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Opinion

Genome Editing (GE) and its Biotechnological Application in Crop Breeding Programs

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Introduction

Molecular genetic studies over past decades have originated new tools for breeding programs of crop plants. Innumerous genetic engineering techniques were developed and applied to generate genetic modified crops varieties with superior agricultural characteristics, including new traits that do not occur naturally in the species. Genetic Modified Organisms (GMOs) become a reality in supermarket shells worldwide. In the last years, molecular techniques promising to develop "transgene free" crops have been introduced in plant breeding programs. These new techniques are called together Genome Editing (GE).GE is changing the way to produce genetic modified organisms since produces specific genetic changes within a genome, with no transgene manipulation. GE refers to platforms that use site-specific nucleases (SSNs) that can introduce DNA lesions at a specific genomic position. Double-stranded breaks (DSBs) created by SSNs at targeted genome sites leads to the activation of endogenous cellular DNA repair mechanisms: non-homologous end joining (NHEJ) or homologous recombination (HR). NHEJ and HR could generate gene knockout or gene replacement, respectively, in the target genome [1]. Several novel GE systems based on SSNs were developed in the last years: Zinc Finger Nucleases (ZFNs) [2,3], Transcription Activator-Like Effector Nucleases (TALENs) [4,5], and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR/Cas9) [6]. Because of its high efficiency and relatively low cost, CRISPR/Cas9-based genome editing system has becoming the most popular choice of plant molecular biologists for functional studies of plant genes. CRISPR/Cas9 have been routinely applied to crop trait development (yield, architecture, disease resistance, and tolerance/resistance to biotic and abiotic stress) in important crops [7,8]. CRISPR/Cas9-based genome editing system has been optimized as a strategy for multiplex genome targeting (MGT) in plants. MGT consist in the simultaneous edition of several genomic sites within a genome, allowing the study/knockout of multiple/redudantgenes, or improvement of different traits in crop breeding programs [7]. Sequential rounds of regular cloning [9], Golden Gate cloning method [10], and polycistronict RNA-gRNA system [11] are some of the novel strategies to assemble multiple target genes into single CRISPR/Cas9 binary constructs [7]. However, limited application for several important field crops is the major limitation of available transgene-free CRISPR/Cas9 methodologies. Evolution in CRISPR/Cas9 protocols through the increasing of its efficiency, specificity, and range of accessible targets promises address this issue in future [1]. GE systems could be greatly boosted by the astonishing advance observed in Next-Generation Sequencing (NGS) platforms in last years. NGS is increasing the availability and accuracy of important plant genomes, including non-conventional food plants.NGS is providing the raw material for actual and emerging GE systems to revolutionize crop molecular breeding in the next decades. In future, farmers and consumers can expect novelties in crop production and consumption through the biotechnological employing of GE in plant breeding programs.

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