New technologies in agricultural biotechnology

András Székács

European Food Safety Authority, Via Carlo Magno 1A, 43126 Parma, Italy

*E-mail: a.szekacs@cfri.hu

Abstract – Technologies that emerged during the last decade as new tools occasionally represent fundamentally new means of genome modification, which, in addition to the scientific novelty, faces legislators with new challenge by giving a new meaning to both the biochemical/molecular biological and legal meaning to genetically modified organisms (GMOs). Emerging plant genetic technologies are categorized as zinc finger nuclease technology; oligonucleotide directed mutagenesis; cisgenesis and intragenesis; RNA-dependent DNA methylation by RNA interference; grafting on genetically modified rootstock; reverse breeding; agro-infiltration; and synthetic genomics. Although all these methods apply biotechnology processes to create new plant varieties, it is debated whether all result in GMOs according to the current legal definition. Official risk assessment of these technologies is a task of outstanding weight of the authority.

Keywords – emerging technologies, genetically modified organisms, zinc finger nuclease technology, oligonucleotide directed mutagenesis, cisgenesis and intragenesis, RNA-dependent DNA methylation by RNA interference, grafting on GM rootstock, reverse breeding, agro-infiltration, synthetic genomics

New applications in agricultural biotechnology not only attempt to utilize the newest results of scientific research in agricultural practice, but also pose a difficult task to scientific evidence based risk assessment. Technologies that emerged during the last decade as new tools occasionally represent fundamentally new means of genome modification, which, in addition to the scientific novelty, faces legislators with new challenge by giving a new meaning to both the biochemical/molecular biological and legal meaning to genetically modified organisms (GMOs).

The 2011 Report of the Joint Research Centre of the European Commission categorized emerging plant genetic technologies (Lusser et al., 2011) as:

(i) zinc finger nuclease (ZFN) technology;
(ii) oligonucleotide directed mutagenesis (ODM);
(iii) cisgenesis and intragenesis;
(iv) RNA-dependent DNA methylation (RdDM) by RNA interference (RNAi);
(v) grafting on genetically modified (GM) rootstock;
(vi) reverse breeding;
(vii) agro-infiltration;
(viii) synthetic genomics.

Although all these methods apply biotechnology processes to create new plant varieties, it is debated whether all result in GMOs according to the definition set in the EU 2001/18/EC Directive (European Parliament and Council, 2001). Nucleases containing characteristic zinc finger domains bind to given, 9-12 base (i.e. 3-4 amino acid) long DNA sequences, cleave both DNA strings there, and result in genome modification by mutation, gene insert or interallelic gene conversion repair, allowing targeted modification (“editing”) by amino acid accuracy, and insertion of desired gene fragments at given points of the genome. RdDM is also capable of creating targeted mutations of a few nucleotides, using 20-100 base long oligonucleotides, slightly (in a single base) differing from the base sequence of the targeted plant genomic sequence, leading to new mutations by nucleotide exchange, or deletion of existing mutations or short gene fragments from the genome. Cisgenesis and intragenesis differ from transgenesis used for the preparation of first generation GM plants in using (multiplying) genes with their introns, regulating elements or gene fragment from the same (cisgenesis) or crossable species (intragenesis), while gene insert is usually not directed. RdDM achieves silencing of genes and their transcriptional expression by methylation of the promoter sequences of targeted genes in the genome. (For this purpose transgenic plants have to be created in the process.) In grafting on a GM rootstock, although the graft is not a GM plant, small RNA molecules may enter into it from the GM rootstock, and the micro-RNAs or interfering RNAs being produced in the rootstock may enter the graft and
cause gene silencing there. During reverse breeding, steps of hybrid preparation are carried out in a reversed order, and genomic modifications are created in the elite heterozygote line. Agroinfiltration is based on the entry of a suspension of a transgenic Agrobacterium, thus leading to transgene expression in the plant affected. Synthetic genomics is a collective term referring to all genomic methods targeted to the formation of components or systems not existing in nature (synthetic or synthetically modified from existing elements).

The European Food Safety Authority (EFSA), operating in close collaboration with national food safety authorities, is the keystone of the European Union regarding food and feed safety. According to its mandate (European Parliament and Council, 2002) its activity includes identification of risk factors related not only to existing, but also to emerging technologies (Article 34). Tools of early identification of new problems (not necessarily incidents or crises), to better anticipate risk assessment needs, include research, data generation and risk assessment methodology development. Accordingly, risk assessment of the eight types of emerging technologies is included in the task of EFSA, but related legislator tasks are out of the scope of the mandate of EFSA, and belong to the corresponding risk managers.

Within these tasks, EFSA announced its statements regarding ZFN-3 based genome editing (European Food Safety Authority, 2012a) and cisgenesis / intragenesis (European Food Safety Authority, 2012b), and concluded that the Guidance documents are applicable for risk assessment, and that using case-by-case evaluation, even lesser amount of data may be needed. To survey the current status of RNAi-based GM plants, EFSA organised an international workshop (European Food Safety Authority, 2014) that updated our knowledge on the biology on RNAi mechanisms in plants, invertebrates and mammals; explored current and future RNAi-based applications; and identified issues unique to RNAi-based GM plants and their risk assessment in three sessions focusing on the three main areas of the GMO risk assessment, i.e. molecular characterisation, food and feed safety, as well as environmental risk assessment. Main conclusions of the workshop stated:

(i) Molecular characterisation and comparative analysis should remain the basis of risk assessment to identify potential safety relevant intended and unintended changes in the RNAi-based GM plant.
(ii) Bioinformatic analyses could play an important role in informing the risk assessment, but more research is needed on interactions between sRNAs and target sequences, and on genomes of target and non-target species.

(iii) Based on the toxicological and pharmacokinetic profile of RNAi molecules, oral toxicity studies with purified RNAi molecules were not considered relevant.

Risk assessment regarding the other above mentioned technologies and additional ones that emerged since (CRISPR/CAS) has not yet been commenced by EFSA. In turn, official risk assessment of these technologies is a task of outstanding weight of the authority.

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