Development of a precise marker excision system in plants

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The use of selectable marker genes such as antibiotic resistant genes, is necessary to isolate transformed cells from among non-transformed cells. Therefore, a suite of strategies has been developed to remove selectable marker genes from plant genomes after selection of transformed cells and plants. In this study, we revealed that animal-derived *piggyBac* transposon can be transposed without leaving footprints at the excised sites and can be utilized for precise marker excision in plants.

Keywords: rice, transgenic plants, *piggyBac* transposon, marker excision

Background

In order to generate and utilize transgenic plants, the selectable marker genes are needed to eliminate following the establishment of transgenic cells. To date, it has been reported that several methods, such as transposition and site-specific recombination, were employed successfully to remove selectable marker genes from transgenic plants. However, marker excision using these methods leaves dispensable sequences such as the residual footprint and recombinase recognition sequences at the excised site. Meanwhile, animal-derived *piggyBac* transposon excises without leaving a footprint at the excised site and has been used for precise marker excision in animal cells. In this study, we investigated whether *piggyBac* can also be transposed accurately in rice cells.

Results and Discussion

1. We designed an assay system that allows visualization of transposase-mediated transposition of *piggyBac* as luminescence derived from reconstituted luciferase (LUC) expression cassettes (Fig. 1A). The reporter construct carrying a LUC expression cassette containing *piggyBac* transposon was introduced into rice calli.
2. Transgenic rice calli harboring reporter constructs were infected with *Agrobacterium* to introduce the control vector or expression vector of transposase PBase. After antibiotic selection, LUC luminescence was detected on PBase-expressing rice calli but not on control calli (Fig. 1B). Furthermore, sequence analysis showed that *piggyBac* was transposed accurately from the reporter construct (Fig. 1C).
3. Regenerated plants were obtained from LUC-positive calli and were subjected to *piggyBac*-excision and re-integration assay. PCR analysis revealed that *piggyBac* transposon was excised from the reporter construct in more than 70% of PBase-expressing plants (Table 1). In addition, the *piggyBac* transposon was lost from the reporter construct in 30% of regenerated plants without concomitant re-integration of the transposon (Fig. 1A and Table 1).
4. Segregation after crossing resulted in T_1 progeny without the selectable marker gene as well as PBase expression.

Future Prospects

1. The *piggyBac* transposon has been shown to transpose efficiently not only in many animal species but also in rice. It is expected that *piggyBac* would also be functional in other plant species and could therefore be widely used as an efficient and precise marker excision system for plant genomes.
2. We are currently developing a reversible transgenesis system in plants in which the delivery of the transgene from T-DNA to genome and sequential excision of the transgene from the genome are both mediated by *piggyBac* transposon.

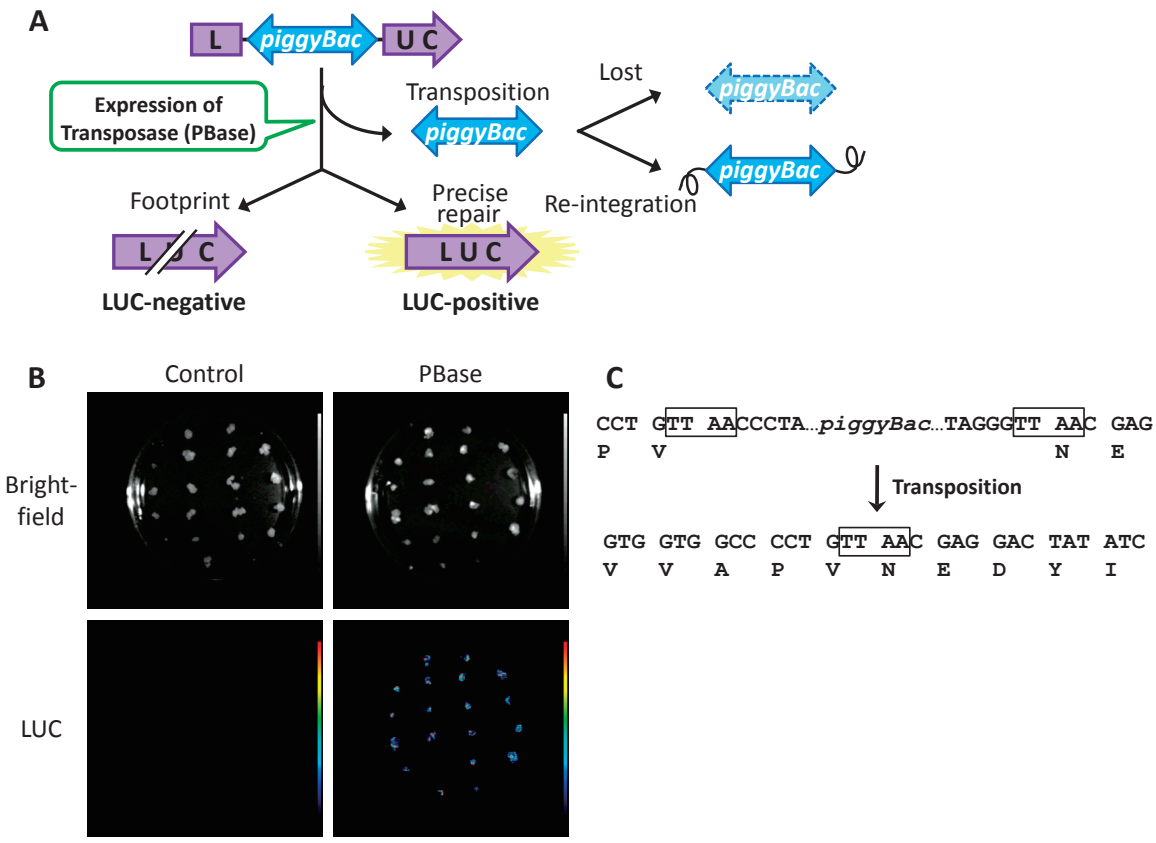

Fig. 1. Analysis of *piggyBac* transposition from reporter constructs in rice calli. (A) Schematic representation of excision assay to detect the transposition of *piggyBac* transposon as luciferase luminescence in rice calli. (B) LUC luminescence images of control (left) and PBase-expressing calli (right) after 4-weeks of selection. The luminescence intensity is shown with the false color scale. (C) Nucleotide sequences of the original reporter construct (top) and *piggyBac* excision site in the LUC reporter cassette (bottom) in PBase transgenic rice calli.

Table 1. PCR analysis of *piggyBac* excision and re-integration events in PBase- expressing T_0 plants

Line no.	No. of T_0 plants analyzed	*piggyBac* excision from reporter locus			Frequency of *piggyBac* excision (%)		
		without *piggyBac*	with *piggyBac*	Total	without re-integration	with re-integration	Total
1	36	4	12	16	11.1	33.3	44.4
2	40	12	22	34	30.0	55.0	85.0
3	20	3	14	17	15.0	70.0	85.0
4	30	24	3	27	80.0	10.0	90.0
5	21	4	10	14	19.0	47.6	66.7
6	20	5	6	11	25.0	30.0	55.0
7	29	10	12	22	34.5	41.4	75.9
Ave.					30.7	41.0	71.7

Collaborator

Keishi Osakabe (University of Tokushima)

Reference

1. Nishizawa-Yokoi A, Endo M, Osakabe K, Saika H, Toki S (2014) Precise marker excision system using an animal-derived *piggyBac* transposon in plants ***The Plant Journal*** 77(3):454-463