Advances in Crop Science and Technology

Sticklen, Adv Crop Sci Tech 2014, 2:2
http://dx.doi.org/10.4172/2329-8863.1000e112

Editorial Open Access

# Crop Genome Editing: Advancing Crops via Editing of Their Genetic Make-Ups

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Crops of the near future will be genome edited via a technology called "Genome editing with engineered nucleases" or GEEN, a technology that can add, remove or repair existing genes that might be not desirable such as genes that make crops susceptible to diseases, insects, drought, heat, cold or other biotic and abiotic stress factors .

For decades, molecular biologists have been using restriction nucleases or restriction enzymes to cut DNA at or near DNA specific recognition sites in order to study the crops DNAs or to genetically engineer crops. Restriction enzymes naturally exist in bacteria, evolutionally developed to provide a defense mechanism against invading viruses by selectively cleaving the viral DNA while the host DNA is protected by the methylase enzymes that block such cleavage in the host DNA. The GEEN technology mostly uses artificially engineered restriction nucleases that have been designed to make specific cleavage called double-stranded breaks (DSBs) in specific recognition sites of a genome.

The GEEN technology has been developed to repair the mutated sequences of human, animals and plant genomes. The double stranded DNA repair system either uses a set of enzymes to directly join the DNA ends of the DSBs or uses homologous sequences as templates for regeneration of missing DNA sequences at the break point of a genome. Therefore using the DSBs technology, one can repair mutated genes or add genes in a genome while using the host's natural gene regulatory systems.

In case of adding genes to a crop genome, unlike traditional genetic engineering that randomly incorporates transgenes into a crop genome, the DSBs technology can precisely add a gene or even add multiple genes to specific site of a genome for trait staking so these multiple desired genes are physically linked assuring their co-segregation during the breeding of transgenic crops [1].

The RNA interference (RNAi) technology has been used during the last couple of decades to eliminate or reduce the harmful effects of mutated genes or reduce the undesired level of expression of a gene using transgene constructs containing the RNAi coding sequences as well as their regulatory systems [2,3]. However in DSBs, there is no need to produce transgene constructs containing the regulatory systems. Plants so far been genome edited include *Arabidopsis* [4-6] tobacco [7] and *Zea mays* [8]. To date, three classes of nucleases including Zinc finger nucleases (ZFNs), transcription-activator like effector nucleases (TALENs) and mega-nucleases have been developed [9-11]. Among these three, the mega-nucleases technology is preferred because unlike the first two, mega-nucleases technology causes less toxicity to the host cells. However, the toxicity of these technologies has been studied to be reduced to a minimum [12].

Certain mega-nucleases naturally exist in a limited number of specific microbes, but mutagenesis and high throughput screening techniques have been used to create different mega-nucleases that recognize certain DNA sequences [13]. Also, hybrid restriction enzymes have been synthesized [14] and a rationally designed mega-nuclease has been developed that can recognize unique sequences to be used as DSBs recognition sites [15].

A protocol was recently developed [16] and used for simultaneous real-time visualization of the whole genome editing processes along with screening for the genome-edited DNA at the same time via a technology called "nanofabricated DNA curtains".

Therefore, it is only a matter of time to advance the promising crop genome editing technology to solving of major crops biotic and abiotic stress problems by adding, removing or repairing of their specific genes [17].

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**Received** February 24, 2014; **Accepted** February 26, 2014, 2014; **Published February** 28, 2014

**Citation:** Sticklen M (2014) Crop Genome Editing: Advancing Crops via Editing of Their Genetic Make-Ups. Adv Crop Sci Tech 2: e112. doi:10.4172/2329-8863.1000e112

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**Citation:** Sticklen M (2014) Crop Genome Editing: Advancing Crops via Editing of Their Genetic Make-Ups. Adv Crop Sci Tech 2: e112. doi:10.4172/2329-8863.1000e112