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Bidirectional Promoter-Based CRISPR-Cas9 Systems for Plant Genome Editing

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Abstract

CRISPR-Cas systems can be expressed in multiple ways, with different capabilities regarding tissue-specific expression, efficiency, and expression levels. Thus far, three expression strategies have been demonstrated in plants: mixed dual promoter systems, dual Pol II promoter systems, and single transcript unit (STU) systems. We explored a fourth strategy to express CRISPR-Cas9 in the model and crop plant, rice, where a bidirectional promoter (BiP) is used to express Cas9 and single guide RNA (sgRNA) in opposite directions. We first tested an engineered BiP system based on double-mini 35S promoter and an Arabidopsis enhancer, which resulted in 20.7% and 52.9% genome editing efficiencies at two target sites in T0 stable transgenic rice plants. We further improved the BiP system drastically by using a rice endogenous BiP, OsBiP1. The endogenous BiP expression system had higher expression strength and led to 75.9-93.3% genome editing efficiencies in rice T0 generation, when the sgRNAs were processed by either tRNA or Csy4. We provided a proof-of-concept study of applying BiP systems for expressing two-component CRISPR-Cas9 genome editing reagents in rice. Our work could promote future research and adoption of BiP systems for CRISPR-Cas-based genome engineering in plants.

Key words: CRISPR-Cas9; bidirectional promoter; enhancer; plant genome editing; rice

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