New plant breeding techniques

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European Commission, JRC-IPTS

Conference "Scientific Support to Agriculture"
Athens, April 23th, 2014
New plant breeding techniques
State-of-the-art and prospects for commercial development

Maria Lusser, Claudia Parisi, Damien Plan and Emilio Rodriguez-Cerezo

Deployment of new biotechnologies in plant breeding

M aria Lusser1,2, Claudia Parisi1,2, Damien Plan2 & Emilio Rodriguez-Cerezo1

The first crops obtained through new plant breeding techniques are close to commercialization. Regulatory issues will determine the adoption of the techniques by breeders.

The global food crisis of 2008reminded us of the importance of innovation in agriculture to address global challenges such as population growth and climate change. The projections presented in a report of the Food and Agricultural Organization of the United Nations (proceedings of a high-level expert forum) show that feeding a world population of 9.1 billion people in 2050 would require raising overall food production by some 70% between 2005/07 and 2050 (ref. 1). Additionally, farmers will have to hit targets for reducing greenhouse gas emissions, improving water use efficiency and meeting the demands of consumers for healthier food and high-value ingredients. In this context, new plant breeding techniques are needed to contribute improvements in crop productivity and sustainability.

Clearly, an important aspect of technology adoption and dissemination is how such approaches relate to regulatory oversight and whether such breeding techniques fall under present or newly modified organisms (GMO) legislation. In the case of EU, the issue is currently being analyzed3. Although studies analyzing new plant breeding techniques from the point of view of risk assessors and regulators are available4,5, data are lacking on the refinement and/or maturation of technology and the extent of adoption in commercial breeding programs (and thus likely contribution to new crop varieties in the short or medium-term).

To close this gap, we have conducted a study on new plant breeding techniques (beyond traditional genetic modification), under the aegis of the European Union’s Joint Research Centre (JRC), that encompasses state of the art technology and their prospects for commercial development, including zinc Finger nucleases (ZFN) technology8,9, oligonucleotide-directed methylation (ODDM)10,11, cas9 genes and tetramethyl12, DNA-dependent RNAi technology (siRNA)13,14, grafting (genetically modified (GM) rootstock)15, reverse breeding16 and agro-infiltration (encompassing agro-infiltration ‘bombed stinging', agro-infiltration and floral dip)17. Our primary focus is on the current development status of these approaches, the main actors exploiting them in R&D (both public and private), the patenting landscape and the current use of these techniques by the commercial breeding sector. We also address the main drivers and constraints for the further adoption of these techniques. Finally, we analyze the possibilities for detecting and identifying crops produced using them (to facilitate regulatory requirements).

Historical backdrop
Since the beginning of the twentieth century, various tools have been introduced to broaden the possibilities for breeding new plant varieties. Chemical and radiation-induced mutations increase the frequency of genetic variations, and hybrid seed technology generates heterozygous plants with improved yield and disease resistance18. Applying the principles of cell biology and tissue culture—micropropagation, embryo rescue and double haploid techniques—allows the rapid production of many uniform plants and the crossing of incompatible plants19.

The latest wave of innovation in plant breeding, dating from the 1980s, came from modern biotech: Molecular marker-assisted selection is now widely used to map and select commercially important agricultural traits19.

1 Genetic modification, also known as genetic engineering, exploits recombinant DNA technology to expand the gene pool available to breeders. The earliest crops produced by genetic modification technologies (pest-resistant and herbicide-tolerant varieties) reached commercial cultivation in the mid-1990s and currently the global area sown with GM varieties measures over 148 million hectares20.

In the past two decades, additional applications of biotech and molecular biology in plants have emerged, with the potential to further enlarge the plant breeder’s toolbox. Several recently described techniques allow for site-directed modifications of plant genes (to knock out or modify gene functions) and the targeted deletion or insertion of genes into plant genomes21,22. Another innovative trend is the use of transgenics solely as a tool to facilitate the breeding process. In this application, transgenics are used in intermediate breeding steps and then selected for removal during later crosses, eliminating them from the final commercial variety. Among these tools are accelerated breeding techniques, where genes that promote early flowering are used to speed up breeding23, and reverse breeding, a technique that produces homogenous potential lines from heterogeneous elite plants24.

The potential of these and other new techniques to produce innovative crop varieties will likely be affected by the regulatory framework of the regions where they are to be introduced. The application of modern biotech in the 1980s resulted in new forms of regulation and governance of certain plant breeding techniques (in particular genetic modification technologies) and of the release of GM crops into the environment. Various legal and regulatory approaches have been adopted worldwide, which include differing definitions of GM crops25.
Comparative regulatory approaches for new plant breeding techniques

Workshop Proceedings

Maria Lusser and Emilio Rodriguez Cereto
Technical innovation plant breeding
Research landscape
Patent landscape and commercial development
Regulatory/policy issues
Technical developments in plant breeding

**New regulatory framework**

**New issues of Intellectual Property (IP)**

**INTRODUCTION OF BIOTECHNOLOGY IN PLANT BREEDING**

- NPBT
- TRANSGENESIS
- TISSUE CULTURE
- HYBRID TECHNOLOGY
- MUTAGENESIS

**INTUITIVE BREEDING**

- 10,000 bC: Plant Domestication

**CROSS BREEDING**

- 1699: Sex in plants
- 1865: Mendel
- 1900
- 1920
- 1930
- 1960
- 1980
- 2000
Innovation: Groups of New Plant Breeding Techniques (NPBTs)

NBTs broadly classified in 3 families

1. Targeted mutagenesis/modification (genome editing)
2. Negative (null) segregants
3. Variants of genetic transformation
New Plant Breeding Techniques

TARGETED MUTAGENESIS TECHNIQUES

- **OLIGONUCLEOTIDE DIRECTED MUTAGENESIS**
- **ZINC FINGER NUCLEASE TECHNIQUE**
- **MEGANUCLEASE TECHNIQUE**
- **TALEN TECHNIQUE**

VARIANTS OF PLANT TRANSFORMATION TECHNIQUES

- **CISGENESIS AND INTEGRATION**
- **GRAFTING ON GM ROOTSTOCK**

TECHNIQUES RESULTING IN "NEGATIVE SEGREGANTS"

- **REVERSE BREEDING**
- **RNA DIRECTED DNA METHYLATION**
1. Targeted mutagenesis/modification (a.k.a. “genome editing”)

- Based on site-specific nucleases (ZFN, MGN, TALEN)
- Based on oligonucleotides (ODM)
- Targeted mutations based on naturally occurring DNA repair mechanisms (ZFN1)

or

- Site specific changes introduced with the help of template molecules (from 1 to x nt)(ZFN2-3)
2. Negative (null) segregants

- A GM event is used in the breeding process but a progeny plant lacking the transgene is released as commercial variety (i.e. early flowering, “reverse breeding”, others)
- RdDM: (RNA-dependent DNA methylation) commercial variety does not contain the transgene but inherits an epigenetic modification
3. Variants of genetic transformation

- Cisgenesis (inserted elements, continuous and unchanged, from same species or x-compatible)
- Intragenesis (as above but re-organisation, new combination possible)
- Grafting non-GM scions on GM rootstocks
Development over time of scientific publications on NPBTs
Country of origin of institutions authoring scientific publications on NPBTs

- North America: 32.7%
- Asia: 12.6%
- Other countries: 12.1%
- EU-27: 42.6%

Sector of institutions authoring scientific publications on NPBTs

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<td>Joint</td>
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Development over time of patents on NPBTs

N. Patents


OMD  ZFN  MEGA  TALEN  CISG-INTRA  RdDM  REV BREED  GRAFT

PATENT LANDSCAPE

European Commission
**Country of origin of patent assignees on NPBTs**

- EU-27: 37.6%
- North America: 50.3%
- Asia: 7.0%
- Other countries: 5.1%

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**Sector of institutions of patent assignees on NPBTs**

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<th>Sector</th>
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<tr>
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<tr>
<td>Joint</td>
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<td><strong>Total</strong></td>
<td><strong>137</strong></td>
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Most active institutions in NPBTs in plants, based on number of patents produced and number of techniques claimed. O:ODM, Z:ZFN, M:MGN, T:TALEN, C: Cisgenesis/Intragenesis, R:RdDM, B:Reverse Breeding, G:Grafting.

<table>
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<td>US</td>
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OECD Meeting on New Plant Breeding Techniques - February 2014

Survey: Is your country developing any crops with NPBT in the private or public sector? Some EU data

Cisgenic apples with scab resistance (Switzerland, the Netherlands).
Fireblight resistant apples through accelerated breeding (Switzerland).
Cisgenic potatoes resistant to late blight (the Netherlands, Belgium, Ireland).
Different food crops developed via ODM (the Netherlands).
Trees with altered lignin composition developed via RNAi (Belgium).
Herbicide tolerant oilseed rape developed through ODM (UK).
Cereal varieties developed through site-directed nucleases (UK, Ireland).
Drivers for commercial development of crops via NPBT

Technical potential of NPBT
Targeted mutagenesis (ZFN, ODM)
Site specific insertion (ZFN 3)
Gene silencing (RdDM)
No transgene integration

Economic advantage:
Faster breeding process

Constraints

Technical constraints of NPBT
Efficiency issues
Regeneration of plants from cuttings, protoplasts, etc.
Method for selection of successfully modified plants

Regulatory costs & market access:
Uncertainty of regulatory status
High costs if regulated
“Comparative regulatory approaches for new plant breeding techniques: workshop proceedings”

12-13 September 2011

Participants & sources of information

- **Argentina** (Biotech. Directorate, M. Agriculture, Livestock, Fisheries)
- **Canada** (Plant Biosafety Office-Canadian Food Inspection Agency)
- **European Union** (DG Health and Consumers, Joint Research Centre)
- **Japan** (University of Tsukuba & National Food Research Institute)
- **South Africa** (Department of Environmental Affairs and Tourism)
- **Australia** (Office of the Gene Technology Regulator)

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**USA** (Donald Danforth Plant Science Center)

Published USDA letters in response to specific inquiries
DETECTION  IDENTIFICATION

ZFN-3
CISGENESIS/INTRAGENESIS
GM ROOTSTOCK

DETECTION  IDENTIFICATION

ZFN-1,2
ODM

DETECTION  IDENTIFICATION

RdDM
REVERSE BREEDING
NON-GM SCION
AGRO-INFILTRATION
New plant breeding techniques derived from biotechnology have quickly developed in the last 10 years.

EU (highest number of scientific publications) and US (highest number of patent applications) key players.

Some techniques are now ready for many crop plants, while others have been tested mainly in model plants.

Breeder’s interest: potential technical and economic advantages, but the extent of use will depend on the regulatory status.

Crops from some NPBTs are very near to commercialisation.
Discussions on regulatory issues associated to NPBTs are taking place in many countries. Expert groups have been set up to advice on classification of the NPBTs & products *vis-à-vis* biotech crop legislations.

In many cases, legislation and definitions are some 20 years old.

Regulatory decisions are still under consideration, and some common elements appear when considering certain NPBTs.

However, discrepancies in eventual regulatory status for certain NPBTs-products are already pointed out and these differences might translate into global trade issues.
Thank you for your attention!

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