New plant breeding techniques – what is that?

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Plant breeding is about changing the genetic composition of our crops, so that they better meet our needs. In recent years, a number of new techniques have been developed, which are called new breeding techniques or new plant breeding techniques. It is not yet clarified how in purely legal terms we shall regulate these new techniques. Regulations will determine who can use the techniques and how.

In this brochure you can learn about the technical aspects of new plant breeding techniques.

What are the "new plant breeding techniques"?
The term covers a number of plant breeding techniques that are relatively new, and which we do not yet know how they will be regulated legally.

As with the existing plant breeding techniques, plant breeders can use the new techniques to change the genetic composition of our crop plants.

One term – many different techniques
The term "new plant breeding techniques" covers a large number of techniques, which work very differently and which produce different results. In addition, new techniques are being constantly discovered, so it is difficult for any discussion to cover all of the new techniques in one go.

We have divided the techniques into three categories:

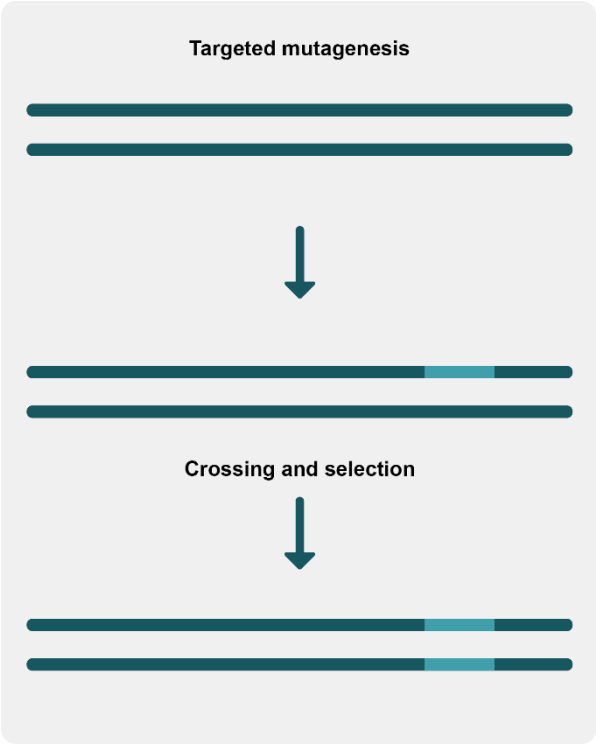
- Techniques for targeted mutagenesis
- Cisgenic Techniques
- Transgenic Techniques

However, it is not clear which techniques belong to which category of techniques. Some of the new breeding techniques can be used in different ways, e.g. CRISPR/Cas9. This means that the same technique can be placed in more than one of the named categories.

<u>Techniques for targeted mutagenesis</u>
Techniques for targeted mutagenesis cause small changes – mutations in the plant's genome. The techniques only contribute to changes that could also occur naturally, and they do not introduce new, foreign genes.

Mutations occur randomly all the time in nature and ensure that genetic variation occurs in plants. Plant breeders have for many years used radiation or chemical treatment to promote random mutations. The problem with random mutations occurring is that they do not necessarily occur where the plant breeder wants them to occur. With the new precision mutagenic techniques, plant breeders can add targeted mutations in specific places in the genome. This makes their work easier and faster.

It is not possible to subsequently detect if a given mutation has occurred naturally or if it was a result of the new precision mutagenic techniques. This is because, as stated previously, these techniques only result in genetic changes that could have occurred naturally.



Enzymatic mutagenesis (Zinc-Finger Nucleases, TALEN, CRISPR/Cas9)

These are techniques where the breeder uses enzymes to cut the DNA strand at the position where the plant breeder wants to create a mutation. The plant will subsequently put the ends back together again, but in some cases the plant cell's repair system will make a mistake, which causes a mutation to occur where the ends meet.

After the procedure, the mutation isinherited in the normal manner.

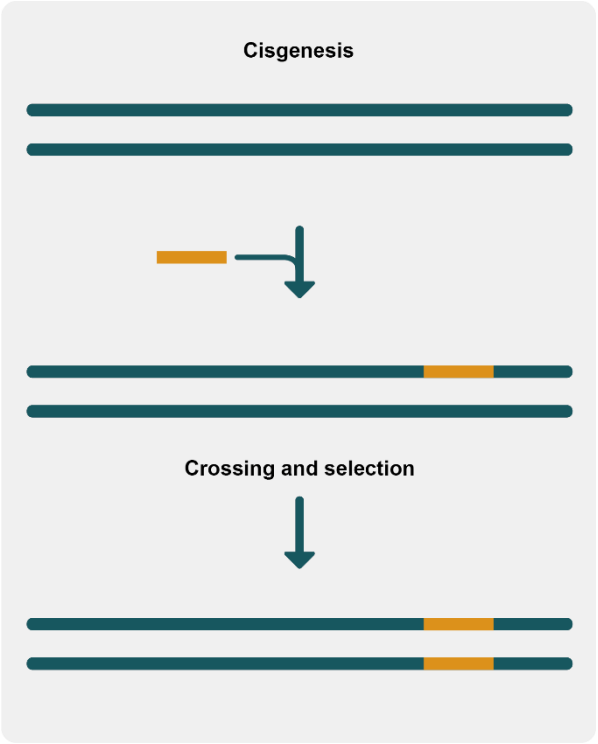
Oligonucleotide-directed mutagenesis (ODM)

In this method, the plant breeder introduces small pieces of synthetic DNA (oligonucleotides) into a living plant cell where it binds to the DNA sequence, which the breeder wants to change. The plant breeder can make the plant replace the synthetic DNA with the plant's own DNA by using the plant's DNA repair system. The desired mutation is produced in this way. Depending on the method that is used to introduce new genetic material or to carry out minor changes (mutations), this method can also be considered as a transgenic technique or a mutagenic technique.

After the procedure, the mutation is inherited in the normal manner.

Cisgenic Techniques

In contrast to mutagenic techniques, cisgenic techniques introduce new genes into the recipient plant. The genes are introduced into the recipient plant by using the same techniques as gene splicing, but the new genes only come from plants that naturally can be crossed with the recipient plant, i.e. plants from the same species or very closely related species.
These types of genes can also be transferred using time-consuming cross-breeding over several generations, but the new cisgenic techniques allow the plant breeder to work much faster.



After the procedure, the the introduced genes are inherited in the normal manner.

In pure technical terms, plant breeders can insert the new genes using several different methods. They can for example, use one of the previously described enzymatic mutagenic techniques, such as CRISPR/Cas9 and during the process supplement this with a gene they want to insert. They can also use traditional gene splicing, e.g. using the soil bacterium (*Agrobacterium*), which we describe in a later section.

In some cases it will be possible to subsequently show that a cisgenic technique has been used to produce a given plant, while in other cases it will not be possible. For example, it will always be possible to show that intragenic techniques (see below) have been used.

Intragenesis

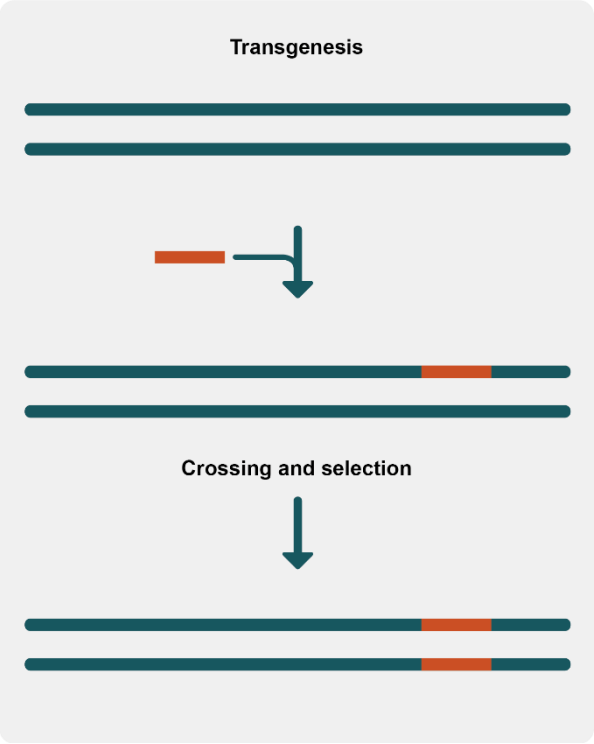
Intragenesis is a variant of cisgenic techniques. In this case, the plant breeders only insert genes from plants

that can be crossed with the recipient plant, but they insert e.g. several copies of genes or in another sequence than occurs naturally.

Transgenic Techniques

The plant breeders can create changes by taking DNA sequences from a second organism and transfer them to the crop plant using gene splicing. This is called transgenesis. The products contain one or several DNA sequences or often several genes that are foreign to the plant species.

Transgenic techniques are characterised by products that are created to introduce DNA from organisms, which the plant does not exchange genes with naturally. The genes can be transferred using a number of transformation techniques, e.g. agroinfiltration, floral-dip, agrobacterium-transformation, etc. It can also occur as an extended form of the previously described enzymatic mutagenic techniques, where in the transgenesis version, a longer DNA sequence is used as a template for the change.



The potential to subsequently show that a given plant was produced using transgenesis is good. It is relatively simple to show that there are genes in the plant that do not occur naturally in the plant.

Reverse breeding

Reverse breeding is used to produce double haploid lines, i.e. plants where the two alleles for all genes are uniform. It is normally very time consuming to develop double haploid lines but using this technique it can be done in a very short time. The technique is based on a modified intermediary stage (produced using RdDM, see later).

Plants which are produced by reverse breeding have no foreign genes and behave and cross breed normally with fellow species. The plant could also have emerged using more time consuming cross breeding, and therefore it is subsequently not possible to show that the plant was produced using reversed breeding.

Grafting on genetically modified root stock

With this technique a GM root stock is made using traditional gene splicing (transgenesis). On this root stock, a non-GM variety scion is grafted. The tecniques could be used in e.g. apples. Even though the root stock is GM, the apples that are harvested will not be GM. Therefore, the authorities cannot detect that the apples in question have been grown using a GM root stock.

Sources:

- New plant breeding techniques. State-of-the-art and prospects for commercial development. JRC Scientific and Technical Reports, 2011.
- New Breeding Techniques: Necessary tools to address forthcoming challenges in plant breeding, Position paper of August 2014 from the GIS BV of All Envi Alliance.

Word definitions

Cisgenesis: Made from two words cis, which means “same” and genesis, which means creation or origin. In this context, the term refers to the DNA sequences being moved between individuals within the same species.

Intragenesis: Made from two words *intra*, which means “within” or “in” and *genesis*, which means creation or origin. In this context, the term refers to the DNA sequences being moved between species from within the same genus.

Transgenesis: Made from two words *trans*, which means “across” or “beyond” and *genesis*, which means creation or origin. In this context, the term refers to the DNA sequences being moved between individuals across species barriers/cross-breeding barriers.

This discussion paper is one of three that sheds light on different aspects of the new plant breeding techniques. The two other discussion papers deal with the techniques’ potential and their regulation with regard to the potential risks involved in their respective use. The discussion papers have been devised in consultation with a broad working group, which the Ministry of Environment and Food of Denmark has established to identify Danish stakeholders’ attitudes to the issue. However, the Danish Agricultural Agency is solely responsible for the discussion papers.