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Editorial Open Access

CRISPR, A New Powerful Weapon for Plant to Combat Viruses

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Editorial

CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9 (CRISPR-associated protein 9) system is the third generation technology for genome editing with the advantages of simplicity and accessibility over the first two generations genome editing tools. It introduces double strand DNA breaks in a sequence specific manner by sgRNA (single guide RNA) and Cas9, and results in gene knockdown. Initially, CRISPR was found in bacteria as part of adaptive immunity system to cope with foreign DNAs, such as viruses. Shortly, it was applied to animals and plants as a genome editing tool. It was reported to edit Arabidopsis genome, rice genome, citrus genome etc [1].

After CRISPR/Cas9 was used to edit genomes, CRISPR/Cas9 was applied in plants to confer plants resistance to DNA viruses [2,3]. Tobacco rattle virus (TRV) was used to deliver gRNA targeting to tomato yellow leaf curl virus (TYLCV) into *Nicotiana benthamiana* overexpression Cas9. Then, challenging the Cas9 transgenic *N. benthamiana* with TYLCV, the plants showed reduced or delayed viral DNA accumulation, and the disease symptoms were abolished or significantly attenuated. It was effective no matter sgRNA targeted coding region or noncoding region. However, the sgRNA targeting stem-loop structure in the origin of TYLCV replication in noncoding region (IR-sgRNA) was more effective. The genomic DNA of virus was cut, and deletion was detected. The same sgRNA targeting to different DNA viruses, including TYLCV, beet curly top virus and merremia mosaic virus could cut the three different viruses and introduced mutations in the viral genomes [2]. Another DNA virus, beet severe curly top virus (BSCTV), was found that it could be edited by sgRNA-Cas9 by transient expression in *N.benthamiana*. Transgenic Arabidopsis and *N. benthamiana* expression sgRNA-Cas9 showed strong resistance to BSCTV. The resistance was related to the expression level of Cas9 [3]. However, until 2017, CRISPR was found that it could edit RNA. Cas13a from *Leptotrichia wadei* (LwaCas13) was the most effective one among the 15 orthologues in the interference assay in *Escherichia coli*. CRISPR/Cas13a was applied to RNA knockdown in animal or plant cells with the similar level of RNA knockdown of RNA interference but better specificity [4]. CRISPR/Cas13b had higher RNA knockdown efficiency than CRISPR/Cas13a. Moreover, catalytically-inactive Cas13 could be used to direct adenosine-to-inosine [5]. CRISPR/Cas9 system from *Francisella novicida* was applied in Arabidopsis and *N. benthamiana* to confer plants resistance to RNA viruses, such as cucumber mosaic virus (CMV) or tobacco mosaic virus (TMV). The plants expression FnCas9/gRNA showed weak disease symptoms and accumulated much less viral RNAs. Moreover, the progenies of transgenic plants with virus-targeting exhibited strong resistance to virus [6]. It is the first report that CRISPR/Cas system to control RNA virus in plant.

CRISPR has the advantage of specificity over RNA interference. Furthermore, CRISPR system has another advantage over RNAi because it can target effectively viruses located in plant nuclei or virus with strong gene silencing suppressor, since RNA interference is less effective to the viruses located in plant nuclei or encoding strong gene silencing suppressor. CRISPR can be used to confer plants enhanced virus resistance by combination of RNA interference. Therefore, CRISPR is a very promising tool to combat DNA virus as well as RNA virus or viroid for plant protection.

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