



Precision Breeding: Strengthening Crop Improvement with TALENs



ISAAA Inc.

“ The benefit of breeding approaches to crop pests and diseases is using a plant’s inherent, strong immune responses, instead of chemicals – packaging protection directly into the seed. ”

**Diana Horvath
President & Co-Founder of 2Blades**





Every crop growing in the field faces a constant battle with different stressors long before it reaches your plate. Biotic stresses, such as insects, fungi, bacteria, and viruses, reduce yield and affect food quality. These threats are especially concerning in large-scale agriculture, where a single outbreak can destroy an entire season's harvest. If left unmanaged, these stressors can significantly impact global food production, leading to economic losses and food shortages.

At the same time, plants must withstand abiotic stresses that are caused by environmental factors like drought, extreme temperatures, soil degradation, and unpredictable weather patterns, making it difficult for crops to thrive and survive. In regions where water is scarce, prolonged drought can cause crops to wither before they reach maturity.

The combined impact of biotic and abiotic stresses not only threatens farmers' livelihoods but also puts immense pressure on the global food supply. The future of food goes beyond simply, "How to grow more food?" but must also ask how do we protect harvests and grow more efficiently.


While traditional breeding has helped improve crops over time, the process often takes years, even decades,

to develop new plant varieties with desirable traits. With the growing global population, researchers have turned to advanced methods and cutting-edge technologies to develop more resilient crops and grow more food using less land, water, and chemicals. By harnessing innovative solutions using precision breeding, scientists aim to create stronger, more adaptable crops that can sustain food production even in challenging conditions.

Gene editing, a revolutionary technology, has emerged as a powerful precision breeding tool to improve crops through targeted modifications to an organism's DNA. Unlike traditional breeding, gene editing allows scientists to target specific genes responsible for traits like disease resistance, drought tolerance, and improved nutritional content. Among the most widely used gene-editing tools are the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR), TALENs (Transcription Activator-Like Effector Nucleases), and ZFNs (Zinc Finger Nucleases).



**What are
TALENs?**



Transcription activator-like effectors (TALEs) are proteins used by plant pathogenic bacteria to control plant genes during infection by binding to specific DNA sequences.

TALE nucleases (TALENs) are composed of a TALE domain that directs the protein to a specific DNA sequence and a nuclease, commonly Fok1, which cuts the DNA. This precise cut triggers the plant's natural repair mechanisms, leading to small insertions, deletions, or the incorporation of new genetic material. Using this method, researchers can fine-tune plant traits in a controlled manner.

TALENs have unique features relative to other gene editing technologies:

- Exceptional precision, surpassing CRISPR in certain applications
- Ability to target any DNA sequence with very few errors
- Ability to distinguish DNA modifications, such as methylation, that influence gene expression
- Capability to edit DNA in mitochondria and chloroplasts

**How can we
use TALENs
to improve
our
crops?**





As global food demand rises and biotic and abiotic threats intensify, scientists are turning to gene editing to develop crops that are more productive, resilient, and sustainable. TALENs enable scientists to make precise modifications to plant DNA, enhancing key traits that improve both the productivity and quality of crops. By fine-tuning genetic sequences, researchers can develop crops that grow more efficiently, resist environmental stress, and require fewer chemical inputs. This level of precision not only benefits farmers by increasing yields but also helps create more sustainable and resilient agricultural systems.

Improved yields

TALENs enable precise gene editing that optimizes plant growth and improves food production by targeting genes that regulate flowering time, seed development, and nutrient absorption. With these improvements, farmers can increase yields without expanding their land use.



Better taste

Gene editing with TALENs allows scientists to modify flavor-related compounds in crops, making them more appealing and enjoyable to consumers by silencing genes that produce undesirable flavors or enhancing genes that contribute to desirable taste. With better-tasting crops, farmers can meet consumer preferences while reducing the need for artificial sweeteners or flavor enhancers.





Resistance to disease and pests



TALENs help create crops that are naturally resistant to harmful pathogens and insect infestations. Reliance on chemical pesticides is reduced by editing plant genes that make them susceptible to diseases and pests, resulting in healthier crops and more sustainable farming practices.

Improved quality of products derived from crops



TALENs create precise modifications that improve the quality and shelf life of food products derived from crops. This technology has been used to make edits that can enhance the texture, nutritional value, and storage stability of various crops.

Resilience to extreme weather conditions, drought, or salinity



TALENs allow scientists to enhance plant tolerance by modifying genes that regulate water retention, root structure, and stress response. Crops edited with TALENs can maintain growth and yield even when water is scarce or soil contains excessive salt.

Applications of TALENs for Crop Improvement

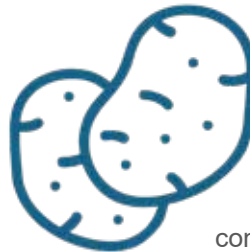
Soybeans

TALENs were used to develop soybean lines with lower polyunsaturated fats by modifying key fatty acid desaturase genes, resulting in healthier high-oleic soybean oil with over 80% oleic acid and less than 4% linoleic acid. This gene-edited oil, launched as Calyno by Calyxt, became the first commercialized product from a gene-edited plant in the U.S. market in 2019.



Potato

TALENs were used to improve the Ranger Russet potato by knocking out the *Vlnv* gene, reducing browning, bitterness, and acrylamide levels while enhancing color and quality. Additionally, TALENs targeting the *SSR2* gene successfully lowered cholesterol-related toxic compounds in potatoes using a transient transformation method, leaving no transgene behind and paving the way for improved genome editing in complex crops.



Rice

TALENs have been used to engineer bacterial blight-resistant rice by modifying the regulatory region of the *OsSWEET14* gene, preventing infection by *Xanthomonas oryzae*. TALENs were also used to disrupt the *OsBADH2* gene, converting non-aromatic rice varieties into aromatic ones by enabling the production of the fragrance compound 2-acetyl-1-pyrroline.





Maize



TALENs were used to create stable, heritable mutations in the *glossy2* (*gl2*) gene in maize, resulting in a distinct glossy phenotype. This demonstrated the potential of TALENs as a powerful tool for genome mutagenesis, gene function discovery, and crop trait enhancement in maize.

Wheat

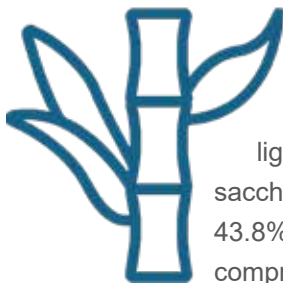


Bread wheat's complex genome, with three pairs of chromosomes, makes gene editing challenging. Scientists used TALENs to target all six copies of the *mlo* gene, successfully engineering nearly complete resistance to powdery mildew, a major wheat disease.

Further Applications of TALENs

Sugarcane

Sugarcane is a top bioethanol feedstock due to its high biomass and sucrose production. Using TALENs, researchers targeted 107 out of 109 copies of the *COMT* gene, reducing lignin by 19.7% and improving saccharification efficiency by up to 43.8%, enhancing biofuel yield without compromising growth or performance.



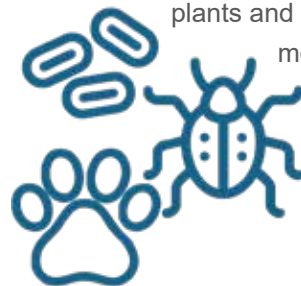
Algae

Algae like *Nannochloropsis* species produce high lipid content for biodiesel, but production costs remain high. Using TALENs, researchers efficiently edited key genes (*NoNR* and *LPAT1*) without off-target effects, demonstrating a successful approach to enhance biofuel production through molecular breeding.



Yeast, insects, and mammals

TALENs have been successfully applied beyond plants and algae, enabling precise gene modifications in various organisms, including yeast, insects, fish, mammals, and even humans. Their versatility has made them a valuable tool in genetic research and biotechnology.



TALE-based editors

TALE-based editors have recently been used to precisely edit mitochondrial DNA within living cells, offering a groundbreaking advancement in genetic research. Scientists identified a chemical that enables the conversion of cytosine to thymine in the mitochondrial genome, allowing for targeted DNA corrections.



By delivering a TALE-based editor capable of crossing the mitochondrial membrane, researchers achieved highly specific genetic changes. This innovation could pave the way for improved models of mitochondrial diseases and potential treatments for disorders linked to mitochondrial mutations.

How Gene Editing with TALENs Works

1. Target selection



Scientists identify the specific DNA sequence they want to modify.

2. Designing TALENs



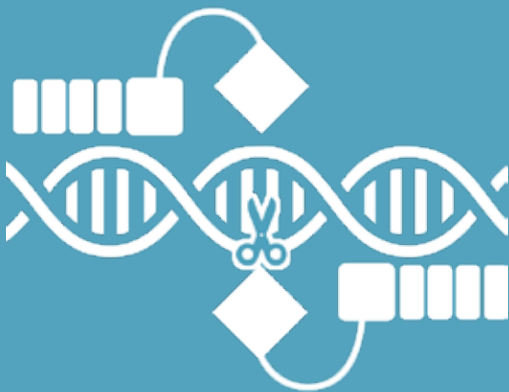
TALENs are created by fusing the TALE with a nuclease, usually Fok1, to cut the DNA.

3. Delivery into cells



The TALENs are introduced into the plant or animal cells using various delivery methods, such as Agrobacterium-mediated transformation, electroporation, or direct injection. Once inside, TALENs bind to the target DNA sequence.

4. Creating the DNA Cleavage



The Fok1 nuclease cuts the DNA at the specific location, creating a double-strand break that signals the cell's natural repair mechanisms to fix the damage.

5. DNA Repair and Modification



The cell naturally tries to repair the cut. During this process, the gene can be turned off or slightly changed or a new piece of DNA can be added to give the plant or animal a desirable trait.

How to access the TALEN technology?

2BLADES
20 YEARS OF CROP INNOVATION

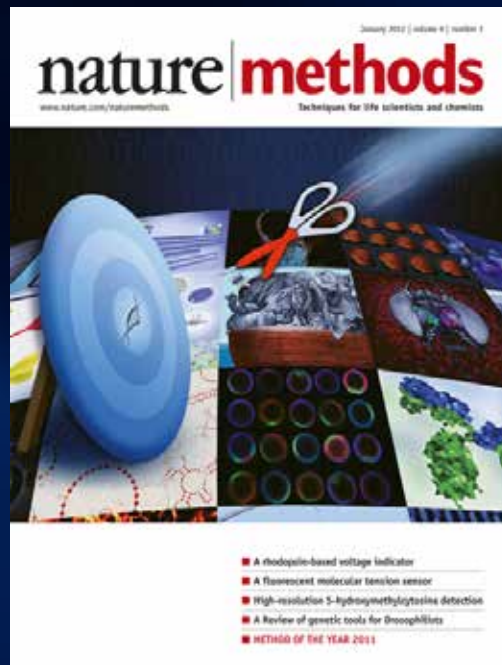


2Blades, a nonprofit agbiotech company, holds exclusive rights for the commercial use of the TALE Code in plants. 2Blades has worked extensively to create simple, broad access to this versatile platform to maximize its benefits for plant breeding. Consistent with its dual-market model, 2Blades creates access to TALENs and the TAL Code for large and small businesses on a tiered basis, including no-cost licenses to non-profit organizations and multilateral entities, such as the International Rice Research Institute (IRRI), to facilitate the improvement of crop varieties and advance agricultural productivity globally.

TALEN technology is empowering researchers to develop crops that can better withstand pests, diseases, and harsh environmental conditions, promising a future with more stable food supplies and reduced agricultural losses.



In 2011, Nature Methods hailed TALENs as the Method of the Year.



EDITORIAL

Method of the Year 2011

The ability to introduce targeted, tailored changes into the genomes of several species will make it feasible to ask more precise biological questions.

In our annual toast to biological research methods and to the scientists who develop them, we have chosen genome editing with engineered nucleases as our Method of the Year 2011. Perhaps the most reliable way to learn about the function of a gene or protein is to specifically perturb it and monitor what happens. This reverse genetic approach is routinely applied in many species, but, with a few exceptions, it is challenging or even impossible to make targeted changes at endogenous genomic loci, arguably the most elegant method of genetic perturbation. Instead, the experimenter must settle for more indirect approaches: overexpressing the modified gene from a heterologous location or knocking it down, often only partially, with an approach such as RNA interference. Engineered nucleases—which can be designed, in principle, to cut at any location in the genome of any species and thus to introduce tailored modifications into the endogenous sequence—are set to change this. We provide a brief Primer on these tools on page 27.

From the perspective of methods development, the trajectory of the engineered nucleases has been a compelling one, as reported in a News Feature on page 21. All three major classes of these enzymes—zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and engineered meganucleases—stand on the shoulders of very basic studies, a testament, yet again, to the necessity of basic research and the difficulty in predicting where technological leaps will come. Engineering the first ZFN to modify an endogenous gene in an organism (the fruit fly, as it aptly turns out), required that three strands of knowledge, gained from many decades of research in many laboratories, be drawn together: how restriction endonucleases work, how DNA breaks are repaired by the cell and how DNA-binding proteins achieve specificity in the vastness of genomic sequence space.

This said, it has undoubtedly been the potential clinical utility of these tools—to correct mutations in monogenic human disease, for instance—that has primarily driven the intense effort put into developing them over the past decades. The fortuitous consequence, however, is that very powerful basic research tools have been generated in the process. Engineered nucleases can be used to knock out or knock in genes, to make allelic mutants, to change gene regulatory control and to add reporters or epitope tags, all in the endogenous genomic context. Matthew Porteus discusses these and other exciting research applications of these tools in a Commentary on page 28.

Although the first ZFN was reported more than a decade ago, the pace of work in this field has picked up remarkably in the past year. This is in no small part because of the development of TALENs. ZFNs remain the best characterized tools, but they are not always easy to design, as Mark Isalan discusses in a Commentary on page 32. The recent discovery of TAL effectors in plant pathogenic bacteria and the realization that their properties—namely their apparently simpler DNA-binding code—should mitigate some of the existing problems with engineering robust tools, has given a real boost to the field. Gene-editing nucleases will achieve their full potential when they can be easily and quickly designed, in practice, to specifically modify any sequence of any genome, having more than one technology available will help achieve this goal.

In addition, commercially available ZFNs are dropping in price at the same time as methods developers are assembling tools to help researchers design their own nucleases. Meanwhile, TALENs and engineered meganucleases are also already commercially available. Which technology will dominate is not yet clear: there are still many unknowns, in particular about TALEN function as discussed in several of the pieces in this issue. But the price at which researchers in regular research labs can obtain good tools is likely to play a role.

You can also hear about genome editing with engineered nucleases in a short video, and we include in this issue, as in previous years, a section of Methods to Watch in the future (p. 35).

To all our readers, a happy, successful 2012!

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A year later, 2Blades' TAL Code technology won the Agrow Award for Best Novel Agricultural Biotechnology in 2012.



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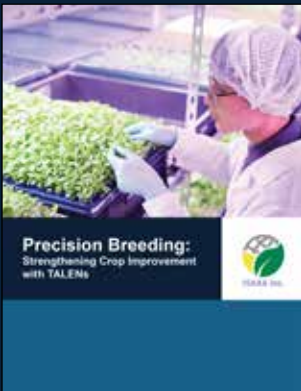
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