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Oligonucleotide-Directed Mutagenesis (ODM)

Classical mutagenesis approaches (*e.g.* chemical/radiation) have been widely employed in the development of numerous commercial crops (*e.g.* see the FAO/IAEA mutant variety database at http://mvgs.iaea.org/AboutMutantVarities.aspx). Such approaches are based on the creation of a large pool of randomly mutagenized plants followed by years of breeding to filter out undesirable mutations and to identify plants with desired improved characteristics (Novak and Brunner, 1992). Over the past decade, a variety of knowledge-based mutational breeding tools, including ODM and Site Directed Nucleases (SDNs), have been developed that result in plant varieties with desirable characteristics in a more precise and efficient manner than classical breeding and mutagenesis approaches.

ODM is a tool for targeted mutagenesis, employing a specific oligonucleotide, typically 20-100 bp in length, to produce a single DNA base change in the plant genome (Beetham *et al.*, 1999; Zhu *et al.*, 1999).The oligonucleotide is identical (homologous) to a unique, predetermined DNA sequence in the plant genome, with the exception of a single base-pair change. When cultured plant cells are temporarily exposed to these short oligonucleotide sequences (repair templates), the repair template binds to the corresponding homologous plant DNA sequence. Once bound, the cell's natural repair machinery recognizes the single base mismatch between its own DNA and that of the repair template. Due to this difference, the cell will repair its DNA sequence by copying the mismatch into its own DNA sequence. As a result, the desired specific change in the plant's genome is produced and the oligonucleotide is subsequently degraded by the cell. Plants carrying the specific mutation are subsequently regenerated by tissue culture techniques and traditional breeding is deployed to efficiently breed the desirable trait into elite plant varities while eliminating undesirable characterizes.

The ODM technique has been successfully applied in several plant crops, *e.g.* to generate herbicide tolerance (Beetham *et al.*, 1999; Zhu *et al.*, 1999; Okuzaki and Toriyama, 2004; Dong *et al.*, 2006). In addition, ODM has the potential to take advantage of the plant's own genome and improve crops through enhanced disease resistance (insect, bacterial, virus), improved nutritional value and enhanced yield without the introduction of new genetic material.

Over the past century, traditional mutagenesis techniques have resulted in over 3,200[1] improved crop varieties in over 175 plant species including rice, maize, wheat, tomato, squash and soybean that have been safely planted and consumed. Due to the long history and its role in production of improved crop varieties, mutagenesis is globally considered to contribute to the production of safe, reliable and sustainable crops. As a result, products developed through mutation breeding are excluded from most country's legislations.

Due to the controlled, precise manner of ODM, random mutations are avoided, the process does not involve recombinant DNA and the final product produced is similar and in many cases indistinguishable from conventionally bred or traditional mutagenesis products. In addition, once developed, traditional breeding (filtering) processes are deployed to efficiently breed the desired mutation into elite plant varieties while eliminating the donor plant genetic background. ODM is conceptually an improved technique over conventional breeding and traditional mutagenesis techniques. Based on these facts, it can be concluded that ODM products should be viewed and treated by regulatory authorities in the same manner as traditional mutational products.

[1] http://www-infocris.iaea.org/MVD/default.htm

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