sources of scab-resistances available
 - *Malus floribunda* *Vf*-gene
- Classical breeding is extremely time-consuming
 - >50 years to breed *Vf*-gene in commercial cultivar

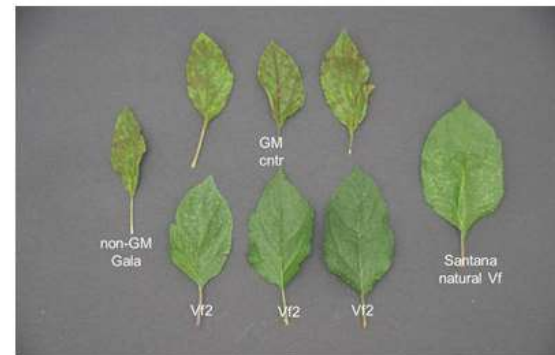


Introduction of *Vf*-gene in apple through cisgenesis

- At WUR-plant breeding: validation of *Vf* as suitable scab-resistance gene
- Introduction in Gala following transgenic approach
- Greenhouse scab-resistance test



Scab-resistance test



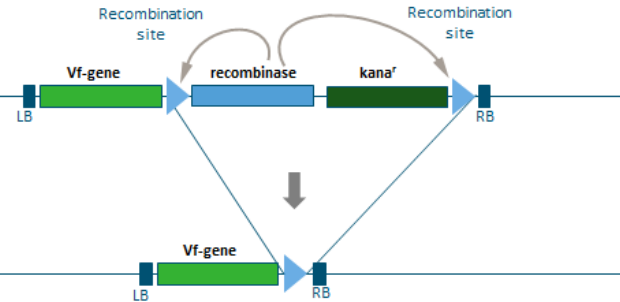
Selection marker removal for cisgenesis



- Introduction of *Vf*-cisgene in apple together with selection gene (kanamycin resistance)
- Selection gene is removed after transformation



Removal of undesired genes by recombination



Recombination Site = 81 bp from non-apple DNA origin
 LB, RB; left, right T-DNA border resp. < 20bp in cisgenic plant



Cisgenic Gala with *Vf*

- Produced in collaboration with ETH, Zurich, Swiss
- Field test at WUR



Durable resistance: gene stacking

- Next step:
 - Combining *Vf* with *Vr2* and *V25*
 - Resistance genes from different origin
 - *Vf* and *Vr2* different type of genes, different mode of action
 - *V25* to be cloned (now)



Cisgenic red-fleshed apple

- Anthocyanins are antioxidants with health beneficial properties
- Red-fleshed apple is natural phenomenon
- Current red-fleshed apples varieties have poor eating quality
- Red-flesh cause by single gene: *Myb10* (from crab apple; isolated by Plant & Food Research; New Zealand)



Introduction of *Myb10* into Gala (transgenic approach; kanamycin R)

- *Myb10* expression visible also in tissue culture
- So, *Myb10* is useful marker for selection of successful transformation events



Gala with *Myb10* (transgenic; KmR)



Grown at Plant & Food Research; New Zealand



Cisgenic Gala with Myb10 (no KmR)



WAGENINGEN UR
For quality of life


Phytophthora-resistant potato

- Similar approach as for apple scab
- Larger number of resistance genes available
- Demonstration of stacking of resistance genes for durable resistance
- Transformation without selection genes + screening using PCR (with DNA markers)

WAGENINGEN UR
For quality of life

Novel sources of *Phytophthora* resistance

▪ Screening of 1,000 *Solanum* accessions



WAGENINGEN UR
For quality of life

Different *Rpi*-genes identified in potato

- 26 *Rpi*-genes in 9 different specificity groups:
 1. *Rpi-R1*
 2. *Rpi-R2*; *-R2-like*; *-abpt*; *-blb3*; *-mcd1-1*
 3. *Rpi-R3a* and *-R3b*
 4. *Rpi-blb1*; *-sto1* and *-pta1*
 5. *Rpi-blb2*
 6. *Rpi-vnt1*; *-nrs1*
 7. *Rpi-mcq1*; *-phu1*
 8. *Rpi-chc*
 9. *Rpi-edn2*; *R9* (most recent cloned genes)

WAGENINGEN UR
For quality of life

Durable resistance

- Durable resistance can be achieved by:
 - broad spectrum R-genes
 - functional stacking of R-genes
- Three genes selected for stacking:
 - Rpi-sto1 (from *S. stoloniferum*)
 - Rpi-blb3 (from *S. bulbocastanum*)
 - Rpi-vnt1.1 (from *S. venturii*)



Triple R-genes against *Phytophthora infestans*



Cisgenesis

- Important technique for crop improvements which are difficult to achieve through conventional breeding
- EFSA Panel on GMOs concluded that similar hazards can be associated with cisgenic and conventionally bred plants



Mutation breeding is important

- From 1930–2007 more than 2540 mutagenic plant varieties have been released
- Flower & fruit colour and shape/size (chrysanthemum, apple, tomato)
- Dwarfing phenotype (wheat)
- Change in oil content (canola)
- Starch type (potato)
- etc etc.
- Also important tool in gene function analysis



Traditional mutation induction

- Ionizing radiation and chemical mutagens
- Untargeted approach: genome saturated with mutations
- Large population needed (10-20.000 individuals)
- Back-cross for fixation of mutations
- Mutation detection:
 - Originally by phenotype
 - Now by sequencing technology
- Removal of unwanted mutations by breeding



New breeding technique: site-directed mutagenesis

- ZFN, Meganuclease, TALENs, CRISPR-Cas9
- Different nucleases, similar in action:
- Allow very precise genome editing
- ZFN earliest development

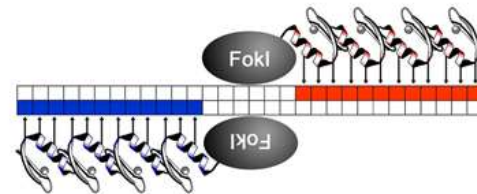


ZFN-protein



ZFN pairs

- FokI is only active as dimer
- So, ZFN are applied as pairs
- Double specificity for DNA-binding domain



What are ZFNs doing?

- ZFN-pairs induce double strand breaks (DSB) at the target site in genomic DNA

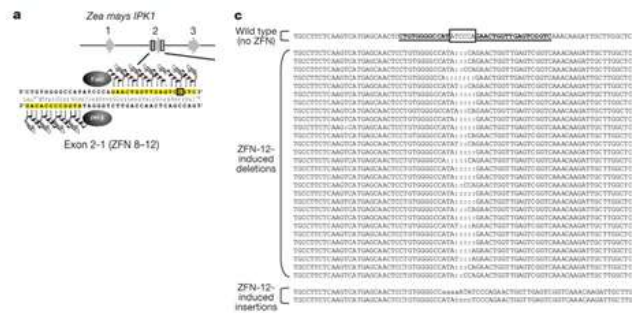


Repair of DSB

- Non-Homologous End Joining (NHEJ)
 - Joining of broken ends by ligation
 - 'Polishing' of broken ends
 - Results in small deletions, sometimes small inserts
 - Deletions/inserts in coding gene sequences can result in frame-shift mutations: Knock-out: loss of function
- Homologous Recombination (HR)
 - Precise repair
 - Requires template-DNA



Targeted mutagenesis in maize via transient expression of an ExZact ZFN and NHEJ



Shukla et al. (2009) Nature 459:437



Project: Targeted mutagenesis in potato

- Collaboration of WUR-Plant Breeding and Dow AgroSciences
- Use of Zinc Finger Nuclease (ZFN) for mutagenesis in potato
- Potato is tetraploid, each gene has 4 alleles!
- For 'loss of function' mutation, 4x alleles have to be targeted
- Research question: can ZFNs induce multi-allelic mutations in potato??




Target gene: potato *SbeII*

- Target: *SbeII*-gene: starch branching enzyme
- ZFNs designed and constructed and validated by DOW/Sangamo
- Combined with strong promoter: high expression level

>Karnico *SbeII*, exon2, ZFN-target

ZFN1
ZFN2

ATTCGAAATCCCRACCTTCTACAGTTGCAGCATCGGGGAAAG



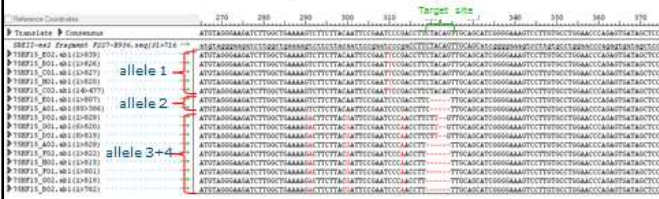
Transformation of potato cv 'Karnico'

- Stable transformation of both *SBEII*-ZFN pairs
 - Selection of ~100 putative ZFN-transformants
 - ZFN plants screened for presence of mutations



Sequence analysis of plants with ZFN

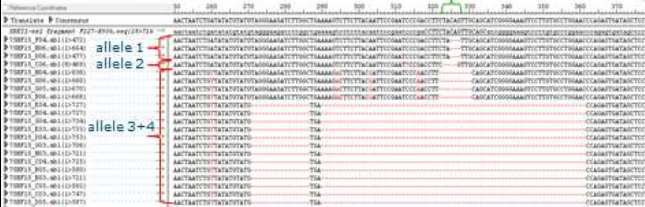
Plant #25-3: 3 alleles deleted, 5x, 2x, 6x bp deletions



Plant #18-4: 3x alleles with large deletions (49x, 145x, 141x bp deletion)



Plant #51-2: deletion in all four alleles!



Mutated ZFN-plants are still transgene

- ZFN-transgenes can be segregated out by crossing
- Final product:
 - GM-sequence not present
 - result of GM activity (deletions) remain present
 - result only detectable with prior knowledge
 - Similar as mutants obtained by 'conventional' mutagenesis, so, non-GM mutants



Recent development CRISPR-Cas9

- CRISPR-Cas9:
 - Different mechanism for DNA-recognition
 - ZFN, TALEN, protein design determines specificity
 - CRISPR-Cas9 makes use of guide-RNA-molecule
 - easy design, few days
 - planning to test at WUR-in wheat, potato, tomato and mushroom



Conclusions

- ZFN can effectively induce targeted mutations in plant genes
- Also effective deletion in polyploid plants
- May be future tool to produce agronomically important mutants!
- Development in this field are going extremely fast!



CRISPR-Cas9 for genome editing is hot

- 1st publications January 2013
- September 2013: 1500 publications, also several applications in plants
- Latest news:

Genome-Scale CRISPR-Cas9 Knockout Screening in Human Cells

Oyuklu Shafiq,^{1,2} Neville E. Sanjana,^{1,2} Ella Hartenstein,³ Xi Shi,^{1,2} David S. Scott,^{1,2} Tapan S. Bhikshani,^{1,2} Dirk Hock,^{1,2} Benjamin L. Ebert,¹ David E. Root,¹ John G. Doench,¹ Feng Zhang^{1,2} *

The simplicity of programming the CRISPR-Cas9 system to target specific genes suggests a new way to interrogate gene function on a genome-wide scale. We show that systematic delivery of a genome-wide CRISPR-Cas9 knockout library targeting 18,080 genes with 64,751 unique guide sequences enables both negative and positive selection screening in human cells. First, we used the GOSK library to identify genes essential for cell viability in control and ribonucleoside-thymine cells. Next, in a melanoma model

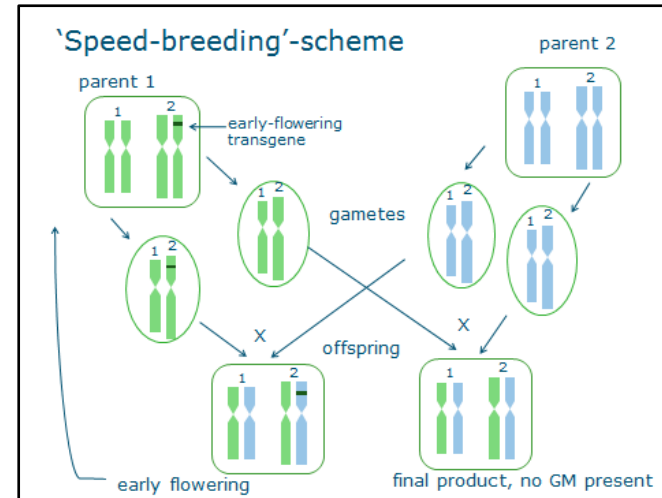
3 JANUARY 2014 VOL 343 SCIENCE www.sciencemag.org

- targeting 18,080 genes with 64,751 unique CRISPR-Cas9 guide sequences!!



Early flowering in trees

- From seed to 1st flowers: 5-10 years (juvenile phase)
- For improvement several generations needed
- Introduction of flowering-time genes: early flowering
- Allows accelerated breeding (> 1 generation/year)



Speed breeding

- Genetic modification using flowering-time genes
- GM is used to facilitate breeding, but after several breeding cycles the GM is crossed out
- Final product:
 - GM-sequence not present
 - result of GM not present
 - GM or result not detectable



2. The Business and Industry Advisory Committee to the OECD (BIAC)

BIAC
The Voice of OECD Business

**BIAC Perspective on
Environmental Risk Assessment
of products developed through
New Plant Breeding Techniques**

Gary Rudgers, PhD
Dow AgroSciences
Indianapolis, IN
OECD – NPBT Workshop
February 10, 2014

BIAC
The Voice of OECD Business

The Global Challenge

Demand Drivers

Population Growth
Nutritional Deficit
Protein Demand

Needed Result

Increased Food Production

Global Constraints

Limited Arable Land
Limited Water Supply

1950: 2.5 billion
2050: 9.3 billion

Food Needs to feed 2 billion
Food Needs to feed 9.3 billion

Food Needs to feed 5 billion

50% In 50 years, the world population will require

100% more food, and

70% of this food must come from efficiency-improving technology

U.N. Food and Agriculture Organization

BIAC
The Voice of OECD Business

Impact of Technologies

Technology helps address sustainability, food security & public health:

- Increase food production / improved nutrition
- Crops tolerant to biotic and abiotic stresses
- Reduced labor and costs

Technology helps contribute to environmental protection:

- Targeted use of crop protection products
- Reduced environmental footprint (land, water, nutrients, waste, etc...)
- Preserves and protects biodiversity

Ability to quickly respond to tomorrow's global challenges:

- Reduced product development time
- More targeted and precise breeding process

BIAC
The Voice of OECD Business

NPBT in Plant Breeding

Plant genomes are naturally variable, dynamic and repetitive...

- Humans have relied on the variability of plant genomes (mutations, rearrangements, gene additions) for millennia to develop today's agricultural crop products
- A growing understanding of biological systems over the past several decades has resulted in advancement of ever evolving breeding tools aimed to meet agricultural and food demands
- Many of these tools are providing a more precise, yet, flexible, set of techniques that allow breeders to make specific, targeted genomic improvements to plant genomes

10,000 YG Today

Drivers

NPBTs foster further innovation in plant breeding...

Although NPBTs may be considered new:

- NPBTs are innovative improvements and refinements of existing breeding methods
- Resulting products in many cases are indistinguishable/similar from existing products produced by traditional breeding techniques
- NPBTs enhance the efficiency and specificity of breeding, with more knowledge and understanding of the final product than ever before
- Adaptable to a variety of crops, including trees and vegetables, by researchers from all sectors (public and private, large and small)

"NPBT"
Snap-shot of today's current, evolving breeding tools:


1. ODM
2. SDN (-1,-2,-3)
3. Cisgenesis
4. Grafting
5. Agro-infiltration
6. RdDM
7. Reverse Breeding

Knowledge Specificity Efficiency ↑
 Time Expense ↓
 Breeding ↑
 Development ↓

Example Cisgenics

Drivers:


- Allows for rapid introduction of desirable traits between two breeding species (*sexually compatible*)
- Crop development can be reduced by decades
- Conventional breeding may result in the introduction of additional undesirable traits in final product (*linkage drag*)



Nature, 20 Aug 2013

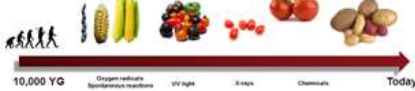
Example: Apple Scab

- Took **85 years** to conventionally breed scab resistant commercial apples
- Fungus (*Venturia inaequalis*) has overcome resistance
- Estimated with conventional breeding it will take **40 years** to breed in resistance
- Cisgenic traits can reduce the breeding process by 50% or more
- Final product does not differ in any meaningful way from existing apple varieties



Example: Mutations (ODM, SDN-1, SDN-2)

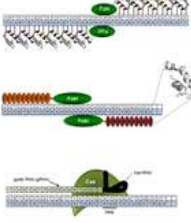
MUTAGENESIS IS FUNDAMENTAL TO EVOLUTION & CROP BREEDING-SELECTION



SITE DIRECTED NUCLEASES

1. Zinc Finger Nucleases
2. Meganucleases
3. TALENs
4. CRISPRs
5. ETC...

DNA binding and restriction proteins which can be designed to recognize a specific DNA sequence



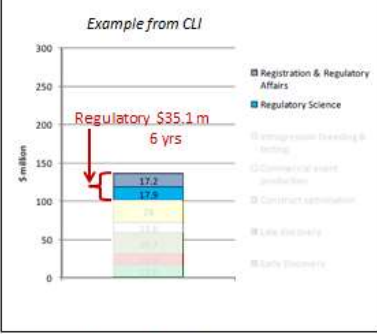
- Mutational products have a long history of safe use. Over 3,200 cultivars have been used commercially and are globally adopted
- SDNs continue the history of improving crop development through modern targeted mutational applications
- ODM/SDN-1/2 allow, for the first time, mutations to be targeted to a specific, desired location in the plant genome

Drivers: Regulation

Excessive Oversight

- Limits development to high value crops
- Asynchronous approvals
- Trading Issue
- Reduce Consumer Confidence
- Limits SME commercialization

Example from CLI



Phillips, M. Dougal. Crop Life International. The cost and time involved in the discovery, development and commercialization of a new plant biotechnology derived trait, 2013.

BIAE
The Value of OECD Business

Products under Development

Cisgenics / Intragenics:
Apple scab resistance, potato late blight resistance, drought/cold tolerant maize, fungal resistant papaya, improved forage ryegrass, a variety of vegetable crops

Grafting:
Citrus trees with transgenic rootstock

SDN (-1/-2/-3):
Improved nutritional quality maize, higher yield tomatoes, disease resistant wheat, improved nutritional quality canola, nematode resistance

ODM:
Herbicide tolerant oilseed rape, herbicide tolerant flax

COUNTRIES

Argentina	Mexico
Australia	Switzerland
Belgium	The Netherlands
Canada	United Kingdom
Ireland	United States
Japan	

CROPS

Apple	Flax
Canola	Maize
Cassava	Papaya
Cereal Grains	Ryegrass
Citrus	Tomato
	Wheat

**DEVELOPERS
USERS**

SMEs	Industry
Academics	Non-for-profit

9

BIAE
The Value of OECD Business

Perspective on ERA:

- Techniques used to develop new plant varieties **do not pose a specific safety hazard** as witnessed by the long history of safe use of plant varieties produced through human domestication and breeding.
- It is the characteristics of the plant that determines its safety**
The need to regulate/assess plants developed through NPBTs should be driven by the characteristics of the **product** rather than by the production method or process used to produce that product.
For example:
 - whether the product is materially different from existing products present in food, feed or the environment.
- Products developed through NPBTs are in many cases similar / indistinguishable to products developed through existing breeding methods
 - Products already have a long history of safe use
 - When applicable, products already have ERA in place
- As some NPBTs offer improved precision and enhanced understanding of the final products, NPBTs reduce questions around safety assessment, including the ERA of the final product

10

BIAE
The Value of OECD Business

Perspective on ERA:

Considerations for an ERA should be...

- Driven by the characteristics of the **product** rather than by the production method or process used to produce that product.
- Based on the degree to which the product is creating **new** potential safety concerns
Strong indicators for the absence of safety effects are...
 - The gene pool used in the process does not differ from that exploited in traditional breeding (sexual compatibility)
 - The product / expressed trait has a **history of familiarity**
 - Genome changes are so small that they are in the order of magnitude of what occurs naturally and in traditional breeding based on sexual compatibility (natural variability)
- Based on sound **scientific principles** (need to know vs. nice to know)
e.g. Does the resulting product raise any additional concerns compared to products produced via conventional breeding for the environment or food/feed chain?

11

BIAE
The Value of OECD Business

Conclusions

- NPBTs are innovative improvements and refinements of existing breeding methods. It is the characteristics of the plant (**product**) that determines its safety
- The public, private and scientists alike have significant opportunities to employ NPBTs in their breeding programs
- The adoption of these technologies will be highly dependent on the regulatory requirements imposed on the products produced through NPBTs.
Non-scientific, unnecessary and non-harmonious oversight / requirements will result in...
 - undue, costly burdens
 - stifle innovation
 - prevent the uptake / limit use of NPBTs
 - Disrupt trade
 - Loss of public confidence
- All governments are encouraged to adopt a globally harmonized approach towards NPBTs, and avoid unnecessary oversight of products developed through NPBTs
- Governments are encouraged to provide predictable, timely guidance regarding the oversight of any NPBT products for developers to foresee appropriate investment and commercialization.

12

3. The Netherlands




National Institute for Public Health and the Environment
Ministry of Health, Welfare and Sport

ERA of Novel Plant Breeding Techniques

Perspective of Dutch CA

Boet Glandorf
GMO Office/RIVM
The Netherlands

ERA of Novel Plant Breeding Techniques | February 10, 2014




Novel Plant Breeding Techniques (NPBT)

From a scientific perspective:

'any technique of biotechnology used in plant breeding resulting in plant(products) that do not contain any 'foreign' DNA'

2 ERA of Novel Plant Breeding Techniques | February 10, 2014




NPBT considered in NL


- ✓ Zinc finger nuclease technology (no ZFN-3)
- ✓ Oligonucleotide-directed mutagenesis
- ✓ Cisgenesis/intragenesis
- ✓ RNA-dependent DNA methylation
- ✓ Grafting (only scion)
- ✓ Reverse breeding
- ✓ Agro-infiltration (no floral dip)

and related techniques

3 ERA of Novel Plant Breeding Techniques | February 10, 2014



Products developed with NPBT in NL




At the moment:
Cisgenic potato and apple trees (disease resistant)

Expected:
Crops obtained by **reverse breeding** and **ODM** (herbicide tolerance, (a)biotic stress, reproduction traits), probably also **ZFN**

No new types of products or phenotypes expected in comparison to other plant breeding techniques in near future


4 ERA of Novel Plant Breeding Techniques | February 10, 2014



ERA required for products obtained with NPBT?

- No clarity on the regulatory status of products obtained by NPBT in EU level so far
- In NL these products are assessed under part B of Directive 2001/18/EC
- ERA is required for each field trial



5 ERA of Novel Plant Breeding Techniques | February 10, 2014



Practical experience with ERA of NPBT?

Practical experience with ERA of cisgenic crops/trees (field trials):



- Late-blight resistant potato (*Rpi* genes from *Solanum* spp.)
- Apple-scab resistant apple (*HcrV* gene from *Malus floribundi*)





6 ERA of Novel Plant Breeding Techniques | February 10, 2014



ERA to protect 'human health and the environment'





7 ERA of Novel Plant Breeding Techniques | February 10, 2014

Environmental risk analysis

<p>hazard</p> <p>invasiveness, persistence</p> <p>selective advantage, disadvantage</p> <p>Gene transfer</p> <p>Target effects</p> <p>Non-target effects</p> <p>Effects on human health</p> <p>Effects on animal health</p> <p>Effects on biogeochemical cycling</p>	<p>likelihood</p> <p>risk</p> <p>risk management</p> <p>overall risk</p> <p>=negligible</p>
---	---

ERA of Novel Plant Breeding Techniques

ERA of late blight resistant potato



- Potato (*S. tuberosum*) with *Rpi* genes from related *Solanum* spp.
- *Rpi* gene: codes for specific receptor for *Phytophthora infestans*

Many cultivated potato varieties already contain these genes through traditional breeding

- Expected: increased survival in potato fields exposed to *P. infestans*. Selective advantage in potato fields only, not in natural ecosystems
- No differences expected as a consequence of the modification on invasiveness, non-target effects, effects on human health and on the soil ecosystem

Conclusion: no environmental safety issue identified

9 ERA of Novel Plant Breeding Techniques | February 10, 2014

ERA of apple scab resistant apple


- Apple (*Malus pumila*) with *HcrV* gene from *M. floribunda*
- *HcrV* gene: codes for receptor for *Venturia inaequalis*
- Only scion is modified

HcrV gene is present in crossable species, commercial apple varieties and in natural apple populations

- Expected: increased survival in apple orchards exposed to *V. inaequalis*. Selective advantage in apple orchards only, not in natural ecosystems
- No differences expected as a consequence of the modification on invasiveness, non-target effects, effects on human health and on the soil ecosystem

Conclusion: no environmental safety issue identified

10 ERA of Novel Plant Breeding Techniques | February 10, 2014




ERA of crops obtained with NPBT

- No special issues foreseen in ERA of plant(products) obtained with other NPBT as compared to ERA of **transgenic** crops.
- No special issues foreseen in ERA of plant(products) obtained with other NPBT as compared to **conventionally** bred crops. Intragenesis?

Products of NPBT are similar to plants with a history of safe use for cultivation, like plants obtained by:

- chemical mutagenesis (ZFN, ODM)
- conventional breeding (cisgenesis)
- natural processes (RdDM)

11 ERA of Novel Plant Breeding Techniques | February 10, 2014



Current approach to ERA of transgenic or other plants is relevant for NPBT?

From a scientific perspective:

- Potential unintended effects of all breeding techniques (GM, conventional, NPBT) are comparable
- Plant products not to be assessed based on the technique used
- Environmental risk should be based on novel plant characteristics
- Only in case products from NPBT lead to novel plant characteristics (other than already obtained by conventional breeding) an ERA is relevant

12 ERA of Novel Plant Breeding Techniques | February 10, 2014

4. Japan

Is it necessary to conduct environmental safety assessment of null segregants*?

(*) Null segregants are products selected for no introduced nucleic acids (transgenes in most cases) through breeding processes.

Background

Two items related to NPBTs in Japan:

1. An example of R&D: accelerated breeding
2. Status of consideration

Research Policy Planning Division, Biotechnology Safety Office
Agriculture, Forestry and Fisheries Research Council Secretariat
Ministry of Agriculture, Forestry and Fisheries of Japan

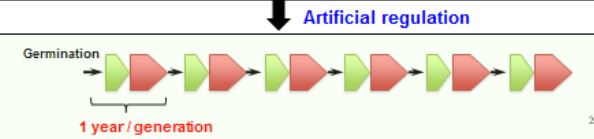
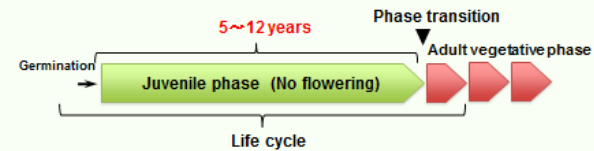
1

1. An example of R&D: accelerated breeding

Promotion of flowering and reduction of generation time in apple seedlings using Apple Latent Spherical Virus (ALSV) vector

Background

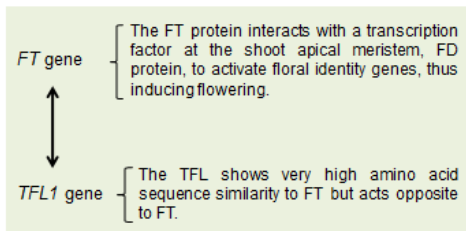
Life cycle of fruit trees (apple and pear)



2

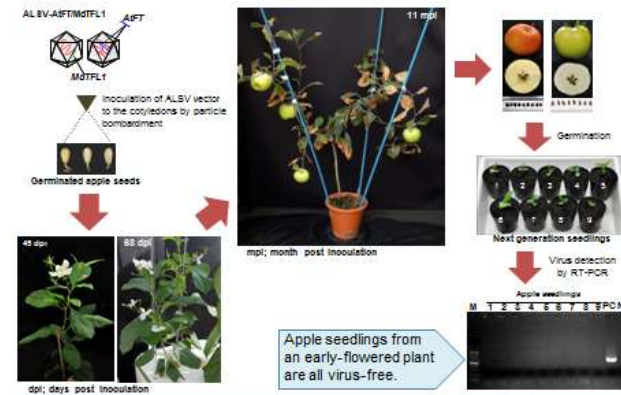
Artificial regulation of genes involved in floral development in apple by ALSV vectors

- 1) Expression of *Flowering locus T (FT)*
- 2) Knock-down of *Terminal flower 1 (MdTFL1)* gene from apple
- 3) Simultaneous expression of *Arabidopsis thaliana FT (AtFT)* and silencing of *MdTFL1*



3

Reduced generation time of apple seedlings to within a year by means of a plant virus vector



Recombinant virus-free apples (null segregants) are produced as end-products

4

2. Status of Consideration

Point of definitions for Living Modified Organisms (LMOs) according to the domestic law*:
 “Living modified organism” shall mean an organism that possesses nucleic acid, or a replicated product thereof, obtained through use of the any of the following technologies (i.e. recombinant techniques)

(* the Act on the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Living Modified Organisms (Cartagena Act)

Stance of competent authorities:
 Whether new varieties fall within the scope of the Cartagena Act is considered on a case-by-case basis

Sole judgment related to null segregants by competent authorities

It was concluded in 2013 that maize derived from Seed Production Technology (SPT) process* using GM maize (DP-32138-1) is not subject to the Cartagena Act.

(* The SPT process was developed by DuPont to enable more efficient hybrid production. In the process, GM maize is used in the intermediate steps but the end-products do not carry transgenes.

Internal Study Group in MAFF

Consisting of 7 experts familiar with environmental safety assessment and international regulatory movement on GMOs

Tentative definition of NPBTs by the Group:
 Breeding techniques in which recombinant DNA techniques are used but which can give rise to agricultural products (plants) that don't carry any introduced gene and are equivalent to those derived from conventional breeding techniques

Convenient classification of NPBTs (not necessarily used by the Group)

1. Techniques through which null segregants are finally produced
2. Site-directed mutagenesis
3. Variants of genetic transformations

Japan thinks much of the first two categories of NPBTs. They can produce end-products that are quite different from conventional GMOs.

■ Views on null segregants - 1

If transgenes exist in the products, they are clearly LMOs that need environmental safety assessment (ESA) for their environmental release in most countries. Therefore, our internal study group considers what should be discussed first in the context of NPBTs is null segregants. Because there are several techniques that produce null segregants, necessity of discussion on the topic is common to them.

The study group suggests that it is basically unnecessary to conduct ESA for null segregants, comparing the extent of environmental risks of them with that of conventionally-bred products. (It also points out that it is necessary to scientifically prove that there are no transgenes in the end-product.)

■ Views on null segregants - 2

If ESA is not needed for null segregants, it basically means they are not regarded as LMOs in case of Japan (and many countries?). Accordingly, it becomes much easier to commercialize products derived from very useful techniques.

Agricultural products derived from NPBTs could be traded internationally. Therefore, it is **desirable to promote international harmonization concerning how to consider null segregants in terms of ESA.**

9

■ Views on null segregants - 3

Relationship between null segregants and mutagenesis using site-directed nucleases (SDNs)

In regard to site-directed mutagenesis of **plant** using SDNs, it is normally **necessary to introduce their genes into plant genome once.** Therefore, discussion on null segregants is also relevant to how to consider products derived from SDN techniques.

Question concerning site-directed mutagenesis

It is appropriate to discuss a criterion on which we can consider whether or not products derived from site-directed mutagenesis are equivalent to those derived from natural mutations or conventional mutagenesis.

However, such a discussion becomes meaningful after it is confirmed that null segregants are not regarded as LMOs.

Question: is it necessary to conduct environmental safety assessment of null segregants?

Thank you

10

5. The United States

Regulation of the Products of Biotechnology in the United States


Sally McCammon
Animal and Plant Health Inspection Service
United States Department of Agriculture




Biotechnology Regulatory Services
Animal and Plant Health Inspection Service

The Coordinated Framework for Regulation of Biotechnology-1986

- The products of biotechnology **do not differ fundamentally** from unmodified organisms or from conventional products;
 - Confirmed by the National Academy of Sciences
- The **product, rather than the process**, should be regulated;
- Regulation should be **based on the end use of the product** and review science-based and conducted on a case-by-case basis; and
- **Existing laws** provide adequate authority for regulation of the products of biotechnology.
 - Broad spectrum of products that cut across many uses regulated by different agencies

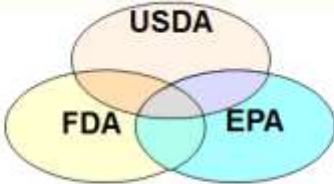



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U.S. Department of Agriculture (USDA)
Environmental Protection Agency (EPA)
HHS-Food and Drug Administration (FDA)

United States Department of Agriculture
Animal and Plant Health Inspection Service



Biotechnology Regulatory Services

Regulation Under the Coordinated Framework

- **Department of Agriculture (USDA-APHIS)**
 - **Plant Protection Act (PPA):** Protecting against damage from plant pests and noxious weeds
- **Environmental Protection Agency (EPA)**
 - **Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA):** Regulating the safe use of pesticides
 - **Federal Food Drug and Cosmetic Act (FFDCA):** Tolerance actions for pesticide residues on food and feed products
- **Food and Drug Administration (FDA)**
 - **Federal Food, Drug and Cosmetic Act (FFDCA):** Food and feed safe and wholesome
- **United States focus is on the product**
 - Taking into account the technologies used



United States Department of Agriculture



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Animal and Plant Health Inspection Service

Examples of biotech products and which agencies would regulate them

New Trait/Crop	Agency	Review
Insect resistance in food crop (Bt corn)	USDA EPA FDA	Agricultural and environmental safety Environmental, food/feed safety of pesticide Food/feed safety
Herbicide tolerance in food crop (glyphosate tolerant soybeans)	USDA (EPA) FDA	Agricultural and environmental safety (New herbicide use) Food/feed safety
Herbicide tolerance in ornamental crop (glufosinate tolerant azaleas)	USDA (EPA)	Agricultural and environmental safety (New herbicide use)
Modified oil in food crop (high oleic acid soybeans)	USDA FDA	Agricultural and environmental safety Food/feed safety
Modified flower color (blue poinsettias)	USDA	Agricultural and environmental safety

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Scope and Definitions: FDA

- FDA (1992)- regulatory status of food independent of technique depends on characteristics of food and intended use. GRAS – voluntary consultation
 - “Manufacturers have an obligation to ensure that the products they are marketing are safe and otherwise in compliance with the law.”
 - applies to food from new plant varieties generally- not just GE varieties
 - Safety or legal questions about food from new plant varieties
 - engage with FDA, even informally, prior to marketing.
 - Can facilitate a smooth market entry.

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Scope and Definitions: EPA

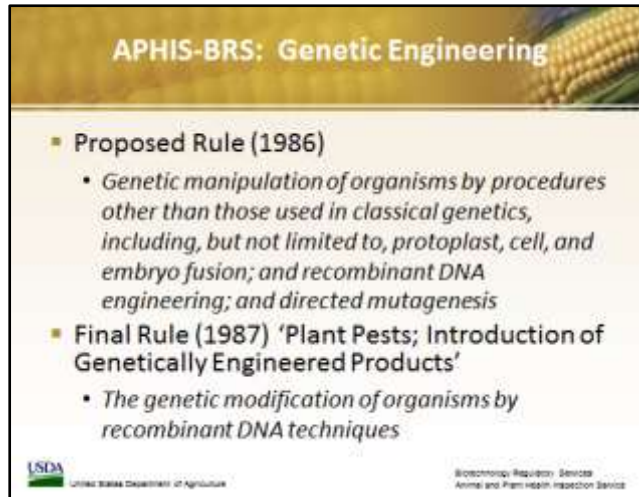
- EPA (2001)- Plant-incorporated Protectants – a pesticidal substance intended to be produced in a plant. Exception for sexually compatible plants
 - rDNA- manipulation *in vitro* using restriction enzymes or other enzymes and introduced into the plant genome

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Scope: USDA-APHIS-BRS

- “Regulated articles” (7 CFR part 340)
 - If the organism has been altered or produced through genetic engineering, **and**
 - The genetic modification of organisms by rDNA techniques,
 - Donor, recipient, or vector organism is a plant pest
 - “Plant pest” is defined by statute
 - organisms that can pose a direct or indirect risk to plants or plant products
 - Imported, moved interstate or released into the environment

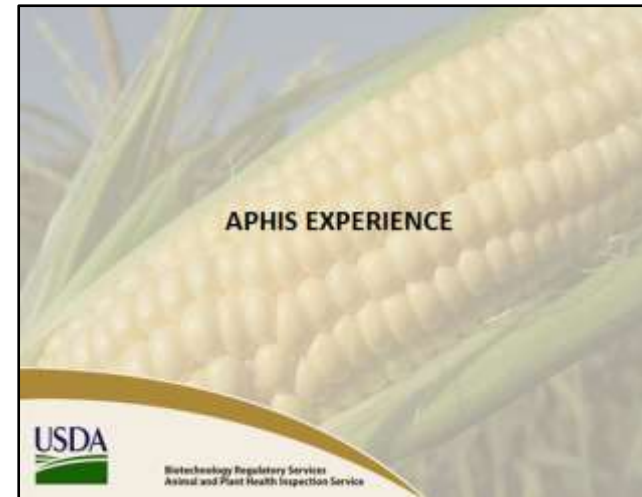
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APHIS-BRS: Genetic Engineering

- **Proposed Rule (1986)**
 - *Genetic manipulation of organisms by procedures other than those used in classical genetics, including, but not limited to, protoplast, cell, and embryo fusion; and recombinant DNA engineering; and directed mutagenesis*
- **Final Rule (1987) 'Plant Pests; Introduction of Genetically Engineered Products'**
 - *The genetic modification of organisms by recombinant DNA techniques*

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APHIS EXPERIENCE

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Am I Regulated?

- **Am I Regulated by USDA/APHIS?**
http://www.aphis.usda.gov/biotechnology/am_i_reg.shtml
- **Letters of Inquiry**
http://www.aphis.usda.gov/biotechnology/reg_loi.shtml

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Am I regulated

- **16 Letters and responses on APHIS website (July, 2011- March, 2013)**
 - Is a plant pest used in development of the organism? *Agrobacterium, CaMV*
 - Is the organism a plant pest?
 - Is the organism to be released into the environment?
 - Is the organism genetically engineered?

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Is Organism Regulated?

- Evaluate plants (intended phenotype), method of transformation, genetic material, genes, and other elements, and donors of components
 - Released into the environment
- Plant pests, unclassified organisms, or organisms whose classification is unknown used
- **Case-by-case, depends on the specific information provided**
 - Only applies to APHIS part 340 regulations
 - PPQ, EPA, FDA

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Plants

- Ornamental plants
- Baby's breath
- Tobacco
- Corn
- Apple
- Grapevine
- Pineapple
- Plum
- Grasses
 - Kentucky Blue Grass
 - Switchgrass
 - St. Augustine Grass
- Sorghum

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Inquiries from Overseas

- Costa Rica
- Israel
- Netherlands
- New Zealand

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Plants

- **Products:**
 - Whole or processed fruit – rose pineapple
 - Cut flowers – pink baby's breath
- **Evaluate**
 - Biology of plant, cannot propagate in the United States.
 - Plant is regulated based upon how created
- **May be regulated by PPQ, EPA, and/or FDA**

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Examples

- Not 'regulated articles' – no plant pest
- Glyphosate tolerant Kentucky Bluegrass
- Early flowering plum-null segregant
- Null segregant tobacco
- RNAi down regulated sorghum
- St. Augustine grass
- Switchgrass


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Cisgenics Used

- Grapevine-anthocyanin (red-berry) **NOT**
 - No different than those produced by breeding or protoplast fusion
 - All genes from grapevine; Protoplast engulfment or biolistics
 - **No plant pest sequences in creation or result;**
- Apple-scab resistant apples-**REGULATED**
 - *Venturia inaequalis* resistance
 - No foreign genes
 - **Agrobacterium tumefaciens used to transform;**


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Cases Not Regulated

- Null segregants from genetically engineered parent plants (6/6/12; 10/27/11)
- Deletion of IPK1 gene in maize using zinc finger nuclease (5/26/12)
- Targeted gene deletions using I-Cre1 meganuclease (1/6/12)
- Centromere-mediated chromosome elimination (10/27/11) - Creates double haploid plants, can manipulate paternal or maternal cytoplasm; transfer CMS traits


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Plants with Targeted Deletions

- Use of ZnF or I-Cre1 meganuclease - **NOT**
 - Naturally occurring DNA repair after targeted break
 - No genetic material inserted into genome
 - Nucleases not from plant pest, no plant pest sequences inserted.
 - No reason to believe genomic changes generated by deletion process would generate a plant pest
- Qualified
 - If plant already a plant pest, different story
 - Nuclease vectored into plant using plant pest


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Case by Case

- DNA insertion or base-pair substitutions:
 - using Zinc finger nuclease (3/8/12)
 - I-Crel meganuclease from green algae- *Chlamydomonas* (5/16/2011)


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Plants: Null Segregants


- Sorghum – RNAi down-regulate native gene
- Tobacco – early flowering – non-GE traits moved
- FasTrack plum- early flowering – reduce generation time


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Null Segregants

APHIS : evaluated breeding method and NS plant lines

- *Agrobacterium* – plant pest
 - No sequences from a plant pest remain
- Select out any transgenic material in subsequent crosses
- Verification by phenotypic and molecular analysis (lack early flowering and have normal growth habit)-no transgenic material present
- Parental lines in environment regulated


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Obama Administration

Executive Order 13563. January, 2011.
Improving Regulation and Regulatory Review

Memorandum for the Heads of Executive Departments and Agencies.
March, 2011
Principles for Regulation and Oversight of Emerging Technologies


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Principles for Regulation and Oversight of Emerging Technologies

- **March 11, 2011 Memo from**
 - John Holdren, Director-Office of Science and Technology Policy
 - Cass Sunstein, Administrator-Office of Information and Regulatory Affairs
 - Islam Siddiqui, Chief Agricultural Negotiator-US Trade Representative
- Nanotechnology, synthetic biology and genetic engineering, among others

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Regulation and Oversight should

- **Ensure the fulfillment of legitimate objectives:**
 - **Protection of**
 - **Safety**
 - **Health**
 - **Environment**
- **Avoid unjustifiably**
 - **Inhibiting innovation**
 - **Stigmatizing new technologies**
 - **Creating trade barriers**

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Benefits of regulation should justify the costs

- **Best and least burdensome tools**
- When no significant oversight issue can be identified, consider not to regulate
- Benefits and risks of new technologies should be **communicated** to the public

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6. South Africa

OECD Workshop on Environmental Risk Assessment (ERA) of Products Derived from Novel Plant Breeding Techniques (NPBT)
10 February 2014
OECD Headquarters, Paris, France

Dr Dean Oelofse
 doelofse@arc.agric.za



OECD questionnaire on environmental risk/safety assessment of plants developed with New Plant Breeding Techniques (NPBT)

- Questionnaire sent to about 20 role players in South Africa, with the Department of Science and Technology (DST) providing contact details of a number of the role players in South Africa.
- Nine provided inputs (of note being the Department of Agriculture, Forestry and Fisheries (DAFF) as the competent authority, BioSafety South Africa, Syngenta South Africa, Pioneer, the ARC Biotechnology Platform and a number of Universities).

Question I: Does South Africa consider NPBT? Which techniques does your country consider as NPBT?

- Yes, we would, but only to the extent that the product generated by an NPBT adheres to the definition of a genetically modified organism as per the GMO Act of South Africa.
- We would view the JRC list as NPBT.
- A number of NPBT are being developed at a research level, however, there is no specific guidance as to what constitutes a NPBT.

Question II: Is South Africa seeing any plants developed with NPBT in the private or public sector (industry and/or academia)?

- No application has been received under the GMO Act which involves NPBT. We do not have access to other non-regulated research developments (which may include the use of NPBT).
- No imminent applications that we are aware of as most activities are early stage research and development work.
- University of Stellenbosch: cisgenic sugarcane with higher sucrose through targeted gene silencing.
- Pioneer: Possibly yes, but not aware of the specific products. Companies do not usually reveal this information until they get to the field trial stage (then public notices are required). For all lab and greenhouse trials, public notices are not a legal requirement so it's hard to tell as to who is doing what in the lab and greenhouse.
- Nothing from Syngenta RSA.

Question III: Does South Africa have any practical experience in performing an environmental risk/safety assessment on plants developed with NPBT?

- *No*
- The regulatory procedure which must be followed will depend on a large degree on whether the NPBT is classified as a GMO or not. This definition has not changed since the GMO Act was first passed. As NPBT have been developed since the passing of the act they are likely to pose challenges to the regulators and an uncertain regulatory environment for developers, as the regulatory requirements are much higher if classified as GMO.



Question III: Does South Africa have any practical experience in performing an environmental risk/safety assessment on plants developed with NPBT?

- The GMO Act has been drafted in a way that enables the Executive Council (EC) to implement a case-by-case risk assessment approach for new technologies. The national approach for NPBT has not been publicly announced, but the EC is expected to deal with this on a case-by-case basis as they are empowered to so by law.
- No environmental risk/safety assessments for plants developed with NPBT been made available to the public because none done to date, thus no relevant reports, guidance documents and links available.



Do you expect plants developed using NPBT will give rise to new issues in environmental risk/safety assessment and, if so, what are those issues?

- *It may be possible, in SA we apply a case-by-case approach implying that not all NPBT would give rise to the same concern.*
- No new issues, except that the regulatory hurdles should be lowered, if not removed.
- The current environmental risk assessment process being followed in the country will be able to cover NPBT. Furthermore, the Executive Council has the authority to request addition risk assessment data if required.



Question IV: Have the public or private sector (academia and/or industry) provided their perspective regarding environmental risk/safety assessment of plants developed with NPBT?

- *No*
- No formal engagement focussing on NPBT has occurred between academia/ industry and regulators that we are aware of.
- Nothing from Syngenta RSA.



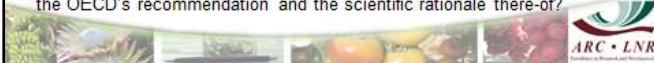
Question V: Are there other questions on NPBTs you consider to be of importance in South Africa?

- *No, we're still assessing the potential implications of NPBT on our current Risk Assessment approach.*
- Regulatory uncertainty and potentially prohibitive costs for risk assessment if crops made from these techniques are classified as GMOs may restrict their development.
- It would be of prime importance if regulatory hurdles, which make it impossible to implement beneficial technologies for the poor, could be removed or 'weakened' to a meaningful extent.
- Increased food production via crop improvement is very important for South Africa and the rest of the African continent, however, we still need to pay equal attention to issues related to food safety.



Question V: Are there other questions on NPBTs you consider to be of importance in South Africa?

- The most important question is whether the NPBT are to be considered for deregulation under existing GMO laws and regulations.
- The risk is that a position is taken that the GMO Act should be extended to cover NPBT, and then the logical extension would be to cover products of any other technique that introduced mutations (chemical or radiation induced mutations for example) and then the further extension would be that all new varieties from conventional breeding or genomic selection programs would be subject to environmental risk assessments, which would then completely inhibit all innovation in crops!
- Pioneer: What is the US/Canada, Argentina and Brazil's approach? What is the OECD's recommendation and the scientific rationale there-of?



Question V: Are there other questions on NPBTs you consider to be of importance in South Africa?

- It would be very undesirable to extend the regulatory space, but at the same time, there needs to be a reasonable process in terms of decisions about what risk assessment is required or desirable.
- Also it is important that we do not get a moratorium on the implementation of NPBT, it would be better to consider them under existing regulation, until there are developments in the legal environment
- It may also be that some of the NPBT are less contentious than others, for example single nucleotide changes introduced by oligonucleotide directed mutagenesis (ODM) would be a lot simpler to deal with than say epigenetic manipulations.



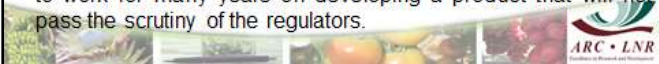
***Question VI: What do you consider to be important objectives and outcomes for the OECD workshop?
Are there NPBT that are of particular interest to South Africa?***

- This is an ideal opportunity to discuss the appropriateness of regulations based on technologies, e.g. genetic modification, vs. regulations based on novel traits or trait combinations, irrespective of how these were introduced. The fact remains that the "unit of risk" is the novel living organism (the product) and not the technology through which it was established. In other words, it is NOT the NPBT that should be regulated per se, but rather their products if they are novel. In this case the definition of "novel" would be crucial. It will also result in less complicated regulations and risk assessment frameworks and ultimately a more efficient and effective regulatory environment.



***Question VI: What do you consider to be important objectives and outcomes for the OECD workshop?
Are there NPBT that are of particular interest to South Africa?***

- All of the emerging NPBT are important for Africa and should be utilized to develop low-input/high yielding crops.
- An important outcome would be to produce a guidance document for the environmental risk assessment of NPBT (similar to the OECD Blue Book for biotech crops).
- Document detailed enough to be informative to specialists, as well as simple enough to be understood by consumers and the rest of the society. Scientists need to be trained and empowered so that they can approach their scientific experiments with the end user in mind. After all, no one wants to work for many years on developing a product that will not pass the scrutiny of the regulators.



***Question VI: What do you consider to be important objectives and outcomes for the OECD workshop?
Are there NPBT that are of particular interest to South Africa?***

- Consideration needs to be given to whether the regulation should be around the technology, or the phenotypic changes that are introduced. For example, we have both transgenic and mutation based tolerance to herbicides; but the regulation and risk assessment of the GM version is not the same as for the non-GM version, although they are exactly the same phenotypic trait with the same risks.




***Question VI: What do you consider to be important objectives and outcomes for the OECD workshop?
Are there NPBT that are of particular interest to South Africa?***

- Pioneer: How do we avoid increasing entrance barriers for NBT's (especially for public institutions and small start-up Biotech companies that may have limited budgets to conduct comprehensive environmental risk assessments?)
- Is it possible to consider benefits as well, and not only assess the risks so that there is a balanced outcome of the process?



7. Australia

Australian Government
Department of Health
Office of the Gene Technology Regulator




Perspectives from different regulators - Australia

OECD workshop on ERA of products
derived from NPBT

10 February 2014

Dr Heidi Mitchell
Office of the Gene Technology Regulator
Australia

Australian Government
Department of Health
Office of the Gene Technology Regulator



Conclusion

The existing environmental risk
assessment (ERA) protocols are
sufficient for ERA of plants developed
using new technologies

No NPBT category in Australian legislation

Australian Government
Department of Health
Office of the Gene Technology Regulator




Regulation of GMOs in Australia



From laboratory work through to field release

5/18

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Department of Health
Office of the Gene Technology Regulator



Environmental releases of GMOs

Licences issued for 89 field trials, 15 commercial releases

- Most licences have been issued for plant GMOs
- 2 vaccines have been authorised by Therapeutic Goods Administration and OGTR for release against Japanese encephalitis and cholera

All require a detailed Environmental Risk Assessment involving extensive consultation

Australian Government
Department of Health
Office of the Gene Technology Regulator



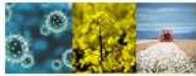
DIR 082/2007

Field trial of GM pasture grass with improved forage digestibility

-> includes 'intragenic' lines

Further details at www.ogtr.health.gov.au

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Department of Health
Office of the Gene Technology Regulator




Risk context


Parent organisms:
perennial ryegrass (*Lolium perenne* L.)
tall fescue (*Lolium arundinaceum* Darbysh. Schreb.)

Location: 1 site in Hamilton, VIC
Size: 800 m²
Duration: 2008 –2010 (since extended)

Aim: Conduct proof of concept experiments using fructan and lignin metabolism genes



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Office of the Gene Technology Regulator



Genetic modifications

Fructan
3 constructs → *L. perenne*

Lignin
3 constructs → *L. arundinaceum*


Cassettes introduced by biolistics so no vector sequences

28 other constructs which are 'transgenic'

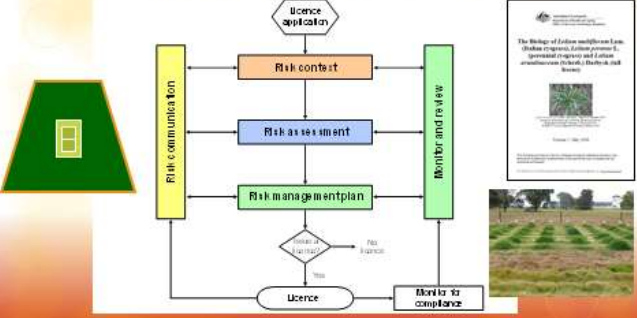
All GM plants also contain selectable marker

Some constructs are intended to cause down-regulation



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Office of the Gene Technology Regulator

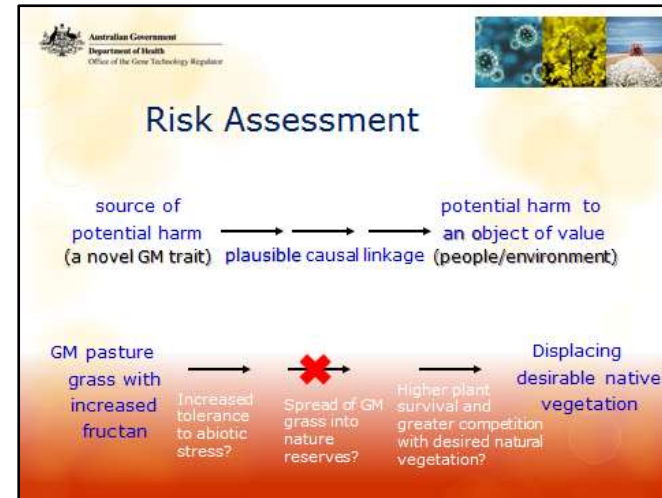
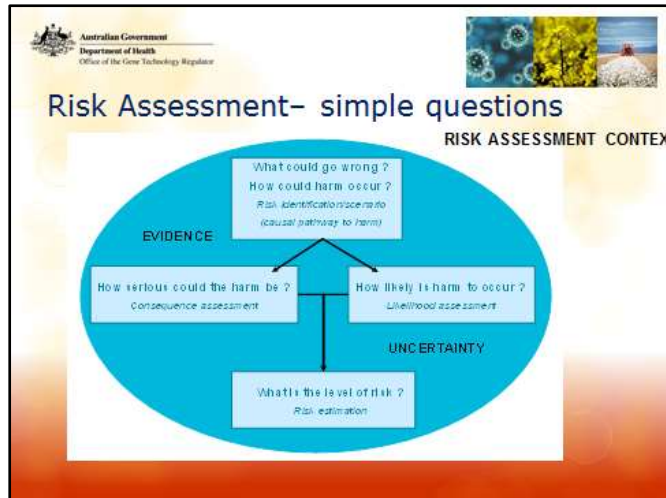


Components of risk analysis



Adapted from ISO standard 31000 on risk management



- Australian Government
Department of Health
Office of the Gene Technology Regulator
- ### Risk management
- No risks identified which require management
 - No additional risks for intragenic plants
 - Field trial licence conditions to maintain context

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Department of Health
Office of the Gene Technology Regulator

ERA - identifying and applying best practice

RISK ANALYSIS FRAMEWORK
www.ogtr.gov.au

NATIONAL POST-BORDER WEED RISK MANAGEMENT PROTOCOL

A collage of three documents related to risk management: 'Risk Analysis Framework 2013', 'ISO 31000 RISK MANAGEMENT', and 'National Post-Border Weed Risk Management Protocol'. The 'Risk Analysis Framework 2013' cover shows a globe and a plant. The 'ISO 31000 RISK MANAGEMENT' cover shows a maze with a person walking through it. The 'National Post-Border Weed Risk Management Protocol' cover shows a document with a 'RISK' stamp.

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Improving risk assessment of GM plants

Incorporation of the Australian Post Border Weed Risk Management Protocol

Australian weeds

Eg.
Kudzu vine
Prickly pear
Patterson's curse

experience
→
knowledge




GM plants

Eg.
Cotton
Canola
carnation

Keese et al 2013 Transgenic Research

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Environmental risk assessment of GM plants – applying WRA methods

Approach

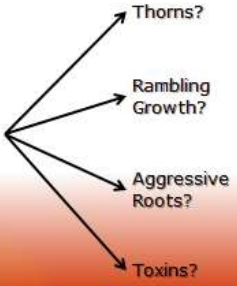
1. Do a weed risk assessment of the parent organism
2. Check if gene technology/new trait identifies increased potential risk according to weed risk assessment criteria
3. Do a more detailed assessment of identified risks (if any)

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Examples of possible phenotypes

tall fescue



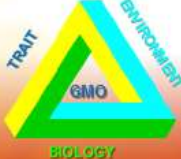

GM tall fescue
Super-weed?

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Summary

- A GM plant is a plant
- Plants developed using NPBTs are still plants
- Phenotype is most important
- Able to identify weediness/invasiveness traits
- Weediness/invasiveness traits encompass all undesirable effects



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8. European Food Safety Authority (EFSA)

efsa
European Food Safety Authority

EFSA opinions addressing the safety assessment of plants developed through (1) cisgenesis and intragenesis and (2) zinc finger nuclease-3 (ZFN-3)

Andrea Gennaro
scientific officer

OECD WORKSHOP ON ERA OF PRODUCTS DERIVED FROM NPBT
Paris, 10 February 2014

efsa
European Food Safety Authority

EC NTWG identified 8 NPBT

8 techs proposed by EC (December 2011)

Diagram illustrating 8 biotech methods: Agrobacterium-mediated transformation, Gene Gun-mediated delivery, Chloroplast transformation, CRISPR/Cas9, Zinc Finger Nucleases (ZFNs), RNA interference (RNAi), CRISPR/Cas9 (continued), and Gene editing (continued).

The views expressed in this report are those of an expert working group and do not necessarily represent those of the European Commission or the European Authority. Only the European Council of Ministers can give a binding opinion on EU law.

New Techniques Working Group
EFSA REPORT

efsa
European Food Safety Authority

EFSA GMO Panel opinions on NPBT

efsa
European Food Safety Authority

Scientific opinion addressing the safety assessment of plants developed through cisgenesis and intragenesis¹

EFSA Panel on Genetically Modified Organisms (GMO)²

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The European Commission requested the EFSA Panel on Genetically Modified Organisms (GMO) to provide a scientific opinion on the safety assessment of plants developed through cisgenesis and intragenesis. The EFSA GMO Panel considered the safety of plants developed through cisgenesis and intragenesis. The EFSA GMO Panel concluded that plants developed through cisgenesis and intragenesis are safe for human consumption and the environment. The EFSA GMO Panel also concluded that plants developed through cisgenesis and intragenesis are safe for the environment and the health of the consumer.

KEY WORDS

Cisgenesis, intragenesis, safety assessment, GM plants

1 Opinion on cisgenesis and intragenesis adopted in the 71st plenary (January 2012)

<http://www.efsa.europa.eu/en/efsajournal/doc/29561.pdf>

2 EFSA GMO Panel opinions on NPBT

ABSTRACT

The European Commission requested the EFSA Panel on Genetically Modified Organisms (GMO) to provide a scientific opinion on the safety assessment of plants developed through zinc finger nucleases (ZFNs) and other site-directed nucleases (SDNs). The EFSA GMO Panel considered the safety of plants developed through ZFNs and SDNs. The EFSA GMO Panel concluded that plants developed through ZFNs and SDNs are safe for human consumption and the environment. The EFSA GMO Panel also concluded that plants developed through ZFNs and SDNs are safe for the environment and the health of the consumer.

KEY WORDS

Zinc finger nucleases (ZFNs), site-directed nucleases (SDNs), safety assessment, GM plants


1 Opinion on Zinc Finger Nucleases (ZFNs) adopted in the 75th plenary (October 2012)

<http://www.efsa.europa.eu/en/efsajournal/doc/29433.pdf>

efsa
European Food Safety Authority

Outline – EFSAGMO Panel opinions

Cis-Intra	ZFN-3
1. Background	1. Background
2. Technical description	2. Technical description
3. Conventional breeding	3. Conventional breeding
4. Hazards	4. Hazards
5. Applicability	5. Applicability

EC Mandate 

1. Trigger:

- EC mandate (February 2011)


2. General objectives:


- To assess the adequacy of **EFSA guidelines** to perform a risk assessment of plants developed through 8 techniques.
- Guidance for risk assessment of food and feed from genetically modified plants (EFSA, 2011)
<http://www.efsa.europa.eu/en/efsajournal/doc/2150.pdf>
- Guidance on the environmental risk assessment of genetically modified plants (EFSA, 2010)
<http://www.efsa.europa.eu/en/efsajournal/doc/1879.pdf>

FF guidance (EFSA, 2011) 



Molecular Characterisation Comparative assessment Toxicological assessment Allergenicity assessment Nutritional assessment

ERA approach (EFSA, 2010) 



Problem formulation Hazard characterisation Exposure characterisation Risk characterisation Risk management strategies Overall risk evaluation

Persistence & invasiveness, VGT
HGT
Interaction with TO
Interaction with NTO
Crop management
Biogeochemical processes
Human and animal health

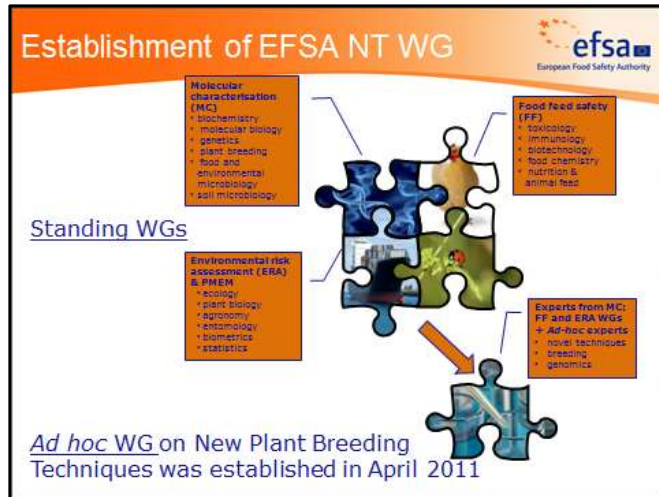
EC Mandate: 2 questions 

3. Specific objectives:

Q1. Determine whether there is a need for new guidance or whether the existing **guidance** on risk assessment should be updated or further elaborated, in anticipation of the placing of products on the market through the application of the listed techniques.

Q2. What are the **risks** in terms of impact on humans, animals and the environment that the techniques could pose?

- compare plants obtained by these new techniques with plants obtained by **conventional plant breeding techniques** and secondly with plants obtained with currently used **genetic modification techniques**.



The EFSA New Techniques Working Group

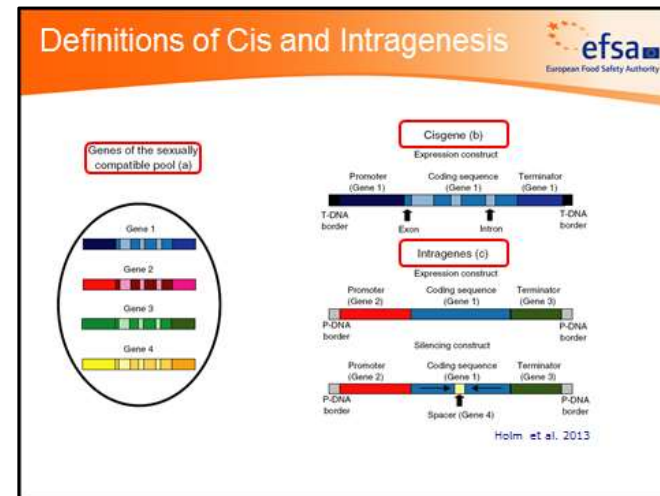
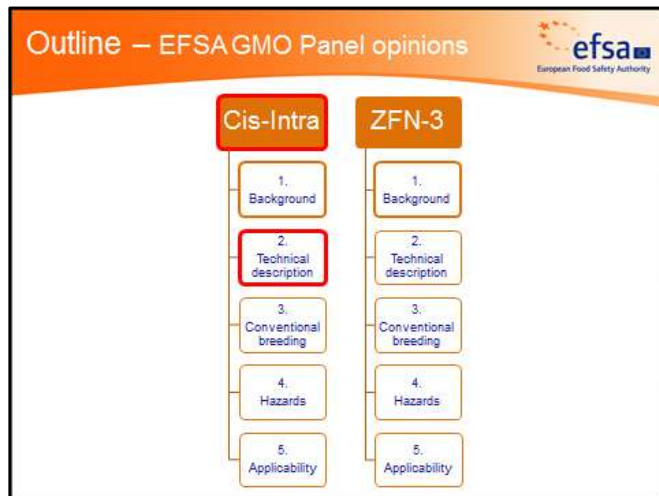
Experts:

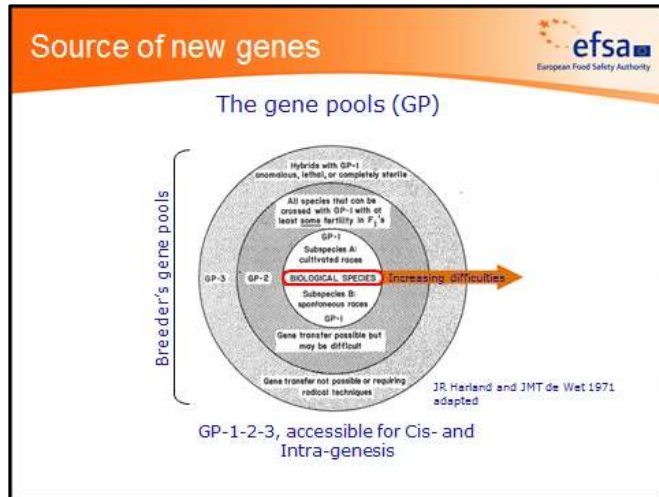
- John BRADSHAW
- Josep CASACUBERTA
- Andrew CHESSON^a
- Howard DAVIES (Chair)
- Huw JONES^a
- Frank HARTUNG
- Gijs KLETER
- Harry KUIPER
- Sirpa KÄRENLAMPI
- Michele MORGANTE^b
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- Yann DEVOS^a
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- Nancy PODEVIN

^a Cisgenesis and Intragenesis only
^b Zinc Finger Nuclease 3 only
^c Hearing expert





- ### Source of new genes
- **Cisgene inserted**
 - Same gene could be bred into the commercial varieties from primary, secondary or tertiary gene pool (breeder's gene pools)
 - Similar hazards related to cisgene
 - No linkage drag in cisgenesis
 - **Intragenes inserted**
 - Intragenesis and transgenesis offers more possibilities

Outline – EFSA GMO Panel opinions

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Relevant components of conventional plant breeding

EXISTING genetic variation

Sexual crosses
Bridge crosses
Embryo rescue
Somatic hybridisation
Translocation breeding

NEWLY CREATED genetic variation (Mutation breeding)

Spontaneous mutations

ATCG → ATCG
TACG → TACG

Induced mutations
 Chemical & physical mutagenesis

Somaclonal variation

Alterations to the genome (2/3)



Changes in the genome induced by the insert

- Novel DNA is integrated using plant DNA repair mechanism
 - Random integration site
 - Also the case for translocation breeding
 - Disruption/deletion/rearrangement of endogenous genes and regulatory sequences possible
 - Similar effects due to movement of transposons, translocation breeding
 - Creation of novel open reading frames at the junction
 - Definition used: from stop-to-stop
 - Any introduction of sequences will create ORFs
 - » Also with movement of transposons, translocation breeding
 - » During transgenesis foreign DNA might lead to ORFs which could not be created using conventional breeding/cisgenesis

Alterations to the genome (3/3)



Changes in the genome not linked to insert

- Somaclonal variation
 - Has been exploited by plant breeders for crop improvement
 - DNA changes
 - Single-base pair changes, or very small insertions/deletions
 - Chromosomal rearrangements and changes in chromosome number
 - Variation in DNA methylation



Ilavský et al. 2008. Somaclonal variation in *Nicotiana glauca* regenerated from 1-year-old embryogenic callus cultures

Presence of non-plant sequences

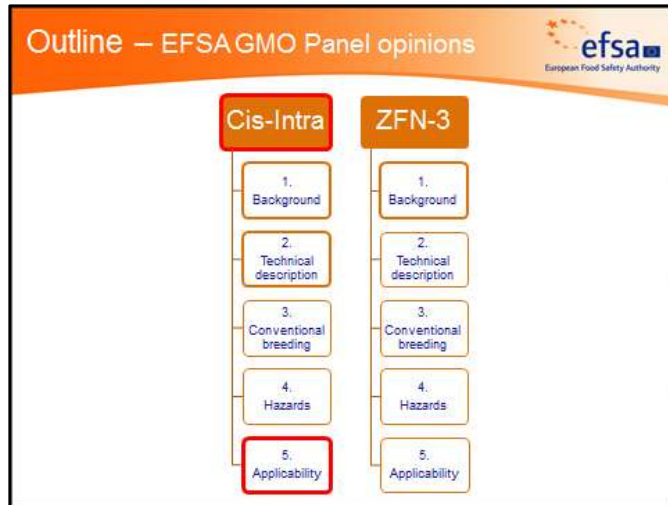


- No vector backbone can be present in cisgenic plants
 - Not relevant for conventional breeding
 - Can be present in transgenic plants
- Selectable markers can only be from breeders' gene pool
 - Otherwise cannot be present in cisgenic plant
- Small remnants of the transformation vector
 - Evaluation of T-DNA border repeat (22 bp) has been done
 - Similar sequences present in plants
 - Similar short sequences can be created via insertion of filler DNA
 - » Statistical probability Lusser et al., 2011
 - Max of 8 AA

Modification of gene expression



- Full promoter
 - Impact on surrounding genes
 - Similar effects by transposon integration
 - Altered expression of cisgene
 - Position effect
 - Altered expression of cisgene
- For cisgenic plants similar levels as in donor might occur**
- Expression might fall outside known expression range
 - Expression also differs in traditional breeding when moving the same gene into different genetic backgrounds
 - commercial cultivars differ extremely in the expression of individual genes in the gene pool


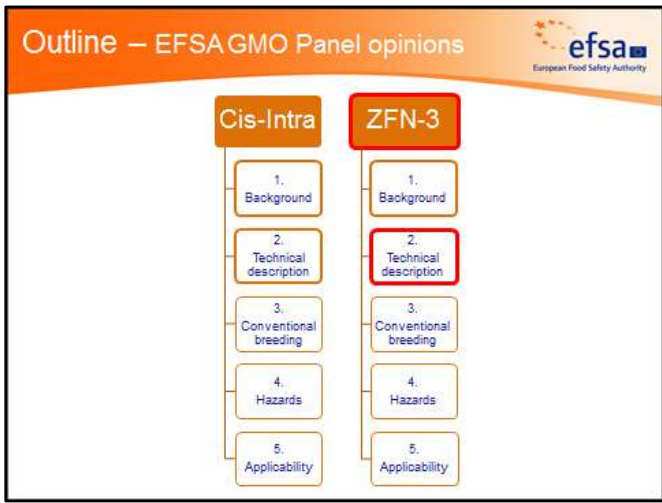



Conclusions on Cisgenesis & Intragenesis (1/2)

- The EFSA GMO Panel considers that the Guidance documents are applicable for the evaluation of food and feed products derived from cisgenic and intragenic plants and for performing an environmental risk assessment and do not need to be further developed.
- It can be envisaged that on a case-by-case basis lesser amount of event-specific data are needed for the risk assessment.

Conclusions on Cisgenesis & Intragenesis (2/2)

- The Panel concludes that similar hazards can be associated with cisgenic and conventionally bred plants, while novel hazards can be associated with intragenic and transgenic plants.





SDN-3 applications (1/2) 

*Insertion of DNA stretches similar to **transgenesis***

The acquire sequence can:


- 1) code for a new protein or to an new isoform (gene insertion)
- 2) its transcriptional product can interfere with a specific target (RNAi)

SDN-3 applications (2/2) 


*Insertion of DNA stretches similar to **cisgenesis or intragenesis***

- 3) a coding sequence (allele) can be replaced with a different one (allele replacement)
- 4) a regulatory region can be replaced to enhance or repress a specific gene product

In both cases the integrated sequences are present in the primary gene pool of the recipient or in a closely related species.

Outline – EFSA GMO Panel opinions 

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Hazard identification



When considering hazards related SDN-3 plants the major considerations by the EFSA GMO Panel include:

1. the source of the DNA and the safety of gene products
2. alterations to the host genome at the insertion site and elsewhere
 - Alteration at the insertion site
As DNA is introduced into an exact, pre-defined location in the plant genome during SDN-3, unintended effects can be minimised
 - Alteration elsewhere in the genome
Due to off-target activity or somaclonal variation
3. the potential presence of non-plant sequences in the insert
4. the expression of the trait and its potential wider implications

Outline – EFSA GMO Panel opinions



Cis-Intra

1. Background
2. Technical description
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5. Applicability

ZFN-3

1. Background
2. Technical description
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4. Hazards
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Applicability of the guidances



The EFSA GMO Panel is of the opinion that the two [EFSA GMO Panel guidance documents for GM plants cover all of the elements](#) and approaches that might be required to risk assess plants developed using SDN-3 approaches.

On a case-by-case basis lesser amounts of data are needed

- **Examples**
 - Where SDN-3 is used for cisgenesis
 - Integration of a well known transgene (e.g. Cry1Ab) into a position in the genome that has been used previously (without indication of unintended effects)