CRISPR-Act3.0 for highly efficient multiplexed gene activation in plants

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RNA-guided CRISPR activation (CRISPRa) systems have been developed in plants. However, simultaneous activation of multiple genes remains challenging. Here, we develop a highly robust CRISPRa system working in rice, *Arabidopsis* and tomato, CRISPR-Act3.0, through systematically exploring different effector recruitment strategies and various transcription activators based on dSpCas9 (deactivated *Streptococcus pyogenes* Cas9). The CRISPR-Act3.0 system results in four- to six-fold higher activation than the state-of-the-art CRISPRa systems. We further develop a tRNA-gR2.0 (single-guide RNA 2.0) expression system enabling CRISPR-Act3.0 based robust activation of up to seven genes for metabolic engineering in rice. In addition, the CRISPR-Act3.0 allows simultaneous modification of multiple traits in *Arabidopsis*, which are stably transmitted to the T3 generations. Based on CRISPR-Act3.0, we elucidate guide RNA targeting rules for effective transcriptional activation. To target T-rich PAMs (protospacer adjacent motifs), we transfer this activation strategy to CRISPR-dCas12b and further improve the dAaCas12b-based CRISPRa system. Altogether, our study has substantially improved the CRISPRa technology in plants and provided plant researchers a powerful toolbox for efficient gene activation in foundational and translational research.