Site-Directed Nucleases

'Give it a break'

Mutations are essential for life as we know it. Without mutations, there would be no evolution and no biodiversity. The variation created by natural mutations has also been the basis for all plant breeding since we started cultivating plants many thousand years ago. Then starting in the 1930s, scientific progress enabled us to use radiation and chemicals to induce mutations and thereby increase the genetic variation available for plant breeding. The discovery of *site-directed nucleases (SDN)* – also called 'molecular scissors' – are now expected to revolutionize the field of mutation breeding. Compared to the randomness of mutations induced by radiation or chemicals, the enzymatic approach of SDN presents an unprecedented level of control and efficiency in plant research and breeding.

Benefits

The precise engineering of genomes now possible with SDN techniques provides a level of control never seen before in the history of plant breeding. Induced mutations through radiation or chemicals have yielded fantastic results over the years, but require time-consuming and expensive screening on large populations and takes several generations to reach the final result. With SDN in the plant breeder's toolbox, we leap from the past trial-and-error to a future knowledge-based precision breeding.

Scientific description

Decades of basic research has greatly improved our capacity for precise engineering of DNA sequences. The common enzymatic 'molecular scissors' were discovered in the 1970s and are now used to break the double-stranded DNA at precise locations. The break triggers the natural DNA-repair process in the cells, which can be exploited to introduce different kinds of mutations. There are several different systems in use today. *Zinc finger nucleases (ZFN)* and *transcription activator-like effector nucleases (TALEN)* consist of two artificially connected proteins that can be delivered to the plant cells either as a stable genome integration event or on a transiently expressed plasmid vector. *Meganucleases* are naturally occurring enzymes that are highly specific with a large DNA recognition site. The recently adapted *CRISPR/Cas9* system is by far the most efficient SDN as it takes advantage of an extremely specific guide RNA to find the target gene. Many other potentially useful enzymatic systems will also likely be discovered. SDNs are versatile and may be used for editing, inserting, deleting or replacing genes.

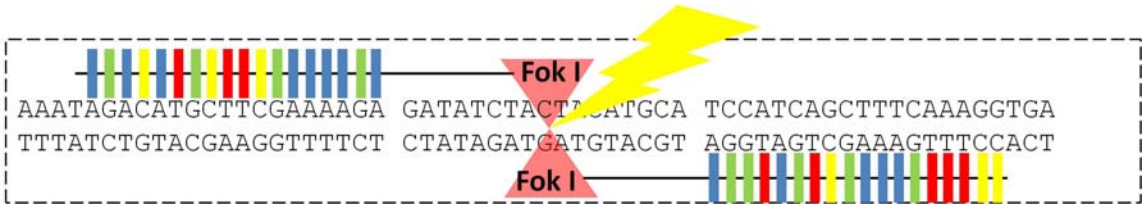

Image: Thorben Sprink, Julius Kühn-Institut

Applications

The field of SDN has literally exploded in the last few years. Many researchers and breeders are adopting these techniques with great enthusiasm. Whereas model plants have often been used in basic research to develop SDN techniques, applications in crop plants are now increasing. ZFN technology is currently being applied in the breeding of maize, rapeseed, soybean and tomato, whereas TALENs have been used in tobacco, rice and potato. Given that SDN applications in plants are relatively recent and many more enzyme systems are expected to be discovered, the full potential of these techniques is enormous.

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