Visit of DDG Mr. Ladislav Miko to KeyGene N. V., Wageningen, the Netherlands on 31st January 2012,11:00 – 16:00

GMO – New Techniques

26th January 2012

Responsible Official:

BACKGROUND BRIEF

1. Keygene N. V.

KeyGene N. V. is a Dutch biotech R & D company with more than 130 staff, focussing in particular on crop plant improvement. Its headquarters a located in Wageningen, the Netherlands, a subsidiary is in Rockville, Maryland, USA (KeyGene Inc.) and a Joint Lab is at the Shanghai Institute of Biological Sciences in Shanghai, China. CEO of KeyGene is

One of the company's special assets is targeted mutagenesis of crop plants using the oligonucleotide directed mutagenesis technique (OdM) under the trade mark KeyBase™. KeyGene co-operates with

Further, KeyGene's portefolio includes tools for molecular breeding, including marker assisted breeding and selection, and KeyPoint™ for screening chemically induced or natural mutations. In addition, the company uses public and private databases to extract key-genes relevant for the trait of interest using bioinformatic approaches. Finally, KeyGene provides a trait platform encompassing biotic and abiotic stress resistances, herbicide tolerances and reproduction traits.

2. Oligonucleotide-directed mutagenesis

OdM employs oligonucleotides for targeted (site-specific) induction of point mutations, which is the replacement of one or a few base pairs or the introduction of short deletions.

Oligonucleotides of approximately 20 to 100 nucleotides are delivered to the cells by methods suitable for the different cell types (including electroporation, polyethylene-glycol-mediated transfection, natural uptake). The technique exploits the sequence specific interaction of the oligonucleotide with the resident DNA of the cells, resulting in gene targeting. This directs the attempted genetic modification to a specific region in the DNA or even to a specific base pair. The genetic modification can be the induction of a point mutation or reversion of an existing mutation which may lead to changes in the expression of a gene.

In the specific method developed byKeyGene (KeyBase™) oligonucleotides contain locked nucleic acid residues (LNAs) for increased target efficiency. These mutagenic oligonucleotides are delivered to plant protoplasts by conventional transfection methods. The availability of efficient protoplast techniques allows the regeneration of fully fertile plants carrying specific mutations after KeyBase™ treatment in tobacco, tomato and petunia. For KeyGene, the method is now routine to produce herbicide-tolerant plants in species for which efficient protoplast techniques are available.

3. Legislative situation

The definition of GMO provided by Directive 2001/18/EC was developed ten years ago. This was before the time of these new plant breeding techniques and raises questions about their legal status (Test attached).

Annex IB of Directive 2001/18/EC lists techniques/methods which are excluded from the Directive, on the condition that they do not involve the use of recombinant nucleic acid molecules or genetically modified organisms, and includes mutagenesis on this basis.

4. Report from the working group on new breeding techniques

Since 2007 a working group of Member States' experts has been studying OdM and seven other new techniques in order to clarify whether these techniques fall under the current EU GM legislation (Directive 2001/18/EC on the deliberate release of genetically modified organisms into the environment and Directive 2009/41/EC on the contained use of genetically modified microorganisms). The report of this working group was finalised end of 2011 and was distributed to Member States' Competent Authorities on January 2012 for discussion.

The working group concluded that there were two possible interpretations about the coverage of ODM by the GMO legislation:

1. ODM is not included on the grounds that oligonucleotides introduced into the cell are not recombinant nucleic acid molecules capable of continued propagation (point 1) and they are not heritable material (point 2). Furthermore, the resulting organisms from ODM are captured by Annex IB above because the technique entails mutagenesis. Mutagenesis is listed as one of the techniques yielding organisms to be excluded from the application of Directive 2001/18/EC and Directive 2009/41/EC. This represents the view of a majority of experts.

2. ODM is similar to techniques listed in Annex IA, Part 1, because ODM is a recombinant nucleic acid technique that (i) leads to a new combination of genetic material resulting in a heritable change in the DNA sequence (point 1) and (ii) it involves the direct introduction of heritable material prepared outside of the organism (point 2). On this basis, ODM falls under the scope of Directive 2001/18/EC and Directive 2009/41/EC. This represents the view of a minority of experts.

There was a discussion in the working group on how many nucleotides could constitute a new combination of genetic material/nucleic acid molecules. A majority of experts concluded that in order to form a new combination, a nucleotide sequence of at least 20 base pairs would be required. A minority of experts were of the opinion that under the current definition, the replacement of only one nucleotide in a nucleic acid molecule could be interpreted as producing a recombinant nucleic acid. Consequently, the experts were divided on whether OdM would be excluded from the scope. A majority argued in favour as OdM is a form of mutagenesis, a minority argued that the exclusion of mutagenesis does not include cases that involve the use of recombinant nucleic acid molecules, adding that OdM is a recombinant nucleic acid technique and it involves the direct introduction of heritable genetic material.

Regarding risks associated with new techniques EFSA has not yet been mandated to provide an opinion on ODM. However, EFSA has started work on cisgenesis and intragenesis, and is

expected to finalise its opinion on these new techniques in the beginning of 2012. The other new techniques remain one of EFSA's work areas for 2012 work

A recent study by the JRC (IPTS) commissioned by DG SANCO concluded that the EU continues leading in the field of innovation in plant breeding: 45% of peer-reviewed scientific research publications in the field worldwide are produced in the EU, followed by North America with 32%. On the other hand, 65% of the total patenting of resulting technologies is carried out by US-based institutions.

The study also found that ODM, cisgenesis/intragenesis and agro-infiltration are the most used techniques (by four companies each) and the crops developed with these techniques have reached commercial development phase. The following crop/trait combinations are likely to be among the first commercial products derived from these technologies: herbicide resistance in oilseed rape and maize (ODM), fungal resistance in potatoes, drought tolerance in maize, scab resistant apples and potatoes with reduced amylase content (cisgenesis/intragenesis). The report concludes that commercial crop varieties could be available in two to three years.

On detection the IPTS Report concluded that mutations that result from ODM can be detected by PCR-based methods as long as certain information on the nucleotides in the vicinity of the mutation is known. On the other hand it is not possible to distinguish, at the molecular level, organisms developed through ODM from organisms bearing the same mutation obtained through other mutation techniques (chemical or radiation mutagenesis).

The main driver for the adoption of new plant breeding techniques is the potential for producing genetic variation, the first step in plant breeding, and the second main driver their economic advantages.

5. KeyGene's view on the regulatory status of oligonucleotide-directed mutagenesis

KeyGene considers the OdM technique used by them (KeyBase™) is out of scope of Directive 2001/18/EC, on the following grounds:

- The "oligo" used by them is not a nucleic acid *sensu strictu* but a chemically altered nucleic acid.
- Modifications are made by the host's own repair mechanisms.
- After mutagenesis the "oligo" is degraded and disappears from the cell.
- The resulting organism only undergoes a small and well defined genetic change at a single predicted location (1 base pair exchange).
- In traditional plant breeding, chemical or irradiation induced mutagenesis can be used without having to apply for an authorisation. The use of these techniques results in a large number of random mutations at many unpredicted locations with in the plant genome
- A mutation achieved using OdM could also occur naturally. It would be impossible to distinguish one from the other at the molecular level. Similarly, no distinction would be possible if the mutation derived from conventional mutagenesis techniques.

A precedent for KeyGene's view of the regulatory status of OdM has been set by the UK Competent Authority for Directive 2001/18/EC, where a crop variety produced by OdM using a technology developed by the company CIBUS was classified as not being covered by Directive 2001/18/EC. In a specific case, the Community Plant Variety Office has recently also been confronted with the question of the regulatory status of OdM. For the time being, the Commission did not respond in substance but referred to ongoing activities to clarify the issue (working group report, future discussions with Member States).

E1 met KeyGene's CEO on 22 June 2011 in Brussels and is currently preparing a note for Commissioner, on a line to take on whether new techniques such as OdM are covered by Directive 2001/18/EC. This line would have to be confirmed by the Legal service.

6. Suggested line to take

- The Commission is analysing, together with the Member States' Competent Authorities, the best way forward to clarify the regulatory status of the various new techniques.
- The opinion of EFSA on the potential risks involved is an important element in this exercise. EFSA will finish a first opinion on cis- and intragenesis beginning of 2012, however, it has not yet started work on OdM.
- In order to decide whether given products/techniques are subject to the GM legislation, a case-by-case basis using concrete examples of particular techniques will be followed by the Commission.
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- Happy to listen to their arguments.

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