Engineering of plant retrotransposon Tto1 for transposon tagging in crop plants

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Transposable elements (TEs) have been used for insertion mutagenesis in many plants. In contrast to DNA transposons, which generate footprints upon excision, retrotransposons stay at the site of integration and therefore allow easy identification of the mutated site. In most successful cases of transposon tagging, the employed transposons are endogenous residents of the plant of choice. However, highly active mobile elements are not known for many plant species of interest. The ideal TE for mutagenesis would be highly active, but then become "dead on arrival" in order to obtain stable mutants. We have engineered retrotransposon Tto1 from tobacco for use as a mutagenesis tool in different plant species, namely Arabidopsis, *Pisum sativum* (in collaboration with Dr. Hans-Jörg Jacobsen, Institut für Pflanzengenetik, Leibniz Universität Hannover) and *Hordeum vulgare* (in collaboration with Dr. Jochen Kumlehn IPK Gatersleben). The endogenous, stress-responsive promoter was replaced by strong constitutive or inducible promoters to circumvent the necessity for callus culture in order to activate transposition. The LTR regions were reduced to a minimum length in order to minimize silencing triggered by repetitive sequences. Finally the element was equipped with monocot- or dicot-specific introns to enhance the expression rate and transcript stability. We successfully identified transposition events of Tto1 engineered in this way in Arabidopsis (Böhmdorfer et al., 2010 Syst. Synth. Biol. 4:133-8). A higher rate of cDNA formation in a *ddm1* mutant background indicates that transcriptional silencing via DNA methylation restricts Tto1 activity, therefore (transient) downregulation of DNA methylation might be an additional tool to enhance mutagenesis efficiency of Tto1 in the future.