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Potential application and current achievements of CRISPR/Cas in rice

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Abstract

CRISPR/Cas is a novel technique in editing a specific region of DNA. It has been applied in many organisms and has gained great achievements, especially in rice which is one of the main crops supplying food for 50% of population in the world. Based on CRISPR/Cas9 technology, a study successfully generated a variety of TIFY1 mutant lines with some desirable changes in the cold-tolerant gene. In addition, the rice blast resistance was improved after editing the target gene OsERF922. The Acetolactate Synthase 1 (ALS1), one of the core enzymes involving herbicide resistance of rice could also be edited using CRISPR/Cas9 system. The effectiveness of CRISPR/Cas was successfully proved through many other genes in rice. Therefore, this technique can be applied in rice to produce high yield, high-quality rice varieties which are strongly tolerant to abiotic and biotic stresses.

Keywords: Cold tolerant; CRISPR/Cas; Herbicide resistant; Gene editing; Rice.

Introduction

Rice is an important crop which contributes more than fifty percent food consumption for humans worldwide, and it is the main food in most of the Asian countries. Therefore, at least 160 million ha in 110 countries have been used for cultivation of rice and production of its products supporting for daily life. In addition, global production of rice is annually 700 million tons whereas the contribution of Asian countries is nearly ninety percents. However, rice production has practically faced many risks of unsustainable development and production. Particularly, unpredictable weather has recently occurred in several rice cultivation areas around the world resulting in a huge yield loss. An illustration of this is salinity intrusion in Mekong River Delta of Vietnam in 2015. The productivity of rice, a major agricultural crop in this region, totally lost due to the high concentration of salt intruding in irrigation water that has never seen before. The adverse impacts of climate change have been predicted to be more severe and gradually increase in the future. Researches on sustainable production of rice, therefore, have been promoted and conducted in many rice research centers and institutions to adopt the worst situation relating to the climate fluctuation.

Resulting from the achievements of the third industrial revolution, applications of biotechnology in rice have been had great outcomes in rice breeding and improvement. Many speed, inexpensive, effective, and non-complex methods have reported and successfully applied to release and enhance new rice cultivars with high quality, high yield, abiotic and biotic stress resistance and tolerance. The typical biological technologies applied in rice include molecular marker-assisted selection, genetic transformation (Xu et al., 2014), and gene editing, and the last technique, especially CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)/Cas (associated protein) system has attracted a great deal of attention by rice researchers due to its effectiveness, power, low-cost and ease of use. In fact, such a method has been empirically tested in many living cells from micro organisms including bacteria and yeasts to

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plants such as liverwort, *Arabidopsis*, *Nicotiana benthamiana*, *Chrysanthemum morifolium*, sorghum, tomato, potato, cotton, maize, sweet orange, soybean, wheat, poplar and rice, as well as animals consisting of *Caenorhabditis elegans*, *Drosophila*, zebrafish, mice, rat and human cells (Kishi-Kaboshi et al., 2017; Jianget al., 2013; Osakabe et al., 2016; Gao et al., 2017; Wang et al., 2016). In rice, the modification of genes related to traits of biotic and abiotic tolerance, herbicidal resistance, yield has been carefully tested using CRISPR/Cas9 (Xu et al., 2017). In this paper, remarkable achievements of CRISPR in rice were highlighted to encourage scientists to continue to apply this method in producing and developing more novel rice varieties.

Typical achievements

Cold tolerance

Rice is extremely vulnerable to low temperature, especially at the seedling stage. Therefore, it is practical to enhance the tolerant level of rice at this stage. TIFY1b, a transcription factor, is one of the cold tolerant involving genes discovered in rice (Huang et al., 2017). CRISPR/Cas9 was employed to edit this gene and its homology gene in Nipponbare rice. The results showed that the highest mutagenesis frequency was more than 85% in T0 transgenic lines. The mutation principally occurred in insertion and deletion of one nucleotide. CRISPR/Cas9 changed the DNA sequences at targeted sites and also affected the function of those genes through protein analysis. With CRISPR/Cas9 technique, the study successfully generated a variety of TIFY1 mutant lines in rice.

Rice blast disease resistance

Blast is considered one of the highly severe diseases affecting the sustainable production of rice, especially causing dramatic yield losses. Many experimentations have been conducted to produce and develop high yield and resistant rice cultivars to the disease using a variety of molecular biological techniques (Yan et al., 2017).The CRISPR/Cas9 method initially obtained some encouraging achievements. In particular, the rice blast resistance was improved in the Kuiku131 rice variety after editing the target gene OsERF922 involving in the resistant ability (Liu et al., 2012). The knockout OsERF922 gene results in T0 generation showed that the highest frequency of mutant individuals was induced by C-ERF922S1S2S3 with ninety percents of recovery, following by C-ERF922S1S2 and C-ERF922 with 70 and 42% (Wang et al., 2016). Interestingly, the blast resistance of T2 generation was considerably improved by the mutation at the two stages consisting of seedling and tillering. The finding suggested that CRISPR/Cas9 is an effective technique for improving rice blast resistance.

Herbicide resistance

The main advantage of the development of the herbicide resistance biotechnology in rice has significantly benefited the management and application of agrochemicals, which have been initiated due to the complex regulatory process and public health (Li and Jennings, 2017a, b). Since human and environmental health are heavily impacted by the use of agrochemicals (Li, 2018), herbicide resistance biotechnology of rice is one of the most effective ways to manage its application.

Rice has been developed the herbicide resistance to retain the growth under treatments of a range of herbicides. The Acetolactate Synthase 1 (**ALS1**) is one of the core enzymes involving herbicide resistance of rice. Sun et al., (2016) carried out mutations using multiple discrete points in the gene ALS of rice under applying CRISPR/Cas9 technique. They used two gRNAs for guiding the system to change both amino acid residues including W584 to L and S627 to I. The obtained results showed that CRISPR/ Cas9-mediated homology-directed repair was successful. This indicated that the method could create double-stranded breaks at the targeted sites where CRISPR/Cas9 aimed to edit. Additionally, herbicide resistance tests were in line with the mentioned results in which the mutant plants were resistant to bispyribac sodium (100 μM) after 10 days of the foliar application while the non-edited plants died after treatment of the herbicide at the same concentration.

Another experiment was conducted on the rice Bentazon Sensitive Lethal gene (**BEL**) that relates to bentazon and sulfonylurea herbicide resistance (Xu et al., 2014). The authors targeted the second exon of the BEL gene region of Nipponbare rice cultivar. The sequencing results indicated the effectiveness of sgRNA: Cas in rice with fifteen deletion and replacement mutations detected. The phenotypic screening supported the results of genetic mutants. Once again, the CRISPR/Cas9 was conclusively proved to be potential in editing rice genes.

Others

The CRISPR/Cas9 technique has also been proved to capably-edit other genes in rice. Ten target genes including OsPDS (albino), OsPMS3 (photo-period sensitive male sterile), OsMYB5 (MYB family transcription factor), OsEPSPS (lethal), OsYSA (albino young seedling), OsMSH1 (pleotrophic phenotype), OsROC5 (abaxial leaf rolling), OsDERF1 (drought tolerance), OsSPP (early seedling leaf chlorosis), and OsMYB1 (MYB family transcription factor) were selected for a gene-editing experiment in the study of Zhang et al., (2014). The results showed that the mutation rates ranged from 21.1 to 66.7% in T0 plants. Five target sites with more than 50% of mutants consisted of OsMYB1, OsYSA (sgRNA1), OsROC5, OsYSA (sgRNA2), and OsDERF1. Additionally, the mutants were successfully inherited to the T1 generation according to the Mendelian law. Briefly, CRISPR/Cas9 system was demonstrated to have potential in editing rice genome.

Conclusions

Five years of invention, CRISPR/Cas has been experimentally tested in plenty of genes of many living cells, and most of the trials obtained encouraging results. Among important crops, rice is regarded the most crucial crop because of its high percentage of consumption worldwide. To adapt to climate change, the essential genes relating to abiotic and biotic stress resistance such as cold tolerance, blast disease resistance and herbicide resistance have been targeted for editing using CRISPR/Cas system to generate novel strong, high-yield and quality rice varieties for sustainable production. As a result, transgenic rice with desirable traits can be expected to produce in the near future by application of CRISPR/Cas technology.

References

1. Gao W., Long L, Tian X, et al. Genome editing in cotton with the CRISPR/Cas9 system. Frontiers in Plant Science.2017; 8: 1364.

2. Huang, X-Z., Zeng, X-F., Zhao D-G., et al. Construction and analysis of tify1a and tify1b mutants in rice (Oryza sativa) based on CRISPR/Cas9 technology. Journal of Agricultural Biotechnology. 2017; 25: 1003-1012.

3. Jiang W., Zhou H., Fromm M., et al. Demonstration of CRISPR/Cas9/sgRNA-mediated targeted gene modification in Arabidopsis, to-

bacco, sorghum and rice. Nucleic Acids Research. 2013; 41: e188.

4. Kishi-Kaboshi M., Aida R., Sasaki K. Generation of gene-edited Chrysanthemum morifolium using multicopy transgenes as targets and markers. Plant and Cell Physiology. 2017; 58: 216–226.

5. Li Z. and Jennings A.A. Worldwide regulations of standard values of pesticides for human health risk control: A review. International Journal of Environmental Research and Public Health.2017; 14: 826.

6. Li Z. and Jennings A.A. Implied maximum dose analysis of standard values of 25 pesticides based on major human exposure pathways. AIMS Public Health.2017; 4: 383-398.

7. Li, Z., (2018). Health risk characterization of maximum legal exposures for persistent organic pollutant (POP) pesticides in residential soil: An analysis. Journal of Environmental Management.2018; 205: 163-173.

8. Liu D.F., Chen X.J., Liu J.Q., et al. (2012). The rice ERF transcription factor OsERF922 negatively regulates resistance to Magnaporthe oryzae and salt tolerance. Journal of Experimental Botany.2012; 63: 3899-3912.

9. Osakabe, Y., Watanabe, T., Sugano, S.S., et al. Optimization of CRISPR/Cas9 genome editing to modify abiotic stress responses in plants. Scientific Reports.2016; 6: 26685.

10. Sun Y., Zhang X., Wu C., et al. Engineering herbicide-resistant rice plants through CRISPR/Cas9-mediated homologous recombination of Acetolactate Synthase. Molecular Plant. 2016; 9: 628–631.

11. Wang F., Wang C., Liu P., et al. Enhanced rice blast resistance by CRISPR/Cas9-targeted mutagenesis of the ERF transcription factor gene OsERF922. PLoS ONE. 2016; 11: e0154027.

12. Qin R., Wang L., Yang J., et al. Gene targeting using the Agrobacterium tumefaciens-mediated CRISPR-Cas system in rice. Rice. 2014; 7: 5.

13. Qin R., Wei P., Yang J., et al. Generation of targeted mutant rice using a CRISPR-Cpf1 system. Plant Biotechnology Journal. 2017; 15: 713-717.

14. Yan L., Yan B-Y., Peng Y-L., et al. Molecular screening of blast resistance genes in rice germplasms resistant to Magnaporthe oryzae.Rice Science. 2017; 24: 41-47.

15. Zhang H., Zhang J., Wei P., et al. The CRISPR/Cas9 system produces specific and homozygous targeted gene editing in rice in one generation. Plant Biotechnology Journal, 2014; 12: 797-807.