Targeted breeding technologies – the way of the future

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A modern era of targeted crop breeding is upon us. Traditional plant breeding aims to change traits/genes in a specific crop to obtain the desired characteristics in the offspring. This targeted trait is often linked to a specific change (mutation) in the parental plants' genetic code (DNA), which the breeder then attempts to develop progeny from, containing target market characteristics.

Though this process seems relatively straight forward it is not, since some traits are 'hidden' (in the form of recessive genes), while other traits are transferred to the progeny in large groups (linked) that may include undesirable traits as well (linkage drag).

Unwanted traits are also randomly transferred to the progeny, which means as the number of desired traits increases, the number of progeny required to obtain an individual with all the desired traits and development cost, increases dramatically. This numbers game becomes even more complicated when breeding with grain crops. This is especially the case for wheat, with its three large complex genomes, having multiple copies of a single gene that originated from the ancestry donor backgrounds.

The breeding process always aims to produce cultivars faster and therefore currently uses tools such as molecular selection (marker-assisted breeding), embryo rescue and double haploid generation and speed breeding to accomplish this.

This ultimately results, after many years (eight to twelve years) of breeding and selection cycles (including traditional trait screening and molecular selection), in the release of higher yielding and adapted cultivars.

New plant breeding technologies targets traits better

However, what if a breeder could actually 'target' a desired trait with precision, thereby transferring only the new desired trait into an elite line without other unwanted characteristics? Well, some of the new breeding technologies in the breeder's toolbox will now allow just that: The ability to transfer a specific trait by targeting the specific genetic code or gene region responsible for it.

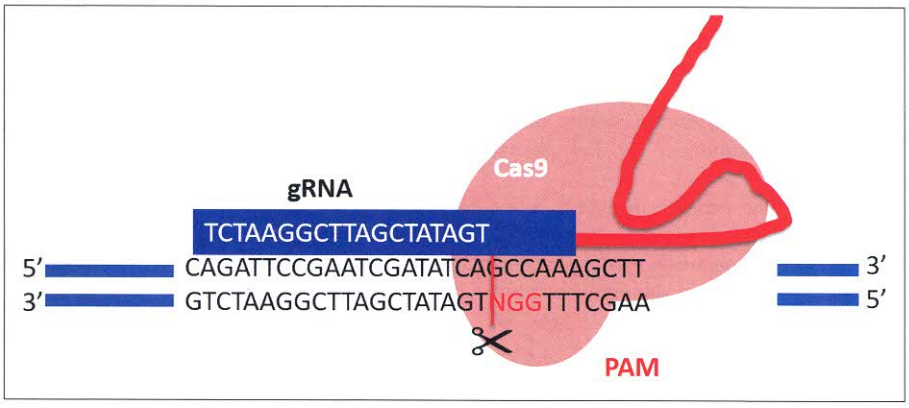

Figure 1: The CRISPR associated nucleases (Cas9) binds to a guiding molecule, the guide RNA (gRNA), which has a complementary sequence to the DNA being targeted in the genome. This ribonucleoprotein (Cas9-gRNA complex) moves along the DNA of the organisms in search of the complementary target sequence. Once found, the Cas9-gRNA ribonucleoprotein complex binds to the DNA in the presence of a PAM (protospacer adjacent motif) sequence (in red – NGG), thereby aligning the nuclease to cut the DNA at a specific site. By changing the gRNA's sequence (white text in blue box) another site in the DNA could easily be targeted. Multiple gRNAs allow multiple DNA sites being targeted at once – though efficiency does decrease.

These new plant breeding tools include a wide variety of technologies, ranging from directed nucleases for targeted mutagenesis to technologies that transfer the trait of interest but does not result in permanent DNA changes.

The tools in the new breeding toolbox that are really making a huge impact are those belonging to the directed nucleases group. Nucleases are enzymes that can cut DNA. Some of these nucleases recognise and cut only specific DNA sequences (e.g. meganucleases), while others use engineered proteins to target specific DNA for cleaving. The usefulness of meganucleases are limited since they can only target and cut at their specific DNA recognition sequences, which will very rarely be within the target trait region desired by the breeder. These technologies require expensive, time-consuming protein engineering skills by experienced individuals. All these directed nucleases have been around for a number of years, but not widely adopted due to their limitations. This all changed with the discovery of the CRISPR/Cas system – a system awarded the *Science Discovery of the Year* in 2015.

CRISPR/Cas: Get familiar with it

CRISPR/Cas9 (clustered regularly interspaced short palindromic repeat)-Cas9 is a multipurpose system for targeted genetic engineering that uses a 'guide molecule' (guide RNA) to direct a DNA nuclease (Cas9 or other similar endonuclease) to a specific target site where it cuts the DNA in a specific manner.

The guide can easily and quickly be changed and synthesised as DNA (or RNA) using current technologies, making this system not only cheaper, but also faster to implement, with less expertise required. The usage of the system is restricted by a Cas nuclease specific protospacer adjacent motif (PAM) – a short, Cas enzyme specific sequence, required to be in the target DNA for successful binding and cutting (**Figure 1**).

This PAM sequence differs between different Cas nucleases, thereby increasing possible cutting sites and allowing easier targeting of a different desired DNA sequence. The availability of complete genome sequence, target gene sequence/mutation and appropriate in vitro (tissue culture) delivery system, are some of the major limitations of the system currently.