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CRISPR/Cas9-Mediated Gene Editing of the *OsESP4-1* and *OsESP4-2* Genes in Rice

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[Introduction]

In recent years, genome editing technologies have been successfully used to genetically modify plants. Among them, CRISPR/Cas9 system can efficiently introduced several mutation types (base substitution, insertion, deletion, inversion of a large chromatin fragment). Also, the CRISPR/Cas9 system can edit multiple target sites of one gene for increasing mutation efficiency and also edit several genes simultaneously for knockout of redundant gene family.

[Materials and methods]

The targeted sequence used to generate sgRNA expression cassettes were selected with the assistance of an online tool called CRISPR-P(http://crispr.hzau.edu.cn/CRISPR2). The CRISPR/Cas9-related vectors (toolbox with multifaceted multiplexed CRISPR/Cas9 reagents) are purchased from addgene. The assembly is based on Golden Gate cloning and multigateway recombination methods with no PCR required.

[Results and Discussions]

Recently, we identified a novel knock-out mutant (*hpd* mutant) involved in phosphate (Pi) starvation signaling in *Arabidopsis*. The *hpd* mutant exhibited enhanced phosphate uptake, induced *PSI* gene expression, and altered root architectural under Pi starvation compared to wild type. Expression analysis using auxin-responsive *DR5::GUS* reporter gene indicated that both auxin biosynthesis and auxin translocation under Pi starvation are suppressed in *hpd* mutant plants. Mis-regulation of auxin translocation in *hpd* mutant was further confirmed by analysis of expression patterns of auxin efflux carrier proteins, PIN-FORMED (PIN) 1, 2, and 3 fused with GFP. Molecular genetic analysis of *hpd* mutant revealed that the mutant phenotype is caused by the lesion in *ENHANCED SILENCING PHENOTYPE4* (*ESP4*) gene whose function is proposed in mRNA processing. The results indicated that loss of *ESP4* function can induce tolerance to Pi deficiency in plants. We intend to generate rice transgenic plants tolerant to Pi starvation by using CRISPR/Cas9-mediated suppression of rice ESP4 activity.

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