

## Pocket K No. 59: Plant Breeding Innovation: TALENs Transcription Activator-Like Effector Nucleases

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Plants are constantly exposed to various biotic and abiotic stresses. Thus, scientists continue to develop tools that help improve the field of plant breeding. These new tools and practices are referred to as plant breeding innovations, which include both precision IT-based tools and new molecular breeding techniques.

Precision breeding tools merge innovative digital devices— like sensors, detectors, and robotics—with management technologies for precise and more efficient production system control.<sup>1</sup> New molecular breeding techniques use gene-editing tools such as clustered regularly interspaced short palindromic repeats (CRISPR), transcription activator-like effector nucleases (TALENs), zinc-finger nucleases (ZFNs), and homing endonucleases or meganucleases.

CRISPRs have become the most popular of the genome editing techniques. Yet, TALENs are extremely precise and have additional capabilities, such as targeting any DNA sequence, discriminating between methylated and unmethylated DNA targets, and modifying DNA within organelles such as mitochondria.

### What are TALENs?

Transcription activator-like effectors (TALEs) are proteins made and used by plant pathogenic bacteria to control plant genes during infection. In nature, TALEs bind to plant DNA sequences and activate genes. Binding is targeted to specific DNA sequences through amino acid repeats of the TALE protein that recognize specific DNA bases by a set of biochemical rules or "code". Researchers can use this code to customize TALEs and TALE protein fusions to bind to any desired DNA sequence. TALENs are protein combinations composed of two parts: one part is the TALE that targets the protein to a specific DNA sequence and the second part is a nuclease (N) that cuts DNA. Fok1 is a nuclease that is commonly used in TALENs (Figure 1).

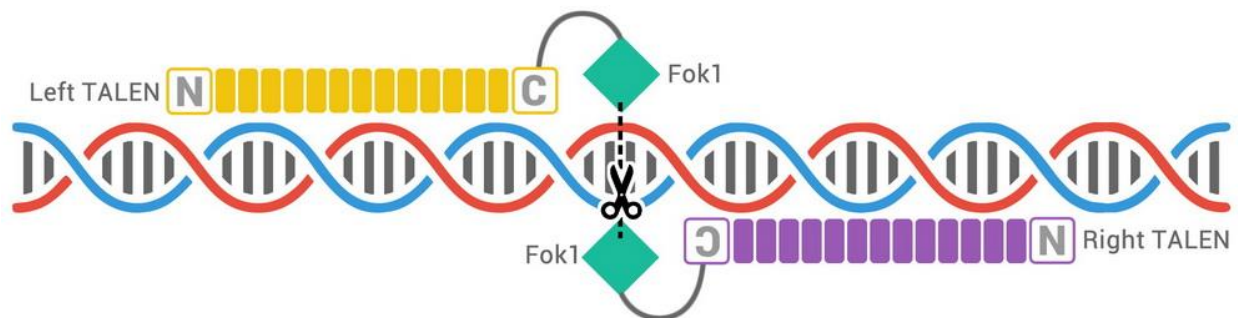


Figure 1. TALENs

TALE-nuclease fusions cut the DNA, making a precise double-strand break. This break initiates the plant cell's existing DNA repair mechanisms which may remove DNA bases around the cut

or incorporate new DNA at the position of the break. The outcome is either small insertions or deletions that create sequence diversity, as often found in nature, leading to new functions or inactivating existing ones. Additionally, new genes can be inserted.<sup>2,3</sup> These molecular scissors provide incredibly precise means to fine-tune traits in a targeted, predictable fashion anywhere in the genome.

Similar to ZFNs, TALENs are modular proteins, but the structure of TALEN DNA binding domains makes them simpler to design.<sup>4</sup>

In 2009, the discovery of the TAL Code was first published in *Science* by Ulla Bonas, Jens Boch, Thomas Lahaye, and Sebastian Schornack at the Martin Luther University in Halle, Germany.<sup>5</sup> The 2Blades Foundation, a US non-profit organization, worked with the scientists to oversee patent protection and licensing for research and commercial applications. Together, the scientists and 2Blades exclusively licensed biomedical rights to Life Technologies (now ThermoFisher) for commercialization and all rights for research and reagent uses while 2Blades Foundation led the licensing for use of TALENs in plants for agriculture.<sup>2</sup>

New uses and improvements to TALENs continue to emerge. For example, in 2020, a rapid TALEN preparation protocol was developed based on the widely used Golden Gate TALEN and TAL Effector Kit 2.0. The protocol involves the use of a set of linear monomers, a Fok1 backbone plasmid, and a straightforward pipeline to assemble the ready-to-use TALEN expression. This TALEN construction pipeline has high reproducibility, reliability, and an efficiency of over 80%. Furthermore, it was shown that these customized TALENs performed with better editing efficiency than CRISPR.<sup>6</sup>

According to Global Market Insights, Inc., the TALENs technology segment reached approximately USD 650 million in 2019. With increasing government support to genome editing, market growth is projected to exceed USD 10 billion by 2026.<sup>7</sup>

### **Applications for Crop Improvement**

TALENs can be applied to help protect plants from the effects of climate change. Genes that play a role in resistance to pests, diseases, or protection from harsh environmental conditions, such as drought and salinity, can be edited to enhance resilience. TALENs have also been used to improve the quality of products derived from crops.

#### **Soybeans**

Oils with low polyunsaturated fats are considered to be a healthier alternative to those high in polyunsaturated fats which can be hydrogenated and produce unhealthy trans-fatty acids. Soybean lines with low levels of polyunsaturated fats were developed using TALENs by introducing stacked mutations in two fatty acid desaturase 2 genes (*FAD2-1A* and *FAD2-1B*) which confer changes in fatty acid desaturase 3A (*FAD3A*). This led to oleic acid levels of over 80% and linoleic acid levels under 4%.<sup>8</sup>

Now gene-edited soybean plants produce premium quality high-oleic soybean oil sold as Calyno by Calyxt. It became available in the U.S. market in 2019, making it the first commercialized product from a gene-edited plant.<sup>9,10</sup>

## Rice

TALENs have been used in rice, one of the world's most important food crops, to engineer resistance to the destructive disease, bacterial blight, caused by *Xanthomonas oryzae*. The rice gene *OsSWEET14* encodes an essential function for *Xanthomonas* to infect and cause bacterial blight. By editing the regulatory region of the gene, researchers generated heritable disease resistance.<sup>11</sup>

Additionally, non-aromatic rice varieties were transformed into aromatic rice varieties using TALENs. TALENs were engineered to target and disrupt the *OsBADH2* gene, resulting in the production of the major fragrance compound 2-acetyl-1-pyrroline.<sup>12, 13</sup>

## Potato

The commercial potato variety, Ranger Russet, suffers from browning, bitter taste, and high levels of potentially carcinogenic acrylamides. TALENs were used to knockout the vacuolar invertase gene (*Vlnv*) producing improved properties. The amounts of reducing sugars were undetectable, and chips produced from these potatoes had a reduced level of acrylamide and a more desirable light color.<sup>14</sup>

To reduce cholesterol and related toxic steroidal glycoalkaloids, TALENs targeting the *SSR2* gene were delivered into potato using a transient *Agrobacterium* transformation method. In the resulting potato lines the *SSR2* gene was inactivated and no TALEN transgene remained in the plant. This new method can help to accelerate the use of genome editing technology to benefit crops like potato that do not have parent lines with homozygous genomes, such as inbred lines.<sup>15</sup>

## Maize

To develop TALEN tools for corn (maize), scientists made stable, heritable mutations at a visible marker, the *glossy2* (*gl2*) locus. Transgenic lines containing mutations were obtained and three of the novel alleles were able to confer a glossy phenotype. The use of TALENs in maize is a powerful tool for genome mutagenesis, discovery of gene function, and trait improvement.<sup>16</sup>

## Wheat

Whereas many organisms have one set of chromosome pairs, bread wheat varieties carry three pairs, increasing the complexity for gene editing. Scientists attempted to engineer resistance to powdery mildew, a widespread disease, by testing both TALENS and CRISPR-Cas9 on the MILDEW-RESISTANCE LOCUS (*mlo*) gene. They succeeded in making TALEN-induced mutations

in all six sets of *mlo* genes, resulting in plants with essentially complete resistance to the wheat powdery mildew pathogen.<sup>17</sup>

### **Access to TALENs**

The 2Blades Foundation holds exclusive global rights for uses of the TAL Code and TALENs in plants. 2Blades has worked extensively to create simple, broad access to this versatile platform to improve the efficiency and precision of plant breeding.

For commercial applications it has licensed on a non-exclusive, tiered basis to a wide range of users, from large commercial seed companies to smaller agribiotech companies. 2Blades also gives no-cost TALEN rights to non-profit and multilateral entities, such as the International Rice Research Institute, to facilitate the improvement of crop varieties and support innovation to benefit food security.<sup>2</sup>

### **Further applications**

#### Biofuel: Sugarcane

Sugarcane is known as one of the most efficient feedstocks used for bioethanol production because of its superior production of biomass and accumulation of sucrose in stems. To further enhance its biofuel yield, researchers successfully targeted 107 out of 109 copies of sugarcane's lignin biosynthetic gene *caffeic acid O-methyltransferase (COMT)* using TALENs. This led to mutants with 19.7% lignin reduction and up to 43.8% improved saccharification efficiency without affecting biomass production and maintaining good agronomic performance.<sup>18</sup>

#### Algae

Algae, such as *Nannochloropsis* species, accumulate large amounts of lipids through photosynthesis. These lipids can be utilized as feedstocks for sustainable new energy products called biodiesels. However, production of algal biodiesel is more expensive than fossil fuel production.

All-in-one expression vectors for Platinum TALENs targeting the nitrate reductase gene, *NoNR*, and the acyltransferase gene, *LPAT1*, were transfected into *Nannochloropsis oceanica*. High genome-editing efficiency was achieved without any off-target editing. Edits in both genes were obtained through the simultaneous introduction of TALENs targeting each gene. The loss-of-function phenotype of *NoNR* was also confirmed. The findings could help develop techniques for molecular breeding of *Nannochloropsis* species for biofuel production.<sup>19</sup>

Yeast, insects, and mammals

Outside of plants and algae, TALENs have been used successfully to modify genes in yeast, fruit fly, roundworm, crickets, zebrafish, frog, rat, pig, cow, silkworm, and humans.<sup>20,21</sup>

Recently, a new tool—TALE-based editors—were used to successfully edit the mitochondrial DNA within living cells in a precise manner. Scientists found a chemical that enabled them to change a cytosine base in the DNA code to thiamine, enabling the correction of specific DNA bases in the mitochondrial genome. This was achieved by delivering a TALE -based editor that can cross the mitochondrial membrane, resulting in the precise desired change. The novel approach may eventually become a tool that scientists use to make better models of mitochondrial diseases, and TALE editors can provide the means to treat mitochondrial associated diseases.<sup>22</sup>

Because of its powerful applications to improve plants and other organisms, TALENs have been recognized by various entities. In 2011, *Nature Methods* hailed TALENs as the Method of the Year.<sup>23</sup> The TAL Code was recognized as the Best Novel Agricultural Biotechnology by Agrow Awards in 2012 and was a finalist in 2017.<sup>2,24</sup>

With the need to double food production to feed the projected population of 10 billion by 2050, TALENs and other new breeding innovations are essential tools to ensure food security.

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