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Genome editing and plant transformation of solanaceous food crops

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During the past decade, the ability to alter plant genomes in a DNA site-specific manner was realized through availability of sequenced genomes and emergence of editing technologies based on complexes that guide endonucleases. Generation of targeted DNA breaks by ZFNs, TALENs, and CRISPR/Cas9, then mending by repair mechanisms, provides a valuable foundation for studies of gene function and trait modification. Genome editing has been successful in several food crops, including those belonging to the *Solanaceae*, which contains some of the most widely used, economically important ones such as tomato and potato. Application of new breeding technologies has the potential to not only address deficiencies of current crops, but to also transform underutilized species into viable sources to diversify and strengthen our food supply.

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Introduction

The ability to modify genomes in a site-directed manner to affect traits for crop improvement could have once been described as the holy grail of plant breeding and genetic engineering. However, during the past ten years site-directed genome modification has been realized through development of sequence-specific nuclease-based technologies that include Zinc Finger Nucleases (ZFNs) [1], Transcriptional Activator-Like Effector Nucleases (TALENs) [2], and most recently, Clustered Regulatory Interspaced Short Palindromic Repeat (CRISPR)-Associated Protein System (CRISPR/Cas9) [3]. Genome editing (GE) strategies were first shown to be effective in bacteria and mammalian cell lines, but they have been rapidly adapted for plant genome modification.

GE has been demonstrated in many plant species, but where it will have the greatest impact is improvement of food crops such as maize [4–6], rice [7,8], soybean [9], and wheat [10]. GE has also been demonstrated in some members of the *Solanaceae*, which is a source of diverse food crops including tomato, potato, pepper, eggplant, tomatillo, and Cape gooseberry [11–14]. Several of these represent major food crops relied on worldwide that are also economically important. Potato is the fourth most important staple crop in the world and on an economic basis, the total US dollar values for fresh market and processing tomatoes in 2015 was 1.2 and 1.4 billion, respectively [15]. As is the case with other food crops, solanaceous ones hardly resemble their wild progenitors, which underwent modifications through natural and induced-mutations, selection, and plant breeding. With the advent of editing technologies, namely CRISPR/Cas9, it has been proposed that perhaps wild species could in a sense be 'domesticated' to generate viable new food crops [16].

Prerequisites for GE of plants are availability of genome sequences and efficient transformation systems for delivery of editing reagents into cells with the subsequent recovery of modified plants. There are genome sequences available for many plant species and the number continues to increase because the evolution of sequencing technologies has reduced the time and cost for even large, complex genomes [17]. GE would not be possible without the ability to do genetic engineering or transformation. Plant genetic engineering has been achieved through direct DNA uptake into protoplasts [18], delivery of DNA by biolistics (particle bombardment) [19], and by *Agrobacterium*-mediated transformation [20]. Each of these approaches has advantages and disadvantages that need to be considered when choosing a gene transfer method.

There are numerous reports and reviews of GE in plants, especially CRISPR/Cas9. Therefore, the purpose of this review is to focus on solanaceous food crops along with information related to transformation methodologies. While GE has been primarily reported for tomato and potato, it is just a matter of time until we see reports for others such as pepper, eggplant, tomatillo, and Cape gooseberry.

Genome editing technologies

Zinc finger proteins are sequence-specific DNA-binding proteins that contain tandem arrays of amino acid

sequences that bind by inserting an alpha-helix into the primary groove of the double helix [1]. For GE purposes, they were designed to make double-stranded breaks (DSBs) in genomic DNA through fusion to the cleavage domain of the *Fok* I endonuclease and have been used for modification of various organisms for more than 20 years [21]. The first report in a major food crop was in maize where an herbicide-tolerance gene, *IPK1*, was precisely integrated at an intended site [4]. Reports of ZFN-mediated genome modification in solanaceous food crops were not found, however, it has been used for targeting the *ACETOLACTATE SYNTHASE* (*ALS*) gene in tobacco, also a *Solanaceae* family member [22].

Transcription activator-like effectors (TALEs) are a class of DNA-binding proteins that are key virulence factors that function as transcription factors in the bacterial plant pathogen, *Xanthomonas* [23]. They induce gene expression by binding to promoters in a sequence-specific manner based on the TALEs' central domain of tandem amino acid sequences and each repeat confers recognition of one nucleotide. As with ZFNs, TALEs were designed to mediate editing through fusion to the cleavage domain of the *Fok1* endonuclease and designated TALEN [24]. The first report of TALEN-mediated editing in a solanaceous food crop was in potato where the *ALS* gene was targeted [13]. Following that report, TALENs were used to study cold storage, processing traits, and gene insertion (Table 1). Lor *et al.* demonstrated TALEN editing in tomato by targeting the *PROCERA* gene, a negative regulator of gibberellic acid (GA) signaling [11]. Fifteen percent of the transgenic lines contained heritable mutations with homozygous lines exhibiting the expected phenotype of enhanced GA response.

CRISPR/Cas9 is the most widely adopted GE technology, which is a complex based on guidance of short RNA sequences to complimentary target DNA that is then cleaved by the Cas9 endonuclease. The realization of the potential of this type II prokaryotic adaptive immune system for editing in organisms beyond bacteria caused a genome engineering revolution [3]. Early reports in plants demonstrated editing in *Nicotiana benthamiana*, a *Solanaceae* family member, that serves as a model species [25,26]. These reports were shortly followed by demonstration in tomato [12,27] (Table 1). CRISPR/Cas9 has proven to be an especially powerful tool for the elucidation of genes and mechanisms that influence tomato meristem development, plant architecture, and fruit characteristics such as ripening, parthenocarpy, and size [28–32] (Table 1). To study the efficacy of using CRISPR/Cas9 to generate disease resistant plants, researchers at the Sainsbury Laboratory designed constructs to target MILDEW.

RESISTANT LOCUS O because it confers susceptibility to the fungus that causes powdery mildew and they recovered resistant lines [33•]. As for the stability of CRISPR/Cas9-induced mutations, Pan *et al.* disrupted the PHYTOCHROME INTERACTING FACTOR gene [34]. Mutations were transmitted from primary transformant plants (T0) to the T1 and T2 generations with some plants containing the mutation alone without any T-DNA present.

Table 1

Genome editing of solanaceous food crops

Crop	Genome editing approach	Delivery method	Gene target/insertion	Reference
Potato	TALENs	PT[a]	*StALS*	[13]
		PT	*StVInv*	[71]
		ATM[b]	*mStALS*[d] (GI)[e]	[72]
	CRISPR/Cas9	PT	*StGBSS*	[14]
		ATM	*StIAA2*	[35]
		ATM	*StALS1*	[36]
	Geminivirus-mediated	ATM	*StALS1*	[52]
Tomato	TALENs	ATM	*PROCERA*	[11]
	CRISPR/Cas9	ARM[c]	*SlSHR*	[27]
		ATM	*SlAGO7*	[12]
		ATM	*SlIAA9*	[28]
		ATM	*SlCIV1, SlCLV2, SlCLV3, SlRRA3a*	[29]
		ATM	*SlSP, SlSP5G*	[30]
		ATM	*SlBOP1, SlBOP2, SlBOP3, SlTFAM1, SlTFAM2*	[31]
		ATM	*SlRIN*	[32]
		ATM	*SlMlo1*	[33•]
		ATM	*SlPDS, SlPIF4*	[34]
	Geminivirus-mediated	ATM	*SlANT1*	[50•]

[a] PT: protoplast transfection, direct DNA uptake into protoplasts.
[b] ATM: *Agrobacterium tumefaciens*-mediated transformation.
[c] ARM: *Agrobacterium rhizogenes*-mediated transformation.
[d] *mStALS*: mutated (promoter removed) *StALS*.
[e] GI: gene insertion.