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.

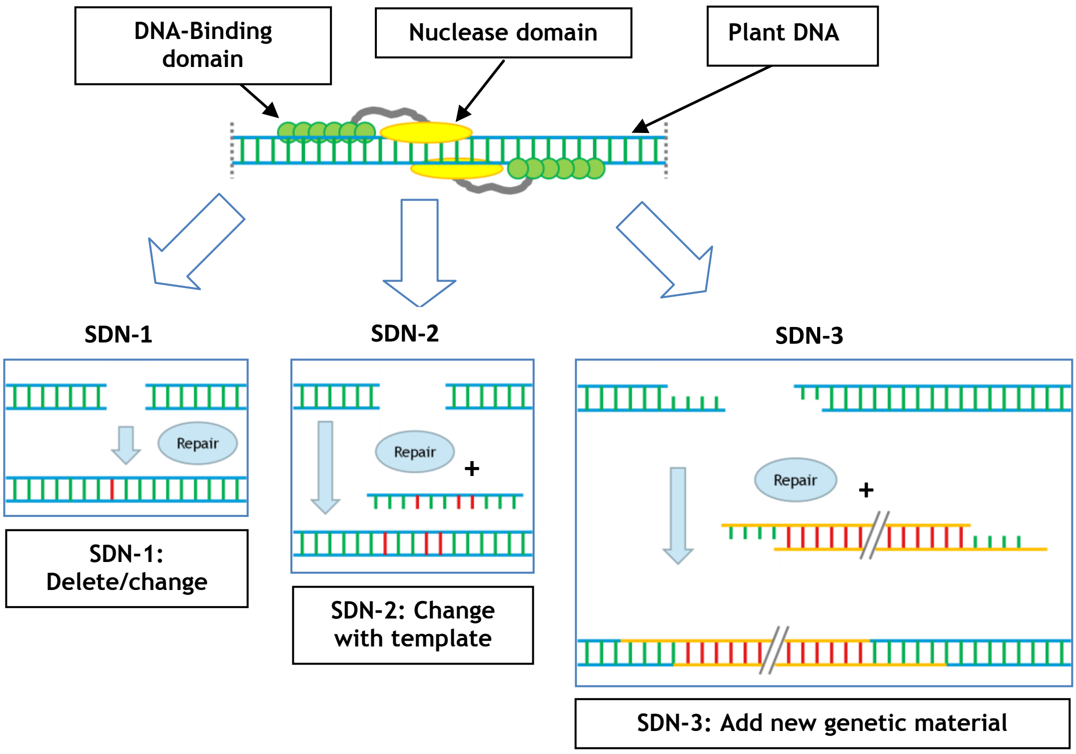

Figure 1. Simplified overview of the main SDN techniques. In all three techniques, the nuclease domain of the SDN-complex causes a double strand break in the DNA, after which one of the three techniques applies. Nucleotide colour-coding: green equals endogenous, occurring in the plant before the technique 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.

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.

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