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Commentary

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# Advantage Gene Targeting: A by-product of Genomic Double Strand Break

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## Introduction

Despite Modern agriculture has created a demand for plant biotechnology products that provide durable resistance to insect pests, tolerance of herbicide applications for weed control, and agronomic traits tailored for specific geographies. These transgenic trait products require a modular and sequential multigene stacking platform that is supported by precise genome engineering technology. Designed nucleases have emerged as potent tools for creating targeted double strand breaks. Exogenously supplied donor can repair the targeted by a process known as gene targeting, resulting in a desired modification of the target genome. The potential of technology has not been fully realized for trait deployment in agriculture, mainly because of inefficient transformation and plant regeneration systems in a majority of crop plants and genotypes. This challenge of transgene stacking in plants could be overcome by Intra-Genomic Homologous Recombination that converts independently segregating unlinked donor and target transgenic loci into a genetically linked molecular stack. The method requires stable integration of the donor into the plant genome followed by intra-genomic mobilization. The complements conventional breeding with genetic transformation and designed nucleases to provide a flexible transgene stacking and trait deployment platform. The Green Revolution in the 1960s combined advances in breeding and agricultural practice, and provided food security to millions of people. Given an increasing global population, there is a projected need to increase world food production by 40 percent in the next 20 years. In addition to a growing population, climate change, degrading natural resources and changing food preferences have raised food and nutritional security to the level of the biggest challenge of the twenty-first century.

## Integration of Multiple Transgenes

Additionally, genetically modified trait technology in the mid-1990s made a major impact in meeting the world food demand and there has been a rapid adoption of the technology. These first generation trait products involved simple herbicide and insect traits that required introduction of a single gene. Control of the broad range of insect pests and weeds desired today requires multiple insect and herbicide tolerance genes. In addition, modern genomics and gene networking tools have revealed that many agronomic traits depend on different genes and complex interactions of proteins reacting to various external stimuli. The next generation trait products, therefore, require integration of multiple transgenes and would also benefit from a flexible and modular trait stacking platform that would accommodate development of increasingly complex future products. Conventional breeding has been successfully employed for trait stacking, but this method requires substantial time and resources for sorting and deregulation of multiple unlinked transgenes and a limited number of independent loci can practically be stacked. Designed nucleases have become a powerful gene targeting tool to create targeted nuclease double strand breaks at specified genomic locations, which stimulate the cell's repair machinery leading to integration of exogenously supplied transgenes into a specified genomic site. While designed nuclease-mediated targeted mutagenesis is becoming routine in plants site-directed transgene integration remains elusive, mainly due to low transformation and regeneration efficiencies in the majority of plant species and genotypes. A method that requires minimal transformation effort would be very attractive to address this challenge. This review focuses on conventional Intra-chromosomal somatic homologous recombination work in plants and its recent application using designed nucleases that can provide solutions to some of the challenges associated with the deployment of technology for transgene stacking in crop plants.

## Significant development of Desired Nuclease

The Genomic system can arise spontaneously, may be induced by ionizing radiation and chemicals or recently by designed nucleases. Genomic system could be negatively mutagenic or lethal to cells if not repaired efficiently. In plants, these are repaired by homologous recombination or non-homologous end joining mechanisms are conserved in eukaryotes however, the efficiency of these pathways differs not only between species but also between cell types. System repair pathway that requires sequences homologous almost identical to those flanking. System is the predominant recombination pathway during meiosis in higher eukaryotes including plants. This mainly involves ligation to unrelated sequences or to sequences with micro-homologies, resulting mostly in non-precise repair with small insertions or deletions at the site. System is the primary nuclease repair pathway in the somatic cells, while genes mainly occur during phases of the cell cycle. Targeted system has been previously described for mutagenesis, deletions or imprecise insertions. In contrast, a more precise mode of DNA repair is preferred for system. Gene targeting through requires simultaneous introduction of the nuclease to create targeted at desired genomic location and donor DNA containing flanking homologies, acting as a template for repair of the donor. The next significant development in the field was the application of designed nucleases for excision of the stably integrated transgene. In tobacco, a transgenic line containing a single copy of the codA gene flanked by cleavage sites specific to nuclease was created. After induction of systems by transient expression of gene was successfully removed from the calli and plants lacking the codA gene were regenerated. Similarly, tobacco plants containing a stably integrated uidA gene cassette flanked by designed nuclease sites were crossed with plants expressing the corresponding nuclease. The complete deletion of a 4.3 kb sequence comprising the uidA gene cassette was obtained in F1 progenies. These reports were later followed by deletions of large endogenous genomic sequences in different plant species using designed nucleases.

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