



## New Plant Breeding Techniques: CRISPR/Cas9 System

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Conventional plant breeding for the most part involves the hybridization of two parents — a recurrent parent that has otherwise desirable attributes except for one or two traits, e.g. susceptibility to a disease; and a second parent called a non-recurrent parent that possesses the desirable trait missing in the former.

After the initial hybridization, the offsprings with the new desirable trait are backcrossed to the recurrent parent to recover the rest of the desirable genome. By the 6th backcross generation, 99% of the recurrent parent genome would have been recovered.

This backcrossing procedure makes conventional plant breeding time consuming, expensive and imprecise. For an annual crop like rice with a generation cycle of four months and twice a year planting, the process would take at least three years. However, for a perennial like coconut with a generation cycle of 6–8 years, the process could take 40–50 years.

In addition to the time and cost limitations, conventional plant breeding does not allow transfer of genes between species that are genetically distantly related, and sexually incompatible.

With the advances in modern biotechnology, new plant breeding techniques have emerged, which not only allow transfer of genes from unrelated species to produce transgenics or genetically modified organisms (GMOs), but also allow introduction of

precise, predictable modifications in an elite genetic background, avoiding the mess and cost associated with sorting out the tens of thousands of genes mixed up in sexual hybrids in conventional plant breeding.

One such novel genetic technique is the CRISPR/Cas9 system that has wide applications in plant and animal breeding as well as in drug development and human gene therapy.

CRISPR stands for Clustered Regularly Interspaced Short Palindromic Repeats. They are odd repetitive deoxyribonucleic acid (DNA) sequences at the end of the genes of many bacteria including the ubiquitous *Escherichia coli* found in the intestines of humans. The DNA sequences are not only repetitive, they are also palindromic i.e. they read the same forwards and backwards.

The repetitive DNA sequences are separated from one another by equally strange “spacer” gene sequences, which were later found out to be not bacterial in origin, but actually snippets of DNA of viruses that are known to attack or invade bacterial cells.

The CRISPRs are associated with certain families of proteins that turned out to be DNA endonucleases i.e. enzymes that naturally cut up/break DNA molecules. One such protein, which was found more effective than others, was Cas9.

For a long while (80s to early 2000), scientists did not know what

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the CRISPRs and their associated proteins do inside the cell. In 2005, three independent research laboratories came to the same conclusion that CRISPRs and the associated proteins were in fact acting as a bacterial immune system that serves to defend the microorganism against invading viruses.

## HOW THE CRISPR/CAS9 SYSTEM WORKS

When a virus invades a bacterial cell, the Cas proteins bind to the viral DNA and help cut out chunks of DNA. These chunks of viral DNA somehow get incorporated in the bacterial cell genome where they are inserted as “spacers” in the CRISPR systems.

The “spacers” in the CRISPR serve as a record of the bacterial encounter with that specific virus.

Since the CRISPR system is part of the bacterial genome, this property is handed down from one bacterial generation to the next by successive cell divisions.

The next time viruses invade a descendant bacterial cell, the ribonucleic acid (RNA) in the CRISPR binds with the DNA of the invading viruses. If there is a match with a spacer(s), the accompanying enzyme (Cas) cuts up the viral DNA, rendering the virus inactive.

This phenomenon is parallel to the human immune system. Vaccination with a weakened version of the pathogen stimulates production of antibodies to resist future attacks. However, the effect is transient because we do not pass the acquired immunity to our offsprings.

In the CRISPR system in bacteria, the resistance is hardwired in the bacterial DNA as a “spacer” and is automatically passed on when the bacteria divide into daughter cells. Later studies confirmed that the CRISPR adaptive immune system is common in bacteria (40%) and in Archaea (90%), the two domains of lower forms of living organisms which are unicellular and which do not have defined nuclei. Plants, animals including humans belong to the third Domain, the Eukarya, whose cells contain defined nuclei and other organelles.

Mutations are brought about by deletions, additions, inversions, and translocations of gene sequences. But before this can be done, there must be breaks in the DNA. Breaks in the DNA can be induced externally by physical means (gold particle bombardment), and inside the cells, by naturally occurring enzymes called “endonucleases”. Nucleases cut up DNA at so many different places more or less at random. The cutting of the DNA can be made more precise if the nucleases can be guided to the precise locations in the genome.

The CRISPR/Cas9 system in bacteria provided the inspiration.

With advances in molecular biology, it was now possible to artificially synthesize the RNA part of the CRISPR which will guide the Cas cutting enzyme to the site in the genome where the break is desired. The guide RNA could also be constructed to contain the gene sequences which are intended to be inserted/added in the DNA breaks.

The CRISPR/Cas system has been found to work on the model plant *Arabidopsis* as well as on a wide range of economic plants like tobacco, tomato, rice, maize, wheat, sorghum, and sweet orange. This has led globally to a mad scramble on research on CRISPR/Cas and their actual applications on the development of novel crop varieties.

Dupont, one of the chemical and biotech giants, predicted that CRISPR-developed plants will be on dinner plates in five years. Among the crops that Dupont is working on are drought-resistant maize and wheat with a drastically changed flowering habit (cross pollinated instead of self-pollinated) that will facilitate the development of wheat hybrids.

## CONCLUSION

There is no stopping of scientific innovations in all human endeavors, including food, agriculture, and medicine. The novel CRISPR/Cas system described above, which is a natural immune defense system found in lower forms of organisms like bacteria, has been tweaked to work in higher plants, animals, including man as a precise, relatively quick and inexpensive method of genome editing.

This new technique is another tool in the growing arsenal of plant breeding technologies to complement with conventional plant breeding. We need to acquire and master this nascent technology to advance our national purposes.



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