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Comparative regulatory approaches for new plant breeding techniques

Workshop Proceedings

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Abbreviations

CFIA	Canadian Food Inspection Agency
DG AGRI	Directorate General for Agriculture and Rural Development
DG SANCO	Directorate General for Health and Consumer Protection
DG RTD	Directorate General for Research and Innovation
DNA	Deoxyribonucleic acid
DSB	Double Strand Break
EC	European Commission
EFSA	European Food Safety Authority
EU	European Union
GM	Genetically Modified
GMO	Genetically Modified Organism
IHCP	Institute for Health and Consumer Protection
IPTS	Institute for Prospective Technological Studies
JRC	Joint Research Centre
NPBT	New Plant Breeding Technique
NTWG	New Techniques Working Group
ODM	Oligonucleotide-Directed Mutagenesis
ORF	Open Reading Frame
PNT	Plant with Novel Trait
RdDM	RNA-dependent DNA Methylation
RNA	Ribonucleic Acid
ZFN	Zinc Finger Nuclease

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1 Introduction

Recombinant DNA (deoxyribonucleic acid) techniques have been used in plant breeding since the 1980s. In many countries the existing legislation was not regarded as sufficient to regulate transgenic crops (and other genetically modified products) and so new legislation on biotechnology and genetically modified organisms (GMOs) was introduced in the 1980s and 1990s. It generally provides for an authorisation process for experimental and commercial release, import and marketing and use of these new crops including a comprehensive risk assessment.

Since the 1980s, many new plant breeding techniques (NPBTs) have been developed. Many of these new approaches deploy biotechnology. Although the applied methodology and changes achieved in the genome of the crops differ from earlier transgenic approaches the question still arises (in countries where GMOs are regulated under specific legislation) as to whether crops derived by these techniques should be classified as GMOs.

2 The report

In 2010 the IPTS together with the Institute for Health and Consumer Protection (IHCP), another institute of the JRC, conducted a study on "New plant breeding techniques: State-of-the-art and prospects for commercial development"[1]. As a follow-up to the 2010 study and as part of the 2011 IPTS work programme, it was decided to organise an international workshop to discuss the regulatory approaches for NPBTs in different countries worldwide. This report provides a summary and evaluation of the presentations and discussions from the workshop.

Chapter 3 of this report presents the participants in the workshop and discusses the geographic coverage. A short overview of the regulatory framework for biotechnology derived crops in six countries, mainly focusing on the legislation and GMO definitions, is provided in chapter 4. Chapter 5 presents the approaches for NPBTs in the six countries, and chapter 6 provides a summary of considerations and decisions for specific groups of NPBTs.

[1] Lusser, M., Parisi, C., Plan, D. & Rodríguez-Cerezo, E. New plant breeding techniques. State-of-the-art and prospects for commercial development. JRC Technical Report EUR 24760 EN. *European Commission. Joint Research Centre* (2011). ftp://ftp.jrc.es/pub/EURdoc/JRC63971.pdf

3 Participants in the workshop and geographical coverage

The workshop brought together experts from seven countries. The names and affiliations of all participants are listed in Annex 1 of this report. The European Union (EU) was represented by staff of the European Commission (EC), from the JRC, the Directorate General for Health and Consumers (DG SANCO), the Directorate General for Agriculture and Rural Development (DG AGRI), the Directorate General for Research and Innovation (DG RTD) and the European Food Safety Authority (EFSA).

Experts from six further countries were invited: Argentina, Australia, Canada, Japan, the USA and South Africa. Argentina was represented by two experts from the Ministry of Agriculture who are involved in the regulatory process of GMOs in their country. The Australian participants came from the Office of the Gene Technology Regulator and academia (La Trobe University, Victoria). Canada was represented by one staff member from the Plant Biosafety Office of the Canadian Food Inspection Agency (CFIA) and one from the Canadian representation to the EU. The Japanese experts work for the National Food Research Institute and Tsukuba University respectively and are both involved in the risk assessment of GMOs. The participant from the USA came from academia and explicitly stressed that he did not represent the US regulator. South Africa was represented by one member of staff from the Department of Environmental Affairs and Tourism. A further invited expert from the Department of Agriculture, Forestry and Fisheries was not able to participate in the end but contributed to the preparation of the presentation from South Africa.

Presentations and discussions covered the regulatory approaches for crops derived through biotechnology for all represented countries with the exception of the USA. Experience with the regulation of crops derived by new plant breeding techniques is very limited in a few countries and discussions are only just starting in the other countries. The information provided in this report represents current views and therefore in many cases is provisional or indicative.

4 Regulatory framework for biotechnology derived crops

When recombinant DNA techniques were adopted by plant breeders and the first GM plants reached the stage of cultivation (in the 1980s and 1990s), countries decided on different legal approaches for the regulation of the cultivation and marketing of these crops. While a few countries like the USA and Canada used existing legislation to regulate crops derived by the recombinant DNA technique, many other countries introduced specific GMO legislation.

Participants in the workshop provided comprehensive presentations on the regulatory approaches in the represented countries (with the exception of the USA). The presentations can be accessed through the following link: **http://ipts.jrc.ec.europa.eu/presentations/NPBT.cfm**. The paragraphs below provide brief introductions to the six systems of the countries presented in the workshop.

4.1 Argentina

Argentina introduced a regulatory system for GM crops in 1991. The national legislation relevant for agricultural biotechnology is compiled in the "Marco Regulatorio de la Biotecnología Agropecuaria en la República Argentina"[2]. New biotechnology regulations have recently been adopted in Argentina[3,4]. For the commercial authorisation to cultivate GM crops and/or place them on the market, three favourable reports on (i) biosafety of the agro-ecosystem, (ii) food and feed safety and (iii) impact on trade and production, are required.

Argentina uses two complementary criteria when defining GMOs, (i) the definition of products of "modern biotechnology" as used in the Cartagena Protocol and (ii) the definition of "event" in the Argentinean legislation (for the definitions see Annex 3). In the case of ambiguity, the definition of "event" is decisive. Labelling is not mandatory for foods derived from GM crops.

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[2] http://www.grupobiotecnologia.com.ar/comercio65/html/458423MarcoRegulatorioArgentino.pdf

[3] Resolution N° 763/2011 (Ministry of Agriculture, Livestock and Fisheries, MAGyP). *Commercial approval*.

Resolution No. 701/2011 (Secretariat of Agriculture, Livestock and Fisheries, SAGyP). *Field trials and full dossier review*

Resolution N° 510/2011 (SAGyP). *Economic impact*.

http://www.minagri.gob.ar/site/agricultura/biotecnologia/55-OGM_COMERCIALES/index.php

[4] Resolution No. 661/2011 (SAGyP): *Production of regulated seed.*

http://www.minagri.gob.ar/site/agricultura/biotecnologia/60-SOLICITUDES/___producciones/index.php

4.2 Australia

In Australia, the Gene Technology Act 2000 and the Gene Technology Regulations 2001 were introduced to cover the issues specific to GMOs which had not been already addressed by existing laws. Depending on the use of the product, the biotechnology legislation is applied in conjunction with other legislation such as the Australia New Zealand Food Standards Code and the Agricultural and Veterinary Chemicals Code Act (e.g. for herbicide tolerant or insect resistant crops). As in most countries, risk assessments (environmental and health) and authorisation are required for the cultivation of GMOs and their use as food.

The Gene Technology Act 2000 includes the definition of GMOs (for the definition see Annex 3). The Gene Technology Regulations 2001 lists techniques which are not classified as gene technology (Schedule 1A) and organisms that are not GMOs (Schedule 1). Labelling of GM food is mandatory under the Australia New Zealand Food Standards Code when novel DNA or a novel protein from an approved GM variety is present in the final product.

4.3 Canada

The Canadian Regulatory Framework for Biotechnology (1993) established the principles that apply to products of biotechnology. It was decided to continue using the existing legislation and that products derived through biotechnology are to be treated as any other novel product. This means that regulation is triggered by the novel trait of the product (plants with novel traits = PNTs, novel feeds and novel foods) and not by the process via which the trait is introduced.

PNTs are defined by a law (for the definition see Annex 3) and the assessment of these products is based on science and decided case by case. When PNTs are used as food or feed, the legislation for novel food and feed will also apply. Currently in Canada labelling is required if there is a health or safety issue with the food which might be mitigated through labelling, e.g. if the nutritional value or composition has been changed or if an allergen is present. This applies to all novel food, GM or not. As for GMO labelling, there is a national standard for the voluntary labelling of foods derived through biotechnology.

4.4 European Union

In the EU, GMOs have been regulated since 1990. The legislation was amended and the scope clarified in the year 2001[5]. In 2003 the GMO legislation was expanded to food and feed derived from GMOs in order to achieve an integrated approach covering food, feed and seeds. To guarantee transparency for consumers, labelling rules were introduced[6,7,8]. Crops falling under the GMO definition require risk assessments (environmental and food/feed safety) and authorisation before being marketed, used or cultivated.

The legislation includes the GMO definition and three lists defining (i) techniques which give rise to GMOs, (ii) techniques which are not considered to result in GMOs such as in vitro fertilization, natural processes like conjugation, transduction, transformation and polyploidy induction and (iii) techniques of genetic modification which are excluded from the GMO legislation (for the definition and lists see Annex 3).

4.5 Japan

In Japan the primary law regulating organisms derived through biotechnology is the Act on the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Living Modified Organisms (Cartagena Domestic Law)[9] which is based on the Cartagena Protocol on Biosafety which was signed by the Japanese government in 2003. Depending on the use of the GMO, additional legislation, e.g. on food[10] or feed safety[11], applies.

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[5] Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC - OJ L 106, 17.4.2001, p. 1–39.

[6] Regulation (EC) No 1830/2003 of the European Parliament and of the Council of 22 September 2003 concerning the traceability and labelling of genetically modified organisms and the traceability of food and feed products produced from genetically modified organisms and amending Directive 2001/18/EC - OJ L 268, 18.10.2003, p. 24-28.

[7] Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed - OJ L 268, 18.10.2003, p. 1–23

[8] Although this legislation in many cases covers also GM animals and microorganisms, the discussion in the workshop and consequently also in this report was restricted to plant breeding.

[9] Act on the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Living Modified Organisms (Act No 97 of 2003).

[10] Food Sanitation Law and Food Safety Basic Law.

[11] Feed Safety Law.

The GM definition in the Cartagena Domestic Law follows the definition of the Cartagena Protocol of “living modified organisms resulting from modern biotechnology” (see Annex 3). This means that the Law covers living organisms produced by

i. modern biotechnology such as recombinant DNA technology including self-cloning and/or recombinant DNA technique using genetic material (host, vector and foreign genes) derived from organism between which natural gene exchange is possible ("natural occurrence") and

ii. techniques for fusing of cells of organisms belonging to different taxonomic families ("fusion techniques beyond taxonomic family")

Possible exemptions[12] for organisms obtained by self-cloning and/or "natural occurrence" are assessed and decided case by case (for each produced organism).

Risk assessments (environmental, health and food and feed safety) have to be carried out for each individual GM product in order to obtain authorization for experimental and commercial release and for placing on the market. This process involves many different ministries (depending on the use of the product) and consequently is very complex. GMO labelling is regulated by the Food Sanitation Law and the Japanese Agricultural Standard Law.

4.6 South Africa

The primary South African piece of legislation in the context of the workshop is the Genetically Modified Organisms Act 15 of 1997, as amended in 2006[13]. Additional legal provisions are included in environmental[14] and food safety[15] legislation, which includes labelling provisions. Since 2010 there is also a Consumer Protection Act, the GMO labelling provisions of which are yet to come into force.

The legislation includes the GMO definition and a list of techniques for which the GMO Act does not apply which are similar to the definition and lists in the EU legislation (for the definition and list see Annex 3). Contained use, experimental release, import, export and

[12] Exemption means in this context that the produced organism falls under the Cartagena Domestic Law however the requirements of the law are not applied to the specific organism.

[13] http://www.info.gov.za/acts/1997/act15.htm

[14] National Environmental Management Act 107 of 1998 and National Environmental Management: Biodiversity Act of 2004.

[15] Foodstuffs Cosmetics Act of 1972.

placing on the market for commercial cultivation or use or processing as food or feed of GMOs require authorisation.

5 Approaches for new plant breeding techniques

Experience with the regulation of crops obtained by NPBTs is very limited globally. While initial decisions have already been taken in a few countries, discussions have only just started in others. The obligation of participants to treat certain information as confidential further restricted the information which could be presented and released in the workshop. The information provided in this report represents current views and therefore in many cases is provisional or indicative.

The following summary of the approaches in the six represented countries is based on the presentations on the regulatory approaches provided by the workshop participants (**http://ipts.jrc.ec.europa.eu/presentations/NPBT.cfm**) and discussions during the workshop.

5.1 Argentina

The Argentinean authorities have so far not received any applications for authorisation of crops obtained through the NPBTs discussed in the workshop. However, it is presumed that decisions concerning the regulatory classifications of new plant breeding techniques will be possible on the basis of the current legislation. As mentioned in chapter 4.1, in the case of ambiguity the definition of "event" will be decisive.

A group of experts in Argentina started to study the issue and reached preliminary conclusions for most of the techniques. The details are presented in chapter 6 for the specific groups of techniques and in the presentation provided by the participant from Argentina. According to the workshop participants from Argentina the study will be continued and will in due course lead to refined regulatory criteria. After a consultation process with developers, academia and researchers, decisions will be taken concerning certain techniques' inclusion in or exclusion from the GM legislation.

5.2 Australia

The GMO definition in the Australian law (see chapter 4.2 and Annex 3) also needs further discussion and interpretation when dealing with NPBTs. The Gene Technology Regulations 2001 include a list of organisms that are not regarded as GMOs. Item 1 of this list[16] is specifically relevant in the context of NPBTs.

[16] (1) A mutant organism in which the mutational event did not involve the introduction of any foreign nucleic acid (that is, non homologous DNA, usually from another species).

The Office of the Gene Technology Regulator has given advice on a few occasions on the interpretation of legislative provisions relevant to NPBTs. This advice has been given in response to specific questions, usually from researchers, and has not been made public. It is likely that use of the ZFN-1 technique, applied to achieve a deletion in the genome, would not result in the crops being regarded as GMOs. Also cisgenesis, according to the strict definition used here, when one piece of DNA from the same species without any further modification is introduced, will most likely not be regarded as GMO. However, these matters are still under consideration and will continue to be addressed on a case-by-case basis. The Office is currently considering a specific question regarding ZFN-1 and ZFN-2 techniques.

The Office has not publicly given general guidance for specific new techniques, but rather encourages developers to contact them with specific cases where the regulatory status is not clear. It intends to continue with this approach until it has more experience with NPBTs.

5.3 Canada

As already discussed in chapter 4.3, crops obtained through biotechnology are treated in the same way as any other crops under the Canadian legislation. Crops with novel traits have to pass safety assessments and an authorisation process, independent of the technology used. Novel traits can be introduced by traditional breeding, cell fusion, mutagenesis, recombinant DNA techniques and other techniques. Therefore, the Canadian regulatory process does not need to be changed or specifically adapted for crops derived through NPBTs. The Canadian participant in the workshop discussed as an example herbicide tolerant (HT) crops that present issues such as the transfer of the HT trait to related plants, management of volunteers or emergence of herbicide resistant weeds. According to the Canadian regulatory framework, crops with such traits have to be managed similarly, and therefore trigger regulation regardless of the technique they were developed by. If a crop with the same trait has already been authorised before, it may not be necessary to submit any new data for a follow-up product that fits specified criteria.

The Canadian participant in the workshop presented the case of a sulfonylurea tolerant canola developed through one of the NPBTs, which triggered legislation because of herbicide tolerance trait present (the development method was not considered during the regulatory status determination).

5.4 European Union

In the EU, the discussions on new plant breeding techniques started in 2007. Within the European Commission, DG SANCO, the JRC and EFSA are dealing with the three following aspects of NPBTs in accordance with their competencies.

Regulatory aspects

A working group of experts from EU Member States was established by the EC in 2007 (New Techniques Working Group, NTWG). On the basis of the EU GMO definition (see chapter 4.4 and Annex 3) they are evaluating whether certain new techniques constitute genetic modification and, if so, whether the resulting organisms fall within the scope of the EU GMO legislation. The report of the working group, once finalised, will be presented to the Member States for further discussion and decisions, which will presumably be carried out technique by technique.

Technical and socio-economic aspects

In 2010 the IPTS and the IHCP of the EC's JRC conducted a study on "New plant breeding techniques: State-of-the-art and prospects for commercial development"[17]. It investigated the degree of development and adoption of NPBTs by the commercial breeding sector, discussed drivers and constraints for further development of new plant varieties on these techniques and evaluated the technical possibilities for detecting and identifying crops produced by NPBTs.

Safety aspects

EFSA received a mandate in 2011 to address the safety aspects of new plant breeding techniques. They have been asked to provide an opinion on whether current guidance is appropriate for the risk assessment of organisms derived through new techniques and also on the possible risks of these organisms. The evaluation is carried out technique by technique, starting with cisgenesis.

[17] Lusser, M., Parisi, C., Plan, D., Rodríguez-Cerezo, E., 2011. New plant breeding techniques. State-of-the-art and prospects for commercial development. European Commission, JRC Technical Report EUR 24760 EN

5.5 Japan

In Japan, officials from the six ministries responsible for regulating GMOs meet for the purposes of consulting and coordinating their activities under the Cartagena Domestic Law (see chapter 4.5). They collect information related to NPBT crops and discuss and consider their classification as GMOs or non-GMOs on a case-by-case basis, but they have not reached conclusions so far.

The GMO definition (see Annex 3) under Japanese law follows the definition of “living modified organisms resulting from modern biotechnology” in the Cartagena Protocol. As discussed in chapter 4.5, organisms obtained by self-cloning or recombinant DNA technique using genetic material (host, vector and foreign genes) derived from organism between which natural gene exchange is possible ("natural occurrence") may be exempted from the requirements of the Cartagena Domestic Law. Cisgenesis could be interpreted as falling under these definitions. However, the exemption rule for "self-cloning" and "natural occurrence" is only applied to microorganisms and not to plants or animals. Therefore, for crops derived through cisgenesis an application for the approval of research and development has to be submitted. The Japanese participant discussed grafting on GM rootstocks as a second example. He explained that the chimeric plant (for environmental safety) and the fruits (because of the possible migration of foreign products such as mRNA or proteins) should be treated as GM, whereas the progeny or seeds should be seen as non-GM.

5.6 South Africa

The South African participant in the workshop stated that the experience of her country with NPBTs is limited to some research activities. No regulatory decisions have been taken yet and no applications concerning NPBTs have been received. Initial considerations have started following the invitation to the JRC workshop. As the GMO definition in South African law is similar to the EU definition (see chapter 4.6 and Annex 3), similar difficulties are expected. It is intended to address the techniques case by case, starting with agro-infiltration, grafting on GM rootstock and cisgenesis/intragenesis.

6 Approaches and decisions for specific groups of new plant breeding techniques

The second part of the workshop was dedicated to a discussion on decisions and considerations concerning the regulatory status of groups of NPBTs in the countries represented in the workshop. A presentation on the rationale for the grouping of the NPBTs discussed was given during the workshop and is accessible through the following link **http://ipts.jrc.ec.europa.eu/presentations/NPBT.cfm**. Short definitions of the discussed techniques are listed in Annex 4.

The roundtable discussions on each group of techniques were introduced with presentations on information from the 2010 study on NPBTs[18]. They summarised information on the intended and unintended changes in the genome of crops obtained by NPBTs and the possibility of detecting and identifying these crops. The results from the evaluation of the commercial pipeline for the crops show the urgency for regulatory decisions.

The following discussions apply only for those countries where a specific legislation for biotechnology derived crops exists. The specific situation in Canada is discussed in chapters 4.3 and 5.3.

6.1 Targeted mutagenesis

The following techniques were discussed:

- Zinc Finger Nuclease technologies (ZFN-1, ZFN-2 and ZFN-3)
- Oligonucleotide directed mutagenesis (ODM)
- Meganuclease technique

Experts from most participating countries regard it as very likely that the ZFN-1 technique and meganuclease techniques whereby no template sequences are introduced will be classified as non-GM. The EU has not yet concluded its assessment. Products of the ZFN-3 technique, or meganuclease techniques whereby a long DNA sequence is introduced, are products of recombinant DNA techniques (GMOs) and consequently fall under chapter 6.5. Between ZFN-3 and ZFN-2 or ODM, it generally appears to be unclear which kind, and specifically what size, of change obtained by the technique should decide between GMO and non-GMO. The representatives of Argentina specified that in their country ZFN-2 and

[18] Lusser, M., Parisi, C., Plan, D. & Rodríguez-Cerezo, E. New plant breeding techniques. State-of-the-art and prospects for commercial development. JRC Technical Report EUR 24760 EN. *European Commission. Joint Research Centre* (2011). ftp://ftp.jrc.es/pub/EURdoc/JRC63971.pdf

meganuclease techniques where coding sequences are introduced or open reading frames (ORFs) modified will most likely be treated on a case-by-case basis. The representative of the Australian Office of the Gene Technology Regulator informed that the Office has given advice to indicate that products of the ZFN-2 technique and ODM are likely to be considered GMOs if any nucleotide is changed. Other experts, however, stressed that products obtained by ZFN-1 and ZFN-2 techniques cannot be distinguished from crops derived through mutagenesis induced by chemicals or irradiation (and this also applies to products obtained by ODM) and, therefore, should be regulated in the same way.

6.2 Cisgenesis and intragenesis

All participants agreed that in their countries intragenesis will most likely be treated in the same way as transgenesis. Cisgenesis is also expected to be classified as a technique of genetic modification with the exception of specific approaches of cisgenesis in a few countries. The Australian participant in the workshop stated that cisgenesis with a very narrow definition (introduced gene from the same species and without any rearrangements, no foreign DNA, and no T-DNA border sequences) would probably not fall under the Australian GMO definition. However, the Office of the Gene Technology Regulator has not yet dealt with such a case. The experts noted that applications of cisgenesis falling under this narrow definition (obtained through a biolistic approach) are rare. Also the expert from South Africa indicated that, according to preliminary discussions in her country, some approaches of cisgenesis might be treated as non-GM. The Japanese expert confirmed that crops obtained by cisgenesis are currently treated as GMOs in his country. Also the Argentinean experts group concluded that cisgenesis should not be treated any differently from transgenesis.

6.3 Transgenic construct driven breeding (negative segregants)

A transgene encoding an RNAi construct or a dominant-negative protein is present in (e.g. inserted into the genome of) an inducer line. The expression of the transgene leads to the inhibition of gene expression or the inhibition of a protein function, respectively. This leads to an effect such as suppression of the meiotic recombination or early flowering. The inducer transgene is segregated out during further breeding and is therefore not present in the final product (negative segregant).

The following techniques were discussed:

- RNA-dependent DNA methylation (RdDM)
- Reverse breeding

The regulatory situation of negative segregants appears to be unclear in most countries. The experts from Argentina informed that, according to a preliminary discussion in their expert group, negative segregants should be excluded from the GMO legislation. The participant from Australia stated that a negative segregant would most likely not fall under the GMO definition of his country if no introduced trait is inherited. However, if an introduced trait is inherited (e.g. gene silencing generated by RdDM) then the progeny may fall under the Australian definition of a GMO even when the introduced DNA is not inherited. However, a submitted application concerning this issue has not yet been dealt with. The EU and South Africa have still to conclude on the classification of negative segregants. The participant from Japan stressed the importance of proving the absence of inserted DNA sequences.

The special case of RdDM, where the methylation of certain regions of the DNA remains after segregating out the inserted gene, was also discussed. Here a more general problem is prevalent. The effect of gene silencing fades out in the following generations. The Canadian and Argentinean representatives mentioned that because of this instability of expression it is unclear how crops with such traits would be treated under the current regulatory framework. This question would need to be addressed.

6.4 Others

The other following techniques were discussed:

- Grafting on GM rootstock
- Agro-infiltration "sensu stricto"
- Agro-infection

As for grafting on GM rootstock, the experts stated that the rootstock is clearly GM and that an approval is required for the plant's release into the environment. Scientific questions still need to be answered, especially concerning the possible migration of molecules from the rootstock to the scion. In Japan fruits from such a graft are treated as GMOs (taking into account the possible trafficking of proteins and metabolites). However the progeny (seeds) are regarded as non-GM. The Argentinean group of experts concluded (preliminary opinion) that the fruits of these grafts should be assessed on a case-by-case basis. In Australia, fruits from grafts on GM rootstock will most likely not be regarded as GMOs, but may be classified

under the food legislation (Australia New Zealand Food Standards Code) as "food produced using gene technology" and may therefore require a pre-market safety assessment. In South Africa it was concluded that the use of the fruits should be taken into account for the assessment.

Scientific questions still have to be addressed for agro-infiltration too, for example relating to the absence of *Agrobacterium* or if integration of the gene takes place. In Australia and Argentina progeny of infiltrated plants will most likely not be regarded as GMO if no *Agrobacterium* is present and no gene is integrated. In South Africa, agroinfiltration is used in research and therefore the regulatory status is under discussion. However no final view has been reached. The Japanese participant stressed the interest of researchers and breeders in the technique in his country.

Annex 1: List of Participants

European Commission, Directorates General & Agencies		
A) BOLLMANN	Joachim	Directorate General Health and Consumers (DG SANCO)
b) DELINCÉ	Jacques	JRC, IPTS, Unit Agriculture and Life Sciences in the Economy (Agrilife)
C) HOEGEL	Jens	Directorate General Research and Innovation (DG RTD)
D) LUSSER	Maria	JRC, IPTS, Unit Agriculture and Life Sciences in the Economy (Agrilife)
E) PARISI	Claudia	JRC, IPTS, Unit Agriculture and Life Sciences in the Economy (Agrilife)
F) PLAN	Damien	JRC, IHCP, Unit Molecular biology and genomics
G) PODEVIN	Nancy	European Food Safety Authority (EFSA)
H) RODRÍGUEZ CEREZO	Emilio	JRC, IPTS, Unit Agriculture and Life Sciences in the Economy (Agrilife)
i) VAN DEN EEDE	Guy	JRC, IHCP, Unit Molecular biology and genomics
j) WEILAND	Sigrid	Directorate General Agriculture and Rural Development (DG AGRI)
External participants		
K) BEACHY	Roger	Donald Danforth Plant Science Center, USA
L) BURACHIK	Moisés	Biotechnology Directorate, Ministry of Agriculture, Livestock and Fisheries, Argentina
M) DORMANN	Nataliya	Plant Biosafety Office of the Canadian food Inspection Agency, Canada
N) DUPUIS	Karl	Mission of Canada to the EU, Belgium
O) KAMADA	Hiroshi	University of Tsukuba, Japan
P) KITTA	Kazumi	National Food Research Institute, Japan
Q) MANDIVENYI	Wadzi	Department of Environmental Affairs and Tourism, South Africa
R) SPANGENBERG	German	La Trobe University. Australia
S) TUCKER	Will	Office of the Gene Technology Regulator, Australia
T) ZELASCHI	Fernando	Directorate of Biotechnology, Livestock and Fisheries, Argentina

Annex 2: Agenda and presentations

AGENDA

WORKSHOP "COMPARATIVE SITUATION OF NEW PLANT BREEDING TECHNIQUES"

12-13 September 2011
European Commission
Joint Research Center
Institute for Prospective Technological Studies

Venue: Edificio Expo
c/ Inca Garcilsaso 3
1st Floor, Room 30A
Seville, Spain

Chair: **Maria Lusser, JRC-IPTS**
Co-Chair: **Emilio Rodríguez Cerezo, JRC-IPTS**

Monday 12 September 2011		
Time	**Programme items**	**Speaker**
14:00-14:10	Welcome	Jacques Delincé AGRILIFE, Head of Unit Emilio Rodriguez Cerezo AGRILIFE, AGRITECH Action leader
14:10-14:30	2010 JRC project "New Plant Breeding Techniques"	Maria Lusser JRC-IPTS
14:30-14:40	Challenges for detection of crops obtained by new plant breeding techniques	Damien Plan, JRC-IHCP
14:40-14:50	Discussion	
Session 1: Regulatory Framework for biotechnology derived crops with specific focus on new plant breeding in different countries/organisations		
14:50-15:10	Regulatory Framework for biotechnology derived crops with specific focus on new plant breeding techniques in the European Union	Joachim Bollmann DG SANCO
15:10-15:20	Discussion	
15:20-15:40	Regulatory Framework for biotechnology derived crops with specific focus on new plant breeding techniques in Canada	Nataliya Dormann, Plant Biosafety Office
15:40-15:50	Discussion	
15:50-16:10	Regulatory Framework for biotechnology derived crops with specific focus on new plant breeding techniques in Australia	Will Tucker, Office of the Gene Technology Regulator of Australia
16:10-16:20	Discussion	
16:20-16:50	*Coffee break*	

Monday 12 September 2011 (continued)		
Time	**Programme items**	**Speaker**
16:50-17:10	Regulatory Framework for biotechnology derived crops with specific focus on new plant breeding techniques in Japan	Dr. Hiroshi Kamada, University of Tsukuba
17:10-17:20	Discussion	
17:20-17:40	Regulatory Framework for biotechnology derived crops with specific focus on new plant breeding techniques in Argentina	Moises Burachik, Directorate of Biotechnology, Livestock and Fisheries
17:40-18:00	Discussion	

21:00-23:00	*Dinner*

Tuesday 13 September		
Time	**Programme items**	**Speaker**
Session 1: Regulatory Framework for biotechnology derived crops with specific focus on new plant breeding in different countries/organisations		
9:00-9:20	Regulatory Framework for biotechnology derived crops with specific focus on new plant breeding techniques in South Africa	Wadzi Mandivenyi Department of Environmental Affairs and Tourism
9:20-9:30	Discussion	
9:30-9:50	New Plant Breeding Techniques - Categories	Maria Lusser JRC-IPTS
Session 2: Round table discussions on groups of new plant breeding techniques: stage of development, practical experience, discussions on possible regulatory approaches, etc.		
9:50-11:00	New Plant Breeding Techniques – (1) Targeted Mutagenesis (ZFN 1 and 2 technologies, oligonucleotide directed mutagenesis, meganuclease technique) Short presentations & Round table discussion	Fernando Zelaschi, Directorate of Biotechnology, Livestock and Fisheries Maria Lusser JRC-IPTS
11:00-11:30	*Coffee break*	
11:30-12:30	New Plant Breeding Techniques – (2) Cisgenesis, Intragenesis Short presentations & Round table discussion	Maria Lusser JRC-IPTS
12:30-13:15	New Plant Breeding Techniques – (3) Transgenic construct driven breeding (reverse breeding, early flowering, RNA dependent DNA methylation) Short presentations & Round table discussion	Maria Lusser JRC-IPTS
13:15-14:15	*Lunch break*	
14:15-15:00	New Plant Breeding Techniques – (4) Transgene integration/expression in non germline tissue only (Grafting on GM rootstock, agro-infiltration, agro-infection) Short presentations & Round table discussion	Maria Lusser JRC-IPTS
15:00-16:00	Final discussion and closing	

Annex 3: Definitions of GMOs and related terms in the legislation of different countries

ARGENTINA

Cartagena Protocol on Biosafety

Article 3. Use of Terms

"Modern biotechnology" means the application of:

a. In vitro nucleic acid techniques, including recombinant deoxyribonucleic acid (DNA) and direct injection of nucleic acid into cells or organelles, or

b. Fusion of cells beyond the taxonomic family, that overcome natural physiological reproductive or recombination barriers and that are not techniques used in traditional breeding and selection;

Resolution 701/2011

Art.2, bullet 19.

"Event" means "The joint and stable insertion into the plant genome of ONE (1) or more genes or DNA sequences that are part of a defined genetic construct".

(Unofficial translation from Spanish)[19]

[19] Original legal text: "Evento de transformación individual, también referido como "evento": la inserción en el genoma vegetal en forma estable y conjunta, de UNO (1) o más genes o secuencias de ADN que forman parte de una construcción genética definida."

http://www.minagri.gob.ar/site/agricultura/biotechnologia/55-OGM_COMERCIALES/index.php

AUSTRALIA

The Gene Technology Act 2000

Section 10. Definitions

genetically modified organism means any of the following

(a) an organism that has been modified by gene technology; or

(b) an organism that has inherited particular traits from an organism (the initial organism), being traits that occurred in the initial organism because of gene technology; or

(c) anything declared by the regulations to be a genetically modified organism, or that belongs to a class of things declared by the regulations to be genetically modified organisms;

but does not include:

(d) a human being, if the human being is covered by paragraph (a) only because the human being has undergone somatic cell gene therapy; or

(e) an organism declared by the regulations not to be a genetically modified organism, or that belongs to a class of organisms declared by the regulations not to be genetically modified organisms.

Gene Technology Regulations 2001

Schedule 1A

Techniques that are not gene technology

Item	Description of technique
1	Somatic cell nuclear transfer, if the transfer does not involve genetically modified material.
2	Electromagnetic radiation-induced mutagenesis.
3	Particle radiation-induced mutagenesis.
4	Chemical-induced mutagenesis.
5	Fusion of animal cells, or human cells, if the fused cells are unable to form a viable whole animal or human.
6	Protoplast fusion, including fusion of plant protoplasts.
7	Embryo rescue.

8 *In vitro* fertilisation.

9 Zygote implantation.

10 A natural process, if the process does not involve genetically modified material.

Examples

Examples of natural processes include conjugation, transduction, transformation and transposon mutagenesis.

Gene Technology Regulations 2001

Schedule 1

Organisms that are not genetically modified organisms

Item Description of organism

1 A mutant organism in which the mutational event did not involve the introduction of any foreign nucleic acid (that is, non-homologous DNA, usually from another species).

2 A whole animal, or a human being, modified by the introduction of naked recombinant nucleic acid (such as a DNA vaccine) into its somatic cells, if the introduced nucleic acid is incapable of giving rise to infectious agents.

3 Naked plasmid DNA that is incapable of giving rise to infectious agents when introduced into a host cell.

6 An organism that results from an exchange of DNA if:

(a) the donor species is also the host species; and

(b) the vector DNA does not contain any heterologous DNA.

7 An organism that results from an exchange of DNA between the donor species and the host species if:

(a) such exchange can occur by naturally occurring processes; and

(b) the donor species and the host species are micro-organisms that:

(i) satisfy the criteria in AS/NZS 2243.3:2010 for classification as Risk Group 1; and

(ii) are known to exchange nucleic acid by a natural physiological process; and

(c) the vector used in the exchange does not contain heterologous DNA from any organism other than an organism that is involved in the exchange.

CANADA

Seeds Regulations Part V

“novel trait”, in respect of seed, means a characteristic of the seed that

(a) has been intentionally selected, created or introduced into a distinct, stable population of cultivated seed of the same species through a specific genetic change, and

(b) based on valid scientific rationale, is not substantially equivalent, in terms of its specific use and safety both for the environment and for human health, to any characteristic of a distinct, stable population of cultivated seed of the same species in Canada, having regard to weediness potential, gene flow, plant pest potential, impact on non-target organisms and impact on biodiversity; (caractère nouveau)

EUROPEAN UNION

Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms [20]

Article 2

Definitions

For the purposes of this Directive:

(1) "organism" means any biological entity capable of replication or of transferring genetic material;

(2) "genetically modified organism (GMO)" means an organism, with the exception of human beings, in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination;

Within the terms of this definition:

(a) genetic modification occurs at least through the use of the techniques listed in Annex I A, part 1;

(b) the techniques listed in Annex I A, part 2, are not considered to result in genetic modification;

Article 3

Exemptions

1. This Directive shall not apply to organisms obtained through the techniques of genetic modification listed in Annex I B.

20 Directive 2001/18/EC20 of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC - Commission Declaration - OJ L 106, 17.4.2001, p. 1–39

Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms

ANNEX I A

TECHNIQUES REFERRED TO IN ARTICLE 2(2)

PART 1

Techniques of genetic modification referred to in Article 2(2)(a) are inter alia:

(1) recombinant nucleic acid techniques involving the formation of new combinations of genetic material by the insertion of nucleic acid molecules produced by whatever means outside an organism, into any virus, bacterial plasmid or other vector system and their incorporation into a host organism in which they do not naturally occur but in which they are capable of continued propagation;

(2) techniques involving the direct introduction into an organism of heritable material prepared outside the organism including micro-injection, macro-injection and micro-encapsulation;

(3) cell fusion (including protoplast fusion) or hybridisation techniques where live cells with new combinations of heritable genetic material are formed through the fusion of two or more cells by means of methods that do not occur naturally.

PART 2

Techniques referred to in Article 2(2)(b) which are not considered to result in genetic modification, on condition that they do not involve the use of recombinant nucleic acid molecules or genetically modified organisms made by techniques/methods other than those excluded by Annex I B:

(1) in vitro fertilisation,

(2) natural processes such as: conjugation, transduction, transformation,

(3) polyploidy induction.

ANNEX I B

TECHNIQUES REFERRED TO IN ARTICLE 3

Techniques/methods of genetic modification yielding organisms to be excluded from the Directive, on the condition that they do not involve the use of recombinant nucleic acid molecules or genetically modified organisms other than those produced by one or more of the techniques/methods listed below are:

(1) mutagenesis,

(2) cell fusion (including protoplast fusion) of plant cells of organisms which can exchange genetic material through traditional breeding methods.

JAPAN

Act on the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Living Modified Organisms (Act No 97 of 2003)

Article 2 (Definitions)

(1) In this Act, "living modified organism" shall mean an organism that possesses nucleic acid, or replicated product thereof, obtained through use of any of the following technologies.

(i) Those technologies as stipulated in the ordenance of the competent ministries, for the processing of nucleic acid extracellularly

(ii) Those technologies as stipulated in the ordenance of the competent ministries, for the fusing of the cells of organisms belonging to different taxonomic families.

SOUTH AFRICA

Genetically Modified Organisms Act [No. 15 of 1997]

Definitions

1. In this Act, unless the context otherwise indicates-

…….

i. "genetically modified organism" means an organism the genes or genetic material of which has been modified in a way that does not occur naturally through mating or natural recombination or both, and "genetic modification" shall have a corresponding meaning; (xiii)

.....

Application of Act

2. (1) This Act shall apply to-

   a. the genetic modification of organisms;

   b. the development, production, release, use and application of genetically modified organisms (including viruses and bacteriophages); and

   c. the use of gene therapy.

2. This Act shall not apply to techniques-

   a. involving human gene therapy;

   b. in which recombinant DNA molecules or genetically modified organisms are not employed-

      i. in in vitro fertilisation in humans and animals;

      ii. in conjugation, transduction, transformation or any other natural process: and

      iii. in polyploidy induction;

   c. in which genetically modified organisms as recipient or parental organisms are not employed-

      i. in mutagenesis;

      ii. in the construction and use of somatic hybridoma cells; and

      iii. in cell fusion (including protoplast fusion) of plant cells.

Annex 4: Definitions of new plant breeding techniques

Agro-infiltration:

Agro-infiltration 'sensu stricto': Non-germline tissues, mostly leaves, are infiltrated with a liquid suspension of *Agrobacterium* containing a genetic construct. The genetic construct is locally expressed at high level, without being integrated into the plant genome.

Agro-infection: Non-germline tissues, typically leaves, are infiltrated with a construct containing the foreign gene in a full-length virus vector to facilitate spreading and expression of the target gene in the entire plant.

Floral dip: Germline tissues, typically flowers, are immersed into a suspension of *Agrobacterium* containing a DNA construct in order to obtain transformation of some embryos that can be selected at the germination state. The aim is to obtain stably transformed plants.

Cisgenesis and intragenesis:

A DNA sequence from the species itself or from a cross compatible species is inserted into the plant genome. In the case of cisgenesis, the inserted gene is unchanged with its own introns and regulatory sequences. In the case of intragenesis, the inserted DNA can be a new combination of DNA fragments from the species itself or from a cross compatible species.

Grafting (on GM rootstock):

A chimeric plant is produced by grafting a non-genetically modified scion on a genetically modified rootstock.

Meganuclease technique:

Meganucleases are proteins that specifically recognize target DNA sequences of 12 to over 30 base pairs and create a double strand break (DSB) that activates repair mechanisms and DNA recombination. Similarly to ZFNs, the technique can be used for site specific mutagenesis or for targeted gene insertion by homologous recombination. Newly designed meganucleases can be produced in order to induce site-specific DNA recombination at a chosen locus in plant cell.

Oligonucleotide directed mutagenesis (ODM):

Oligonucleotides target homologous DNA and induce site-specific nucleotide substitutions, insertions or deletions through repair mechanisms. Oligonucleotides such as chimeric

oligonucleotides , consisting of DNA and RNA bases, and single stranded DNA oligonucleotides can be deployed for ODM in plants.

Reverse Breeding:

Homozygous parental lines are produced from selected heterozygous plants by suppressing meiotic recombination. This suppression is obtained through RNA interference-mediated downregulation of genes involved in the meiotic recombination process. Subsequently, double haploid (DH) homozygous lines are produced and hybridised in order to reconstitute the original genetic composition of the selected heterozygous plants.

RNA-dependent DNA methylation (RdDM):

Genes encoding RNAs which are homologous to plant sequences, like promoter regions, are delivered to the plant cells. These genes, once transcribed, give rise to the formation of small double stranded RNAs. They induce methylation of the homologous sequences and consequently inhibit their transcription.

Zinc finger nuclease technology:

ZFN-1: Genes encoding Zinc Finger Nucleases (ZFN) are delivered to plant cells without a repair template. The ZFN binds to a specific DNA sequence and generates a site-specific double strand break (DSB). The natural DNA-repair process through non-homologous end-joining (NHEJ) leads to site-specific mutations, which consist of changes of single or few base pairs, short deletions or insertions.

ZFN-2: Genes encoding Zinc Finger Nucleases (ZFN) are delivered to plant cells along with a short repair template. The ZFN binds to a specific DNA sequence and generates a site-specific double strand break (DSB). Gene repair mechanisms generate site-specific point mutations like changes of single or few base pairs through homologous recombination and the copying of the repair template.

ZFN-3: Genes encoding Zinc Finger Nucleases (ZFN) are delivered to plant cells along with a large stretch of DNA, whose ends are homologous to the DNA sequences flanking the cleavage site resulting from the DNA double strand break. As a result, the DNA stretch is site-specifically inserted into the plant genome.

European Commission

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Abstract

The JRC-IPTS organized a workshop on "Comparative approaches for new plant breeding techniques" in September 2011. In this workshop, the regulatory framework for biotechnology derived crops with specific focus on approaches for new plant breeding techniques in Argentina, Australia, Canada, the European Union, Japan and South Africa was presented by experts from these countries. Additionally, experts discussed approaches and decisions for specific groups of new plant breeding techniques.

Whereas, in Canada products derived through biotechnology are treated as any other novel products (plants with novel traits, PNTs), specific biotechnology or GMO legislation was introduced in the other five countries. Experience with the regulation of crops obtained by new plant breeding techniques is very limited globally. While initial decisions have already been taken in a few countries, discussions have only just started in others. Deviating decisions (between countries and between techniques) have to be expected.

The workshop presentations are accessible through the following link:

http://ipts.jrc.ec.europa.eu/presentations/NPBT.cfm

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