CRISPR-Cas9 Mediated Functional Genomics

Subjects: Plant Sciences

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The genome-editing tool, CRISPR-Cas9, reveals the functional features of several parts of the plant genome. Current developments in CRISPR, such as *de novo* meristem induction genome-engineering in dicots and temperature-tolerant LbCas12a/CRISPR, enable greater DNA insertion precision.

plant stress   abiotic stress   biotic stress   omics   CRISPR-Cas9   crop stress tolerance

1. Introduction

In cells, CRISPR-Cas9 is a cheap, easy, fast, and effective system for gene knockout[1]. For effective genome engineering, CRISPR-Cas9 has been used in animals, plants, and bacteria[2][3][4][5]. Furthermore, CRISPR-Cas9 has been used for high-throughput screening of genes, gene knockout, chromosomal loci live-cell labeling, endogenous gene expression, and single-stranded RNA (ssRNA) edition. The application of CRISPR-Cas9 for studying the function of a gene has generated disease models. However, several queries and challenges need to be addressed. CRISPR-Cas9 will likely enhance our comprehension of disease activity and its management. For targeted genome engineering, detecting programmable nucleases that produce cuts in double-strands has radically changed molecular biology; ZFNs pioneered this success, with TALEN extending the genome modifying capacity[6]. Globally, CRISPR-Cas9 received recognition from researchers for its visible benefits over TALEN and ZFN[7], being its (1) ease of designing target, (2) ability to create mutations by inserting the guided RNA and Cas9 protein, and (3) multiplexing ability to target several genes at one time [8][9]. Figure 1 summarizes the principles of CRISPR-Cas9.

1. SgRNA+Cas9   2. Target gene   3. Target cleavage

4. Gene knockout   5. Repair templates
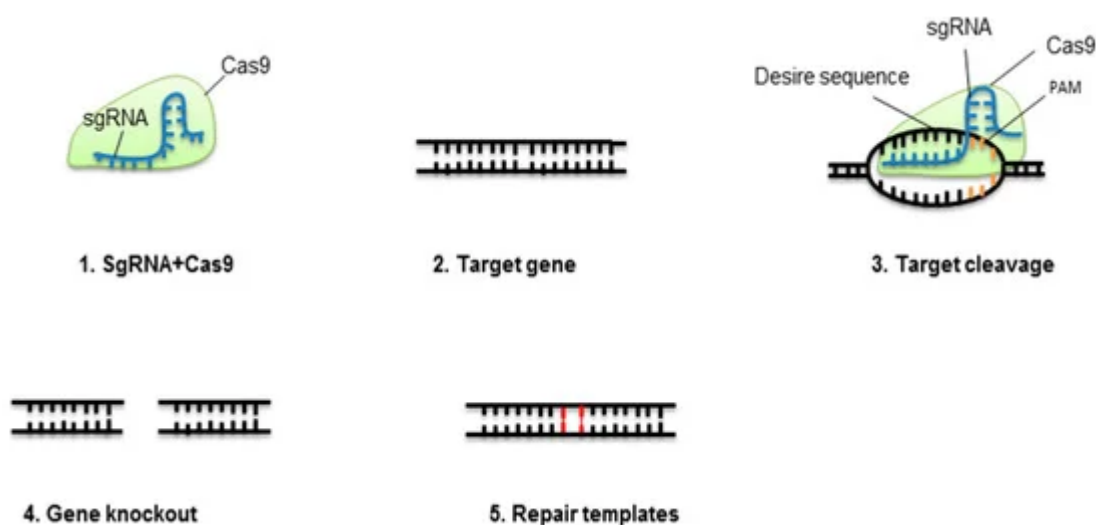
Figure 1. Concept of CRISPR-Cas9-mediated gene elimination. Single guide RNA (sgRNA) containing crRNA and tracrRNA fixes to Cas9 protein. This complex will break at a specific target of the double-stranded DNA molecule. The nonhomologous end-joining pathway (NHEJ) will repair the cleaved location.

2. CRISPR Technology

Due to its robust success, CRISPR-Cas9 is becoming a potential tool for genetically enhancing desirable crop traits, i.e., disease resistance, nutrient content, adaptation to multiple stresses, plant architecture, and yield. In some cases, a specific trait can be improved by negative regulatory gene knockout. Rice grain weight improved with gene modification of some QTL[10]. Maize grain yield under drought increased with genome engineering of the ARGOS8 locus[11]. In woody plants, CRISPR-Cas9 produced mutants in the first transgenic generation; this is significant as woody plant breeding is difficult due to their long lifespan[12][13]. Another study knocked out the *OsGAN1* gene in rice and verified that it regulates root length and plant height[14]. Similarly, *OsABCG26* gene knockout verified that this gene regulates pollen exine and anther cuticle, and *OsTCD10* had a substantial role in chloroplasts of cold-stressed rice[15][16].

3. CRISPR-Cas9 Genome Editing to Biotic Stress Tolerance

Genome editing by CRISPR-Cas9 has been used effectively in several crops, including cotton, maize, rice, and wheat. However, most genome engineering studies have targeted biotic stresses, such as diseases. In wheat, the CRISPR-Cas9 method was used successfully to knock out all three *EDR1* homologs to create plants (Taedr1) with increased tolerance to powdery mildew [17]. In *Arabidopsis*, the knockout of susceptible gene *EDR1* increased resistance to powdery mildew[18]. Recessive resistance genes, *eIF* (eukaryotic translation initiation factor), have been detected in several dissimilar hosts, with *eIF* (iso) *4E* and *eIF4E* genes used with CRISPR-Cas9 to form virus-resistant plants in *Arabidopsis* and cucumber, respectively[19][20]. *CsLOB1* is a susceptible gene of the citrus canker (causative agent; *Xanthomonascitri*); CRISPR-Cas9 was used to edit this gene to develop resistant grapefruit plants [21][22]. Additionally, a negative resistance function *MLO* gene, responsible for powdery mildew susceptibility, was mutated successfully by Cas9 knockouts to enhance resistance against powdery mildew in tomato and wheat [23][24][25]. The application of CRISPR-Cas9 as an antivirus tool cleaved beet severe curly top virus, which decreased the viral infection[26][27]. The rice tungro spherical virus (RTSV), linked to the negatively controlled susceptible *eIF4G* gene, was eliminated using CRISPR-Cas9 to develop resistant rice varieties[28]. From CRISPR-Cas9, the loss of function *VvWRKY52* gene produced resistance against *Botrytis cinerea* in grape (*Vitis vinifera*)[29]. Furthermore, CRISPR-Cas9 has been used to interrupt multiple virus genomes, including $CLCuK_0V$, TYLCSV, and TYLCV[30]. For cucumber mosaic virus and tobacco mosaic virus, a technology to modify RNA virus genomes has been advanced from sgRNA and FnCas9. Hence, molecular immunity to RNA viruses was mediated by sgRNA/FnCas9 expression in *Arabidopsis* and tobacco[31]. CRISPR-Cas9 successfully targeted *OsERF922* against blast fungus resistance in rice[32]. Plant ethylene-responsive factors (ERFs) can control tolerance against various stresses because they are involved in the ethylene (cytokinin) pathway[33]. When taken together, these reports deliver robust indications that CRISPR-Cas9 can enhance biotic stress resistance in plants (Table 2).

Table 2. CRISPR-Cas9 application for crop improvement.

Species	Traits	Target Genes	Reference
Abiotic stresses			
Rice	Improved resistance to arsenic stress	*ARM1*	[34]
	Depletion of Cd into grain	*LCT1*	[35]
	Depletion of Cd into grain	*Nramp5*	[36]
	Drought tolerance	*SAPK2*	[37]
Tomato	Drought tolerance	*SIMAPK3*	[58]
Maize	Drought tolerance	*ARGOS8*	[39]
Arabidopsis	Cold tolerance	*CBF1 CBF2*	[40]
Biotic stresses			
Arabidopsis	Resistance to turnip mosaic virus	*eIF (iso)4E*	[20]
Wheat	Improved resistance to powdery mildew	*TaMLO*	[25]
	Improved resistance to powdery mildew	*EDR1*	[17]
Rice	Increased resistance to blast fungus	*OsERF922*	[32]
	Increased resistance to tungro spherical virus	*eIF4G*	[28]
Barley	Improved resistance to fungal pathogens	*MORC1*	[41]
Orange	Improved resistance to citrus canker	*CsLOB1*	[42]
Tomato	Improved resistance to powdery mildew	*Mlo1*	[24]
	Anthocyanin biosynthesis	*ANT1*	[43]

Species	Traits	Target Genes	Reference
Grape	Improved resistance to *Botrytis cinerea*	*WRKY52*	[29]
Cucumber	Virus resistance	*eIF4E*	[19]

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