# Fact sheets on New Breeding Techniques

This series of fact sheets was produced by the NBT Platform in 2013.

The NBT Platform is a coalition of SMEs, large industry representatives and members of prominent academic and research institutes which strives to bring clarity to the European debate on NBTs. Its aim is to provide policy makers and stakeholders with clear and precise information on NBTs and to generate awareness about their widespread benefits for the European economy and society as a whole.

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# New Breeding Techniques: seizing the opportunity

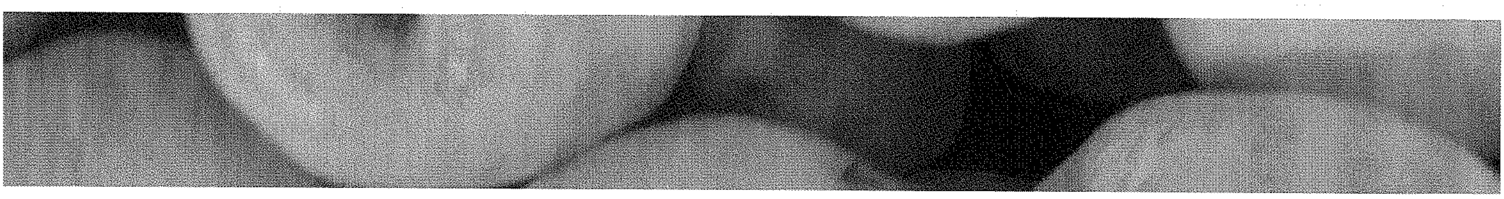
**Globally, we are faced with important challenges relating to food security, sustainability and climate change. Therefore, agricultural productivity must increase, and we must find ways of doing so with more respect for the environment and better use of natural resources. In other words, this means that crops need to produce higher yields, while resisting to pests, disease and stress factors such as drought. In this area, the European plant breeding sector has historically played a leading role in developing highly-innovative and sustainable solutions.**

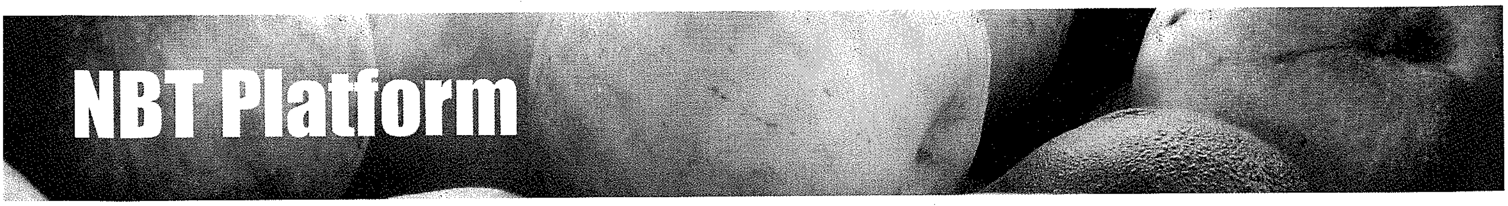
**However, conventional plant breeding relies on techniques that usually require between seven to twenty years to generate the desired characteristics, which - given the need for timely solutions - represents a very long time span. In the last ten years, significant progress has been made in the field of plant genetics. This has enabled scientists in both the private and public sector to develop new methods of plant breeding which can be used to bring in desired characteristics more precisely and efficiently in a wide variety of crops. However, at the EU level, there is some confusion as to if and how these so-called New Breeding Techniques (NBTs) should be regulated. Until legal clarity is reached, the application of these innovative solutions is hampered.**

Developed as a response to the *de facto* moratorium on GMOs that currently exists in Europe, NBTs allow the plant breeding industry to produce plant varieties in a similar - but more precise - manner to that of conventional breeding techniques, and to effectively surpass the limitations we currently face. In Europe, the plant and seed industry is a world leader in terms of innovation, representing a market value of approximately 6.8 billion euro. The innovative nature of this industry, however, is seriously threatened by the fact that in some European Member States, NBTs are being assimilated to the debate on GMOs. While plants resulting from the application of NBTs differ from GMOs, the EU institutions are still in the lengthy process of evaluating whether or not they should fall within the scope of GM legislation.

### New Breeding Techniques: a wide array of advantages

Within the search for solutions to limit the environmental impact of our agricultural activities and to secure a sustainable food supply for a growing world population, NBTs provide a series of advantages. They allow breeders to develop desired plant characteristics at a far more rapid pace. These characteristics include resistance to pests, which results in far less need for pesticides. This not only has a positive impact on the environment and for consumers, but also presents an economic benefit for farmers. Plants that better resist to disease and drought as a result of NBTs also enable a higher yield - more food - and a better use of resources, including water.






## Beneficial technology that is vital to Europe's economy and innovative edge

If the EU can confirm that the products of NBTs do not fall under the scope of GMO legislation, this will give strong impetus to the competitiveness of the European plant breeding sector which, thus far, has carried out almost 50% of the research on NBTs done globally. Indeed, the many small and medium sized enterprises (SMEs) that make up this sector will be freed from the expensive regulatory burden associated with the GMO legislation. This will give them the possibility to focus their resources on research and valorization of innovation - an added value for the European agricultural sector and economy as a whole. Furthermore, this will level the playing field between Europe and other markets, such as the US and Argentina, which are less likely to raise barriers at the breeding level. Indeed, as varieties developed using NBTs are identical in many cases to those developed using conventional breeding techniques, a different regulatory regime could generate issues relating to enforcement.

**New Breeding Techniques (NBTs)**

- NBTs help to improve the precision and efficiency of the plant breeding process, providing more methods for plant breeders to increase food production in a sustainable manner.
- NBTs allow for important breeding objectives - such as increased disease resistance- to be achieved more rapidly and efficiently, which is beneficial for the environment, farmers and consumers.
- NBTs provide impetus to Europe's economy and to the competitiveness and innovation potential of the European plant breeding sector, which represent a 6.8 billion € market.
- The first applications from NBTs could most likely be herbicide resistance in oilseed rape and maize, fungal resistance in potatoes, drought tolerance in maize, scab resistant apples and potatoes with reduced amylase content.

## The NBT Platform: achieving agreement on NBTs at the EU level

The NBT Platform is a coalition of SMEs, large industry representatives and members of prominent academic research institutes, which strives to bring clarity to the European debate on NBTs. Its aim is to provide policy makers and stakeholders with clear and precise information on NBTs and to generate awareness about their potential benefits for the European economy and society as a whole. Ultimately, NBTs provide us with innovative and sustainable solutions for dealing with environmental and food supply challenges, and allow European plant breeders to remain competitive in a global setting.

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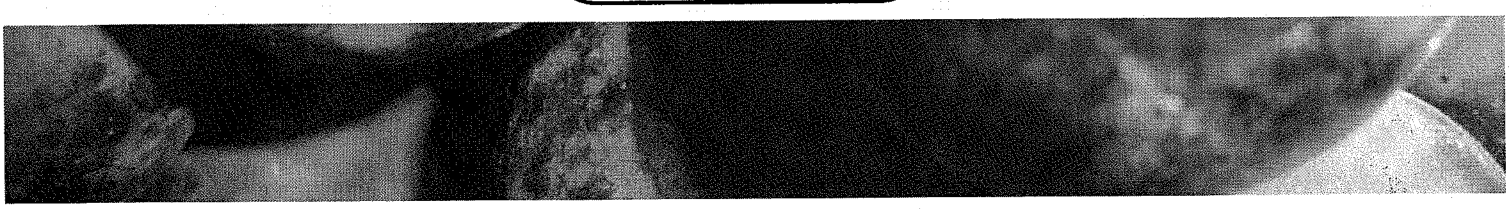

# SDN: Site-Directed Nuclease technology

For many years, plant breeding has been a trial and error exercise, whereby new varieties are produced from a cross between parental plants or through self-pollination. The process is based on identifying a desired characteristic in one plant - for instance higher resistance to a specific disease - and crossing it with another plant which allows the desired trait to appear in the offspring. However, a series of unwanted characteristics is transferred as well, which requires several more breeding cycles in order to be replaced by desired traits. This form of breeding takes many years to accomplish, which represents a very long time span given the need to rapidly address issues linked to climate change and food security. In order to speed up the process and allow for more precision and efficiency, new methods are needed. Several New Breeding Techniques (NBTs) have already been developed, including Site-Directed Nuclease (SDN) technology.

**Obtaining desired characteristics through targeted adaptations**

Three main SDN technologies currently in use include: Meganucleases, Zinc-Finger Nucleases (ZFNs) and Transcription Activator Like Effector Nucleases (TALENs). These technologies rely on biological molecules that have both a DNA-binding domain that recognizes a specific DNA sequence (the site-direction) and a DNA cleavage activity (the nuclease), which, when added to a plant cell, result in a specific, predetermined break in the plant's DNA. The plant's natural DNA repair mechanism recognises this break and repairs the break using enzymes naturally present in the cell.

The goal of SDN technology is to take advantage of the targeted DNA break and the host's natural repair mechanisms to introduce specific small changes at the site of the DNA break. The change can either be a small deletion, a substitution or the addition of a number of nucleotides. Such targeted edits result in a new and desired characteristic, such as enhanced nutrient uptake or decreased production of allergens.

SDN applications are divided into three techniques: SDN-1, SDN-2 and SDN-3 (see Figure 1, opposite):

**SDN-1** produces a double-stranded break in the genome of a plant without the addition of foreign DNA. The spontaneous repair of this break can lead to a mutation or deletion, causing gene silencing, gene knock-out or a change in the activity of a gene.

**SDN-2** produces a double-stranded break, and while the break is repaired by the cell, a small nucleotide template is supplied that is complementary to the area of the break, which in turn, is used by the cell to repair the break. The template contains one or several small sequence changes in the genomic code, which the repair mechanism copies into the plant's genetic material resulting in a mutation of the target gene.

**SDN-3** also induces a double-stranded break in the DNA, but is accompanied by a template containing a gene or other sequence of genetic material. The cell's natural repair process then utilizes this template to repair the break; resulting in the introduction of the genetic material.

SDN-1 and SDN-2 do not use recombinant DNA, do not lead to the insertion of foreign DNA. As such, they do not produce new plant varieties that fall under the scope of the GMO legislation. In the case of SDN-3, the newly developed plant should fall under GMO legislation only if foreign DNA exceeding 20 bp is inserted.

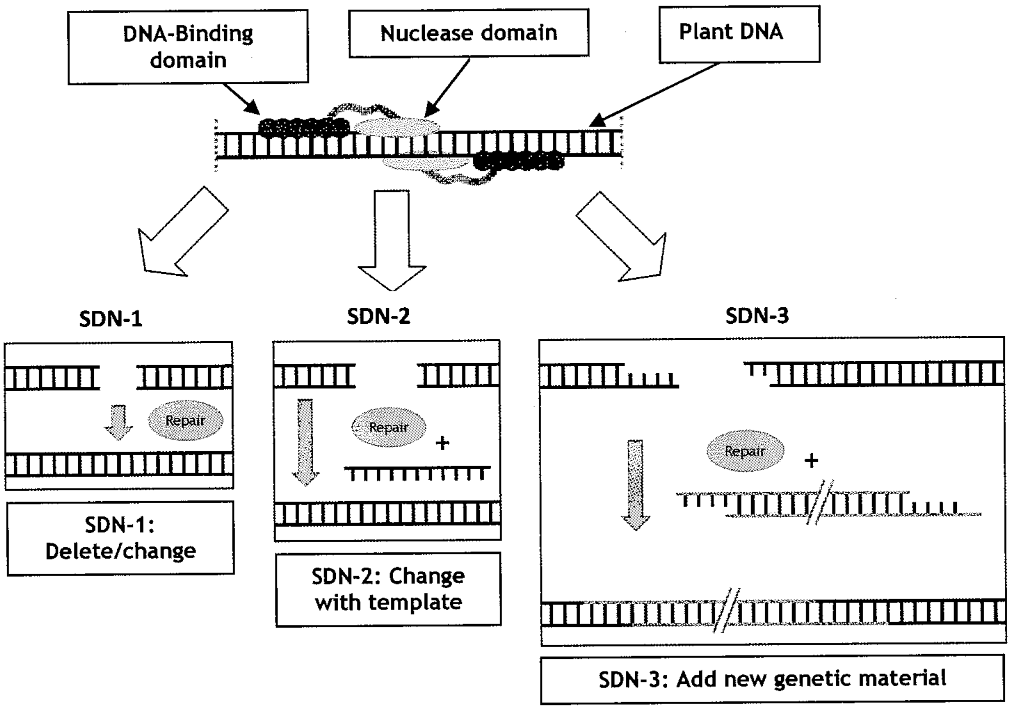


*Figure 1. Simplified overview of the main SDN techniques. In all three techniques, the nuclease domain of the SDN-complex causes a double strand break in the DNA, after which one of the three techniques applies. Nucleotide colour-coding: green equals endogenous, occurring in the plant before the technique is applied; red equals a change in the genetic code. 'Repair' indicates the natural repair mechanism present in the plant.*

### Where can Site-Directed Nuclease technologies be applied?

SDN technologies can create specific and targeted mutations in the genome of a plant, in order to obtain plants with improved characteristics. Random mutations - induced with the help of chemical agents or radiation - have traditionally been used by plant breeders. However these random mutation methods also produce a series of undesired traits which must be eliminated through a series of lengthy breeding cycles. SDN technology allows for specific and targeted mutations, thus enabling new plant varieties to be developed significantly faster than with traditional methods as no further breeding has to be undertaken to eliminate unwanted characteristics.

**Benefits**
Site-Directed Nuclease technology can be used to precisely remove undesirable traits in plants - such as anti-nutrients or allergens, in order to reduce environmental pollution or to enhance the nutritional value of a crop, for instance in maize. It can also modify certain existing characteristics in a plant to respond to consumer needs such as enhanced shelf-life and improved taste or texture, for instance in tomatoes.

### SDN technology: a strong driver for Europe's economy and innovative potential

Small and Medium Enterprises (SMEs) represent a large part of the EU's innovative plant breeding sector. These companies could especially benefit from SDN technology to answer market demands and develop new varieties that are more sustainable, respond to environmental and consumer demands and produce higher yields in a whole range of plants, including fruit and vegetable crops. Before this can happen however, EU Member States must align their position toward Site Directed Nuclease technologies. If they can build on the notion that it allows for new plant varieties to be developed in much the same way as conventional breeding or biological reproduction methods (e.g. asexual reproduction), then the European plant breeding sector can free itself from expensive regulatory burden and enhance its competitiveness. Indeed, companies, and SMEs in particular, will be able to focus their resources on research and valorisation of innovation within Europe rather than having to do so in non-EU countries - an added value for the European agricultural sector and economy as a whole. It will also level the playing field and allow the EU to effectively compete with other markets where the technique could be applied.

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# Oligonucleotide-Directed Mutagenesis: accelerating innovation

For many years, plant breeding has been a trial and error exercise, whereby new varieties are produced from a cross between parental plants or through self-pollination. The process is based on identifying a desired characteristic in one plant - for instance increased resistance to a specific disease - and crossing it with another plant which allows the desired trait to appear in the offspring. However, a series of unwanted characteristics are transferred as well, which require several more breeding cycles in order to be replaced by desired traits. This form of breeding takes many years to accomplish, which represents a very long time span given the need to rapidly address issues linked to climate change and food security. In order to speed up the process and allow for more precision and efficiency, new methods are needed. Several New Breeding Techniques (NBTs) have already been developed, among which Oligonucleotide-Directed Mutagenesis (ODM).

## Producing desired characteristics by single base changes

Nucleotides are organic molecules that form the basic building blocks of DNA, an organism's genetic material. ODM makes use of oligonucleotides - short molecules - to produce a specific single base change within the DNA of a plant. The technique relies on the introduction of an oligonucleotide into a plant cell; the inserted oligonucleotide is identical to part of the plant's genetic material, except for the presence of one intended change. The oligonucleotide acts as a template for the plant's natural DNA repair mechanism, which detects the mismatch between the template and the endogenous genetic material and copies the intended change into the plant's DNA. In this way a desired specific change in the plant's genetic material is produced. The oligonucleotide itself is not inserted into the DNA of the plant; it remains in the plant cell only for a short period of time before it is degraded.

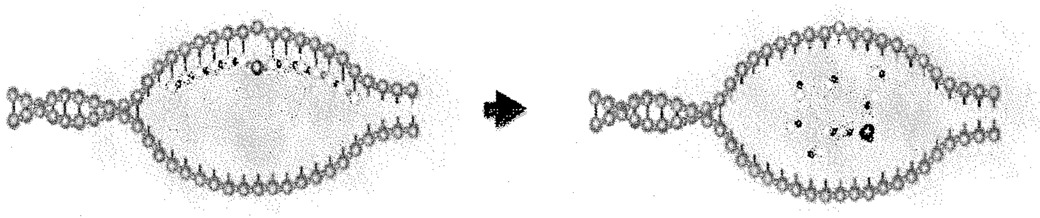

*Figure 1. Simplified illustration of ODM. The left DNA helix (light blue/red) with oligonucleotide template (tan/red) containing one intended mismatch (dark blue). After the endogenous DNA repair mechanism has copied the change (pink) into the DNA, the template is degraded. The strands return to their original form (not shown) and the DNA repair mechanism copies the intended change of one strand into the complementary strand, successfully completing the process.*

**Accelerating plant breeding to produce new plant varieties more quickly**

In practice, ODM consists of mixing plant cells with oligonucleotides, obtaining the desired change in the plant's cells and letting these cells develop into mature plants using regular tissue culture methods. The genetic improvements for useful traits, such as disease resistance, drought tolerance, higher nutritional value, etc. occur without altering the plant's overall genetic makeup or introducing any foreign genes. ODM produces results similar to the natural breeding process, only four times faster and in a controlled precise manner, as the desired trait is the only change generated and no further breeding has to be undertaken to obtain the desired plant.

As ODM does not involve the use of recombinant DNA, or does not lead to the insertion of DNA, the plant varieties that are produced as a result of its application are not covered by GM legislation and are very similar to, and indistinguishable from, those produced using conventional breeding techniques. ODM produces changes that are indistinguishable from those obtained using traditional breeding and mutagenesis, but in a much more controlled and efficient manner.

**Benefits**

ODM can be used to improve a wide variety of plant traits in every plant species, by repairing faulty genes or improve existing ones in a very precise and surgical manner. Examples of traits that can be obtained are for example tolerance to drought or heat (making it possible to grow crops under unfavourable climate conditions), resistance to diseases or insects (resulting in the use of less crop protection chemicals), longer shelf-life, improved nutritional quality or taste, or improved flower colours. The benefits may equally apply to field crops, horticultural crops, ornamentals or even to forestry.

**ODM: a strong driver for Europe's economy and innovative potential**

Small and Medium Enterprises (SMEs), which represent a large part of the EU's innovative plant breeding sector, could in particular benefit from ODM to answer market demands and develop new varieties that are more sustainable or produce higher yields in a whole range of crops. In order for this to become reality, EU Member States must align their position toward Oligonucleotide-Directed Mutagenesis. They must realize that ODM creates varieties similar to those obtained through conventional breeding or natural reproduction and are indistinguishable. Only then can the European plant breeding sector free itself from expensive regulatory burdens and enhance its competitiveness. Indeed, breeding companies, and SMEs in particular, will be able to focus their resources on research and valorisation of innovation within Europe rather than having to move outside Europe - an added value for the European agricultural sector and economy as a whole. It will also level the playing field and allow the EU to effectively compete with other markets where new breeding techniques are already actively applied.

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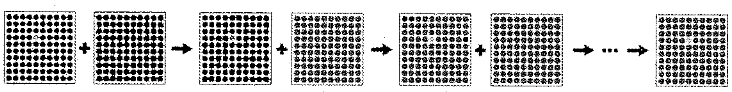
# Cisgenesis: accelerating innovation

For many years, plant breeding has been a trial and error exercise, whereby new varieties are produced from a cross between parental plants or through self-pollination. The process is based on identifying a desired characteristic in one plant - for instance higher resistance to a specific disease - and crossing it with another plant which allows the desired trait to appear in the offspring. However, a series of unwanted characteristics are transferred as well, which require several more breeding cycles in order to be replaced by desired traits. This form of breeding takes many years to accomplish, which represents a very long time span given the need to rapidly address issues linked to climate change and food security. In order to speed up the process and allow for more precision and efficiency, new methods are needed. Several New Breeding Techniques (NBTs) have already been developed, among which cisgenesis.

### A more rapid and precise method of plant breeding

As a technique, cisgenesis is very similar to conventional breeding, but allows for a more specific transfer of genes between closely related crossable plant species. With this technique, a specific trait, such as disease resistance, is transferred from a same or closely related crossable plant species to another - without altering the plant's overall genetic makeup. Cisgenesis allows the natural breeding process to occur up to four times faster and in a controlled manner, as the desired trait is exclusively introduced and no further breeding must be undertaken to eliminate unwanted characteristics in the new plant variety. As with conventional breeding, the donor plant must be crossable with the recipient plant, and the genetic transfer could also occur naturally as a result of crossbreeding. As cisgenesis produces varieties that are comparable to those produced by conventional breeding techniques, the European Food Safety Authority (EFSA) is of the opinion that cisgenesis presents the same level of safety as conventional breeding[1].

**Conventional breeding**



**Cisgenesis**

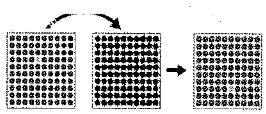


*Figure: Comparison of conventional breeding and cisgenesis for the transfer of a single desired characteristic (yellow dot) from the plant represented in red into the plant represented in blue. Each dot represents a single characteristic. With cisgenesis, a characteristic can be introduced in one step, without losing the properties of the "blue plant".*

### Where could cisgenesis be applied?

Cisgenesis could be used to enhance the durable resistance to diseases in a large number of crops such as potatoes, apples and bananas. This would enable less pesticides to be applied, which would not only have a positive impact on the environment and for consumers, but would also present an economic benefit for producers. Cisgenesis therefore holds great potential for grape cultivators and winemakers. Indeed, as a result of mildew, grapes are one of the most susceptible crops within the EU. Using cisgenesis to introduce mildew resistance in existing grape varieties would significantly contribute to sustainable production within the EU, without changing the characteristics or quality of the grapes. This combination of specific characteristics and resistance cannot be obtained through conventional grape breeding, as the offspring are a genetic mix of two parental varieties and thus do not present the typical qualities and traits - for instance flavour - of the original varieties.

**In 12 years to scab resistant apple trees**

Many apple varieties are susceptible to apple scab and must be sprayed with fungicides 20 to 30 times a year. Cisgenesis allows scab resistant apples to be produced in approximately 12 years - compared to 50 years with conventional breeding techniques. This not only represents substantial cost reductions for farmers, but also benefits consumers and the environment.

**Cisgenesis: added value for Europe's economy and innovative potential**

Small and Medium Enterprises (SMEs), which represent a large part of the EU's innovative plant breeding sector, could especially benefit from cisgenesis to answer market demands and develop new varieties that are more sustainable or produce higher yields in a whole range of crops, including fruit and vegetable crops. Before this can happen however, EU Member States must align their position toward cisgenesis. In January 2012, the EU Expert Working Group on New Breeding Techniques published a report outlining that cisgenesis creates similar varieties to those obtained through conventional breeding techniques or via normal biological reproduction methods. If the EU can build further on this conclusion, the European plant breeding sector will be freed from expensive regulatory burden and its competitiveness will be given a strong boost. Indeed, companies, and SMEs in particular, will be able to focus their resources on research and valorisation of innovation within Europe rather than having to do so in non-EU countries – an added value for the European agricultural sector and economy as a whole. It will also level the playing field and allow the EU to effectively compete with other markets where the technique could be applied.

[1] Scientific opinion addressing the safety assessment of plants developed through cisgenesis and intragenesis (2012). EFSA Journal 10:2561 [33 pp.]. doi:10.2903/j.efsa.2012.2561 http://www.efsa.europa.eu/en/efsajournal/pub/2561.htm

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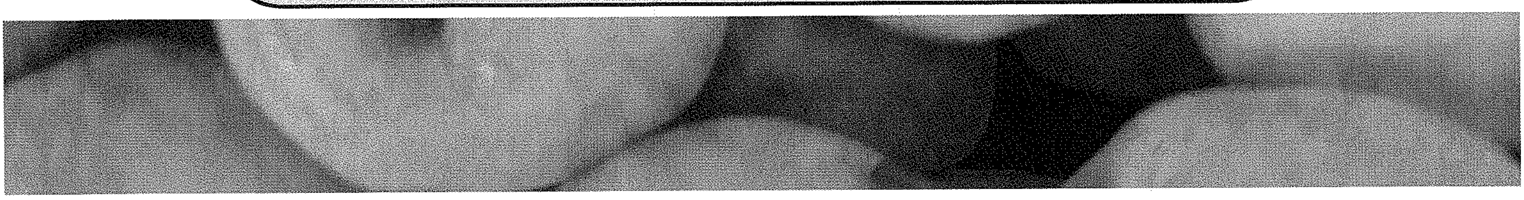


# NBT Platform

**Factsheet Reverse Breeding**

## Reverse Breeding: accelerating innovation

**For many years, plant breeding has been a trial and error exercise, whereby new varieties are produced from a cross between parental plants or through self-pollination. The process is based on identifying a desired characteristic in one plant - for instance higher resistance to a specific disease - and crossing it with another plant which allows the desired trait to appear in the offspring. However, a series of unwanted characteristics is transferred as well, which requires several more breeding cycles in order to be replaced by desired traits. This form of breeding takes many years to accomplish, which represents a very long time span given the need to rapidly address issues linked to climate change and food security. In order to speed up the process and allow for more precision and efficiency, new methods are needed. Several New Breeding Techniques (NBTs) have already been developed, among which Reverse Breeding.**

### A new way of breeding

Reverse Breeding allows production of new hybrid plant varieties in a much shorter timeframe and ambient numbers compared to conventional plant breeding techniques. In Reverse Breeding (illustrated in Figure 1, below), an individual plant is chosen for its elite quality (Fig. 1 A). By suppressing normal genetic recombination (Fig. 1 B&C), homozygous parental lines are derived from this plant. Upon crossing, these parental lines can reconstitute the original genetic composition (Fig. 1 D) of the selected elite plant, from which the lines were derived. During Reverse Breeding, a genetic modification step is employed to suppress genetic recombination (Fig. 1 B), and thus yielding intermediate plants which fall under GMO-legislation (European Directive 2001/18/EU). However, the final selected variety and their parental lines do not contain this genetic modification and thus fall outside the scope of the GMO-legislation.

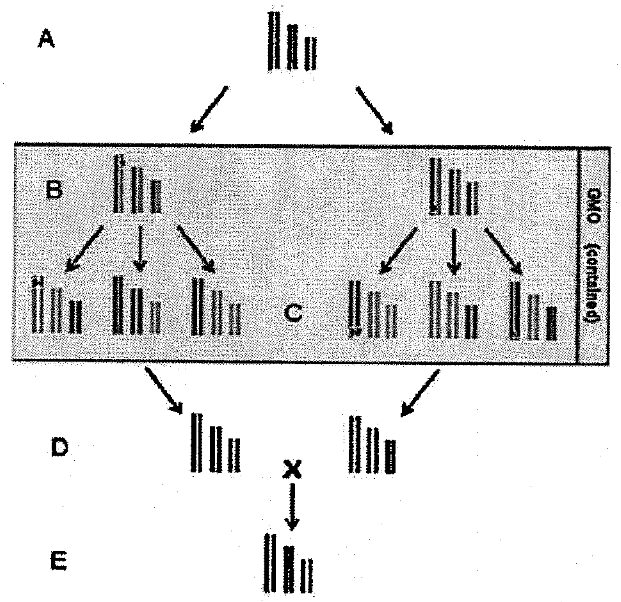
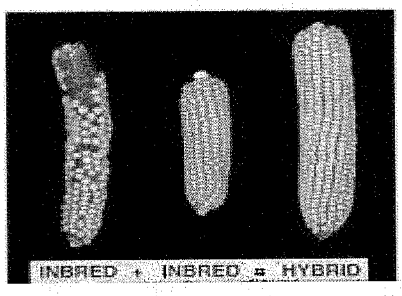



*Figure 1. Schematic explanation of the steps involved in Reverse Breeding. A = Original hybrid (field); B = Transformants (intermediate organism, contained); C = Double haploids (intermediate organism, contained); D = Homozygous parental line (resulting organism, field); E = Reconstructed heterozygous offspring (field)*

**Benefits**
Reverse Breeding accelerates the breeding process considerably and increases the number of available genetic combinations which allows breeders to respond much quicker to the needs of farmers and growers with better varieties.

**Where could Reverse Breeding be applied?**

Practically, Reverse Breeding creates new heterozygous hybrid plant varieties with hybrid vigour (see insert 'Hybrid vigour') which would be difficult and time-consuming to obtain through classical breeding. The classical heterozygous hybrids cannot be stably maintained by breeders due to the natural genetic recombination of the chromosomes. Until now, breeders create elite hybrids afresh by crossing homozygous parental lines (forward breeding). Reverse Breeding can construct homozygous parental lines, that, when mated, perfectly reconstitute the selected heterozygous hybrid plant afterwards. These homozygous parents can be propagated indefinitely by breeders.




**Hybrid vigour**
Hybrid vigour is essential to produce high-yielding varieties in many crops. Reverse Breeding can be used in the crops like; cucumber, onion, broccoli, cauliflower, sugar beet, maize, pea, sorghum, (water-)melon, rice, tomato eggplant and so on. *Source: Nature genetics, volume 44, 2012.*

Although the Reverse Breeding process does involve the use of recombinant DNA, the selected homozygous parental lines and their offspring are <u>non</u> transgenic. The plant varieties that are produced as a result of this application are similar to those that can be produced using conventional breeding techniques.

**Reverse Breeding: added value for Europe's economy and innovative potential**

Small and Medium sized Enterprises (SMEs), which represent a large part of the EU's innovative plant breeding sector, could especially benefit from Reverse Breeding to answer market demands and develop new varieties that are more sustainable or produce higher yields in a whole range of crops, including fruit and vegetable crops. Before this can happen however, EU Member States must align their position toward Reverse Breeding. If they can build on the notion that the technique creates plant varieties which could also be obtained through conventional breeding, the European plant breeding sector will be freed from expensive regulatory burden and its competitiveness will be given a strong boost. Indeed, companies, and SMEs in particular, will be able to focus their resources on research and valorisation of innovation within Europe rather than having to do so in non-EU countries - an added value for the European agricultural sector and economy as a whole. It will also level the playing field and allow the EU to effectively compete with other markets where the technique could be applied.

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# Grafting on GM rootstock: accelerating innovation

For many years, plant breeding has been a trial and error exercise, whereby new varieties are produced from a cross between parental plants or through self-pollination. The process is based on identifying a desired characteristic in one plant - for instance higher resistance to a specific disease - and crossing it with another plant which allows the desired trait to appear in the offspring. However, a series of unwanted characteristics is transferred as well, which requires several more breeding cycles in order to be replaced by desired traits. This form of breeding takes many years to accomplish, which represents a very long time span given the need to rapidly address issues linked to climate change and food security. In order to speed up the process and allow for more precision and efficiency, new methods are needed. Several New Breeding Techniques (NBTs) have already been developed, among which grafting of non-GM scions on GM rootstocks.

## Grafting

The technique of grafting consists of inserting a vegetative part of a plant (termed the scion), usually a shoot or a bud, onto another, root-bearing plant (termed the rootstock), as illustrated in Figure 1 below. Normal vascular flow (e.g. nutrient flow) is established between scion and rootstock if grafting is successful, allowing for the growth and development of the scion. The shoots of the rootstock are usually eliminated, so that all the aerial parts of the grafted plant bear the characteristics of the scion. Grafting is commonly used in horticulture, with fruit trees, vegetables and ornamental plants, for example to combine the quality of the harvested products of the scion with beneficial characteristics of the rootstock, such as resistance to soil-borne disease or more efficient nutrient uptake resulting in a higher yield. Grafting of a non-GM scion onto a GM-rootstock works in the same way, utilising the required and/or beneficial characteristics of a specifically selected GM rootstock.

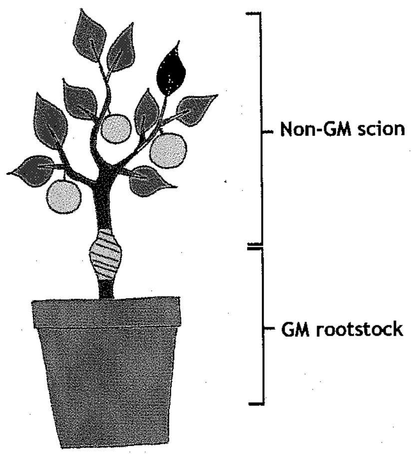


*Figure 1. Simplified illustration of grafting. Here, a fruit bearing non-GM scion has been grafted onto a GM rootstock.*

*Image adapted from: http://ipts.jrc.ec.europa.eu/presentations/documents/15group4.pdf*

Although the rootstock is regarded as GM, and will likely require cultivation approval, it has been established that heritable genetic material is not transmitted from the rootstock to the scion. Signalling molecules can be exchanged that affect the growth and development of the scion or rootstock, but these effects are transient in nature and not heritable. Therefore, any materials harvested from the non-GM scion are regarded as non-GM.

**Benefits**
Grafting can genetically improve rootstocks regarding soil borne diseases and pests. This can prevent treatment with chemical soil disinfectants or steaming of soil that would require a lot of energy. Grafting can also be used to improve the fruit quality of the non-GM scion.

**Where could grafting on GM rootstock be applied?**

GM techniques could be used to confer beneficial characteristics to the rootstock, as mentioned above. For example, GM rootstocks could be used in the future to improve resistance of fruit or forest trees to often devastating nematodes. GM rootstocks could also affect the development of the canopy in a desirable manner (e.g. earlier flowering, dwarfism). Alternatively, GM technology could also be used to create rootstocks with better water use efficiency thereby limiting the plant's need for water.

*Figure 2. Increasing yield of tomato plants with grafting. Higher yield plant (right) is grafted onto a GM-rootstock, while the lower yield plant (left) is grown on its own rootstock.*

**Grafting on GM rootstock: added value for Europe's economy and innovative potential**

Small and Medium Enterprises (SMEs), which represent a large part of the EU's innovative plant breeding sector, could especially benefit from grafting on GM rootstocks to answer market demands and develop new varieties that are more sustainable or produce higher yields in a whole range of crops, including fruit and vegetable crops. Before this can happen however, EU Member States must align their position toward grafting on GM rootstock. In January 2012, the EU Expert Working Group on New Breeding Techniques published a report outlining that grafting on GM rootstock creates products similar to those obtained through conventional breeding techniques or via normal biological reproduction methods, as long as the scion is from a non-GM plant. If the EU can build further on this conclusion, the European plant breeding sector will be freed from expensive regulatory burden and its competitiveness will be given a strong boost. Indeed, companies, and SMEs in particular, will be able to focus their resources on research and valorisation of innovation within Europe rather than having to do so in non-EU countries - an added value for the European agricultural sector and economy as a whole. It will also level the playing field and allow the EU to effectively compete with other markets where the technique could be applied.

**About the NBT Platform**

The NBT Platform is a coalition of SMEs, large industry representatives and members of prominent academic and research institutes which strives to bring clarity to the European debate on NBTs. Its aim is to provide policy makers and stakeholders with clear and precise information on NBTs and to generate awareness about their widespread benefits for the European economy and society as a whole.

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# Agro-infiltration *'sensu stricto'*: accelerating innovation

**For many years, plant breeding has been a trial and error exercise, whereby new varieties are produced from a cross between parental plants or through self-pollination. The process is based on identifying a desired characteristic in one plant - for instance higher resistance to a specific disease - and crossing it with another plant which allows the desired trait to appear in the offspring. However, a series of unwanted characteristics is transferred as well, which requires several more breeding cycles in order to be replaced by desired traits. This form of breeding takes many years to accomplish, which represents a very long time span given the need to rapidly address issues linked to climate change and food security. In order to speed up the process and allow for more precision and efficiency, new methods are needed. Several New Breeding Techniques (NBTs) have already been developed, among which Agro-infiltration *'sensu stricto'*.**

## Increasing availability of natural resistance genes

Until recently, plant breeding has produced plant varieties with new disease resistances through thorough screening of gene pools using classical disease testing in the open field or greenhouses. However, some plant diseases cannot be transmitted to host plants by mechanical inoculation but require an insect as a vector. This poses a serious obstacle to study the level of disease resistance of plant varieties. A New Breeding Technique that has been developed to overcome this obstacle is Agro-infiltration *'sensu stricto'*, which has become an established technology in the field of plant breeding.

Basically, plant parts (e.g. leafs) are brought in contact with cells of the bacterium *Agrobacterium tumefaciens* (in a contained environment, e.g. a greenhouse) which has the capability to transfer and integrate a part of its own DNA into the genome of the plant. This natural capability has been exploited to transfer viral genetic material to a plant cell (see Figure 1, opposite). This transfer effectively mimics a viral infection required to identify plants carrying a viral resistance gene. Resistant plants identified through Agro-infiltration *'sensu stricto'* can be used to produce progeny which is used to develop commercial varieties. Agro-infiltration *'sensu stricto'* is applied very locally on a plant; as a rule, the genetic material is not stably incorporated in the germline and therefore not transmitted to progeny. Plants derived from lines in the absence of a stably integrated event should thus be considered out of the scope of the GM legislation.

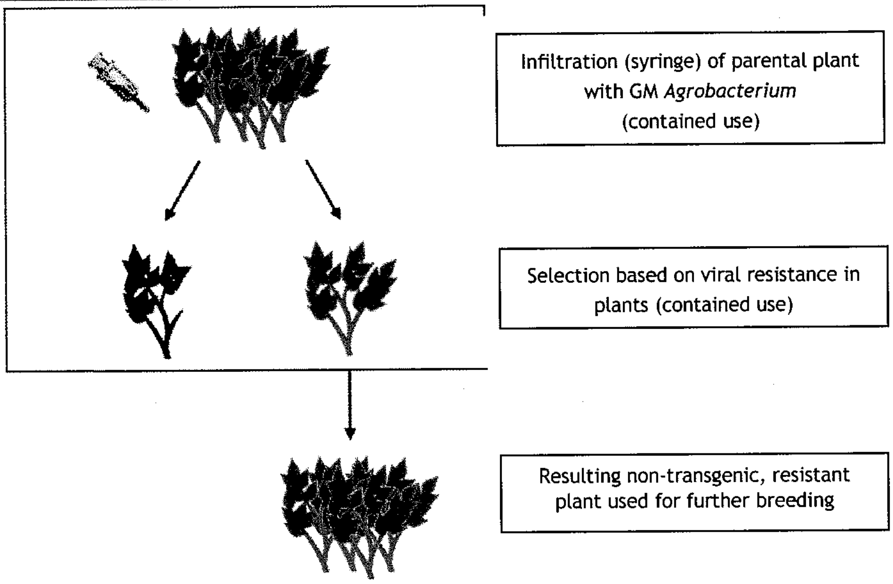


*Figure 1. Schematic representation of Agro-infiltration* 'sensu stricto'*. A syringe is used to apply* Agrobacterium tumefaciens *which contains viral genetic material to mimic a specific viral infection. Resistant variants can be selected after Agro-infiltration* 'sensu stricto'*. The infection is temporary as it is not stably incorporated in the germline and therefore not transmitted to progeny. Therefore, the resulting selected plants are non-transgenic and can be used for further breeding.*

**Where could Agro-infiltration '*sensu stricto*' be applied?**

Plant viruses are responsible for many important plant diseases all around the world. Breeding for natural resistance to plant viruses is a major goal for plant breeding companies. While most resistance tests are easy, some plant viruses cannot be transmitted to host plants by mechanical inoculation. They require an insect as vector, a feature that has heretofore presented a serious obstacle to the selection of resistant plants in plant breeding programs. Utilising insects as vector organisms does not only pose practical problems, it also entails additional biosafety aspects when combining plants, viruses and vector organisms. Agro-infiltration '*sensu stricto*' is proposed as an attractive alternative method for controlled and specific introduction of a plant virus into (parts of) a plant without using an insect vector.

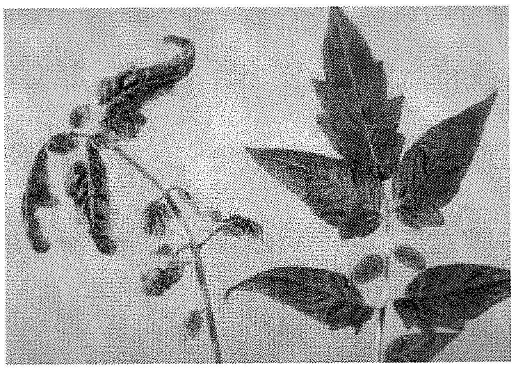

*Figure 2. Tomato yellow leaf curl virus, transmitted by white fly; a susceptible (left) and resistant tomato leaf (right)*

**Benefits**
Disease resistance in plants is essential in practice since it provides protection of crops against pests and pathogens while reducing the need for spraying chemicals. Resistance therefore reduce costs for growers and farmers and are to the benefit of the environment; Agro-infiltration *'sensu stricto'* considerably increases the possibility to find these valuable natural resistances and resistance mechanisms.

**Agro-infiltration '*sensu stricto*': added value for Europe's economy and innovative potential**

Small and Medium sized Enterprises (SMEs), which represent a large part of the EU's innovative plant breeding sector, could especially benefit from Agro-infiltration '*sensu stricto*' to answer market demands and develop new varieties that are more sustainable or produce higher yields in a whole range of crops, including fruit and vegetable crops. Before this can happen however, EU Member States must align their position toward Agro-infiltration '*sensu stricto*'. If they can build on the notion that the technique creates exact the same varieties to those obtained through conventional breeding, the European plant breeding sector will be freed from expensive regulatory burden and its competitiveness will be given a strong boost. Indeed, companies, and SMEs in particular, will be able to focus their resources on research and valorisation of innovation within Europe rather than having to do so in non-EU countries - an added value for the European agricultural sector and economy as a whole. It will also level the playing field and allow the EU to effectively compete with other markets where the technique could be applied.

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