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Expanding the Plant Base-Editing Toolbox with DarT-Mediated Append Editing

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Base editing tools are transforming plant biotechnology, enabling precise single-nucleotide changes for trait improvement and sophisticated functional genomics. However, it remains challenging to introduce these desirable sequence modifications at a specific location within the genome due to efficiency constraints and target sequence limitations. Therefore, further development and optimization of precision editing tools are necessary to enhance their flexibility and efficiency. Append editing is a new base editing technology with the potential to diversify the base-editing toolbox. This system leverages the bacterial ADP-ribosyltransferase DarT fused to a Cas9 nickase (nCas9), enabling targeted modification of thymine (T) bases to adenine (A), cytosine (C), or guanine (G) without introducing double-strand DNA breaks. nCas9 guided by gRNA directs DarT to the target site and creates a nick on the non-target strand, while DarT attaches a bulky ADP-ribose adduct onto the target T in a specific sequence context. During DNA replication, this bulky adduct is reconciled through a combination of translesion DNA synthesis and nucleotide excision repair pathways, resulting in a base change. We are currently optimizing append editing in *Nicotiana benthamiana*, characterizing editing outcomes in plants transiently or stably transformed with different DarT-nCas9 variants. We are evaluating multiple construct architectures, including different Cas9 variants and nuclear localization signal configurations, the incorporation of additional introns into Cas9 and DarT, and DarT proteins derived from other bacterial species. Through optimization of DarT append editing, we hope to increase the efficiency, specificity, and flexibility of the base-editing toolkit. Ultimately, advances in precision genome editing technologies have the potential to accelerate breeding cycles by enabling the direct and targeted modification of genes directly in elite varieties.