Update: Review of the New Plant Breeding Techniques (NBT) from the Viewpoint of Regulation

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New Plant Breeding Techniques (NBT) or Plant Breeding Innovations

- Refers to a continuously evolving suite of tools that facilitate the development of new plant varieties with desired traits (Madre and Agostino, 2017) in a way that is faster and more precise than conventional plant breeding techniques (CBT).

- As NBTs were developed later than GM technology, the main aim of NBTs is to develop products that ultimately, will not trigger GMO regulation.

- However, some NBTs can also be useful in inserting an entire gene; hence, could potentially give rise to a GM crop.
The different NBTs:

1. Site-Directed Nucleases or SDN (Meganuclease, ZFN, TALEN, CRISPR-CAS)
2. Oligonucleotide-directed mutagenesis (ODM)
3. Cisgenesis and Intragenesis
4. RNA-dependent DNA methylation (RdDM)
5. Grafting with GM Material
6. Reverse breeding
7. Agro-infiltration
8. Synthetic Genomics/Biology
The different NBTs:

1. Site-Directed Nucleases or SDN (Meganuclease, ZFN, TALEN, CRISPR-CAS)
   - introduce a mutation (e.g. insertion or deletion) or a gene in a precise location (where there is a double-strand break or DSB created by a site-specific nuclease)
   - May use a repair template in the case of SDN2 and SDN3.
2. Oligonucleotide-directed mutagenesis (ODM)

- Introduces of a non-random mutation (e.g. base change) in a precise location to which the designed oligonucleotide is homologous
3. Cisgenesis and Intragenesis

- Introduce an allele or another version of a gene from the same or cross-compatible species

- Introduce a modified gene (with new combination/arrangement of promoters, exons and introns) from the same or cross-compatible species; can also be used for gene silencing
4. RNA-dependent DNA methylation (RdDM)

- Used to block/suppress the expression/function of a gene by epigenetic control (methylation) of its promoter region
5. Grafting on GM Material

- Produces non-transgenic products from a non-transgenic scion joined to a transgenic root stock, or vice versa
6. Reverse breeding

- Produce homozygous parentals from an existing excellent hybrid variety with unknown parents/lineage.
7. Agro-infiltration

- Non-germline: Provides a way to test a construct or to screen for desirable (e.g. disease-resistant) plants without affecting the host's DNA
- Germline: Insertion of a large DNA fragment such as a gene directly into the genome of immature embryos that will develop into seeds.
8. Synthetic Genomics

- Synthesize minimal or complex genome or gene that does not exist in nature; or to reengineer existing biological elements
Definition of Key Terms (from Cartagena Protocol)

GMO or LMO
- “any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology” (Cartagena Protocol)

Modern biotechnology
“the application of: a) in vitro nucleic acid techniques, including recombinant deoxyribonucleic acid (DNA) or 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 or selection.”
Definition of Key Terms (continued):

Novel Combination of Genetic Material
- not currently defined in the law/regulations!

Proposed definition:

A resultant DNA combination in a living organism that is not possible through conventional breeding.

Living organism – any biological entity capable of transferring or replicating genetic material, including sterile organisms, viruses and viroids.
Overall Decision Process Flow for the Biosafety Assessment of Plant Products

1. Produced through Modern Biotechnology?
   - No
   - Yes

2. With novel combination?
   - No
   - Yes

**GMO (Classic)**
- rDNA technology with trans insert;
- Direct injection;
- Fusion of unrelated cells

**NBT (Case 1)**
- Site-directed Nuclease 1 (SDN1);
- SDN2;
- Grafting with GM material;
- Oligonucleotide-directed mutagenesis (ODM);
- Cisgenesis and Intragenesis;
- RNA-dependent DNA methylation (RdDM);
- Reverse Breeding;
- Agroinoculation of non-germline tissues;
- SDN3 with cis insert;
- Agroinoculation of germline tissues with cis insert;
- Synthetic Genomics (synthetic cis-like insert)

**NBT (Case 2)**
- SDN3 with trans insert;
- Agroinoculation of germline tissues with trans insert;
- Synthetic Genomics (synthetic novel gene)*

**CBT-HGT**
- Natural genetic transformation through horizontal gene transfer (HGT) by some bacteria and viruses

**CBT**
- Mutagenesis (chemical, physical, transposon, retrotransposon);
- Hybrid breeding;
- Tissue culture;
- Fusion of related cells

**GM Regulation**
- Regulation for Non-GM/Conventional Products which are assumed to be safe (e.g. Codex Alimentarius)

* Artificial genomes and nucleic acids are not covered by existing regulations.
The major considerations in the biosafety assessment of plant products are whether or not modern biotechnology is used and whether novel combination of genetic materials occurs as a result of the use of modern biotechnology. It is generally understood that natural plant species and conventionally bred plants that do not use modern biotech are safe and are only subject to standard safety regulations (e.g. Codex Alimentarius). In addition, because conventional plant breeding does not involve transfer of DNA from a different species and because the resulting plants rely on natural sexual compatibility to reproduce, there is no problem about the formation of novel combination of genetic materials. Recently, however, with the advent of advanced genome sequencing techniques, some of these conventional plant species are found to have genes in their genomes that originate from non-sexually compatible species, which suggests that novel combinations can also be formed in nature. The phenomenon behind this is now widely known as horizontal gene transfer (HGT) which is possible due to the inherent ability of some bacteria and viruses to integrate their genetic material into their host cell genomes. The result is a naturally transformed plant that is still considered to be as safe as other ordinary varieties.

On the other hand, modern biotechnology offers a fast and precise way to produce better crops by allowing researchers to breach the natural reproductive barriers existing among species to produce a novel crop or simply to induce targeted mutations that produce mutants indistinguishable from conventionally bred crops. Traditional or classical GM technology using recombinant DNA is used as a last resort when no desired genes are present in the gene pool of a given species. The stable introgression of a novel gene through this process results in novel combination in the final product, hence, producing a GMO. Currently, heavy regulation is being imposed on GMOs because of the perceived risks associated with novel combinations. In contrast, some products of modern biotechnology produced through certain New Plant Breeding techniques, do not have stable introgression of novel genes; if not, any novel genes are bred out and so, there is no novel combination in their final products (NBT Case 1); hence, they cannot be distinguished from their conventional counterparts and could be exempted from GM regulation.

In another case, some of the NBTs mentioned can be used to create a GMO (NBT Case 2) by allowing stable introgression of a gene or other sequence of genetic material from a non-sexually compatible species or even to introduce a novel de-novo synthesized DNA fragment.

Up to this point, the basis for classification discussed here has been the presence of novel combinations. Compared to the rest of the techniques, synthetic biology could go beyond the concept of novel combination as its scope is up to the genome level and so, it is viewed that a separate regulation for plants with artificial genomes will be needed in the future. If not, the existing GM regulation may need to be expanded.
Thank you!