RNA-directed DNA Methylation: 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 can be transferred as well, which require several more breeding cycles in order to be replaced by desired traits. This form of breeding usually 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 RNA-directed DNA Methylation (RdDM).

RNA-directed DNA methylation

In the process of RNA-directed DNA Methylation, short double-stranded RNA molecules (dsRNA) with homology to a target site in the plant genome are introduced into plant cells. This dsRNA is subsequently recognised by the plant's natural defence mechanism, which recruits an enzyme called DICER. DICER subsequently breaks the dsRNAs down into smaller RNA molecules called small interfering RNAs (siRNAs). These siRNA molecules then direct the plant's defense mechanism to methylate the DNA of the target site through a DNA methylation pathway (Figure 1).

Referred to as an 'epigenetic' modification, the plant's nucleotide sequence itself is left unchanged. Rather, the chromatin structure (a complex of nucleotide sequences and proteins) is altered, resulting in decreased activity or even silencing of a specific gene. The resulting plant thus has no change in its genetic material *vis-à-vis* the starting plant material. In most cases these changes are passed from generation to generation in the absence of the original trigger.

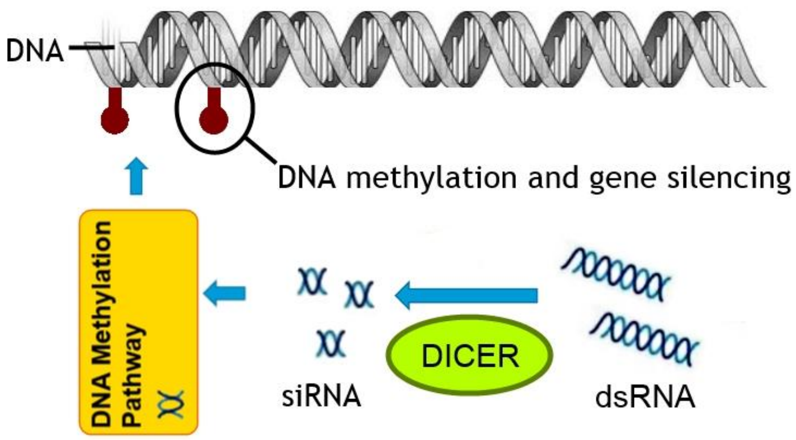

Figure 1: Simplified overview of RdDM - Double stranded RNA (dsRNA) molecules are recognised by the plant's natural defence mechanism. By action of the protein DICER they are cleaved into small interfering RNA (siRNA) molecules. These siRNAs activate the DNA methylation pathway in the plant, causing DNA methylation and silencing of selected genes without altering the DNA sequence.

RNA-directed DNA Methylation can be induced in a variety of ways

The production of the small double stranded RNA molecules used in RdDM can be achieved in different ways. For example, with Virus Induced Gene Silencing (VIGS) the plant is infected with a plant virus that is engineered to produce the dsRNA. Alternatively, it is possible to introduce a transgene that leads to the production of dsRNA.

After the silencing is achieved, the methylation is maintained, resulting in the gene being silenced in future generations, but the genetic material coding for the RNA is lost. In the case of VIGS the original viral genetic material containing the sequences for the production of dsRNA is lost during meiosis; in the case of introduction of a transgene, the gene is removed by crossing with plants that do not possess the transgene. As a result, the final selected variety (the 'product') is not genetically modified since there is no introduction of genetic material into the plant and there has been no alteration of the plant's nucleotide sequence *vis-à-vis* the starting material. The only difference is the specific methylation of a certain stretch of DNA resulting in the desired trait; a common characteristic of all methods of RdDM used for plant breeding. Therefore, the products resulting from the use of RdDM should be outside the scope of Directive 2001/18/EC.

Benefits

RdDM can be used to improve a wide variety of plant traits in most plant species by the down-regulation of an endogenous gene, without alteration of the genetic material. 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 different colours. The benefits apply to field and horticultural crops, ornamentals, forestry, etc.

RNA-directed DNA Methylation: 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 RdDM 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 the use of RdDM. If the EU can embrace this technology, 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 and 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.

Contact us via info@nbtplatform.org