Botanical Studies (2011) 52: 375-381.

A one-time inducible transposon for terminating selectable markers in transgenic plants

Hung-Kuang TAI, Kuan-Te LI, and Yuh-Chyang CHARNG*

Department of Agronomy, National Taiwan University, No. 1 Sec. 4 Roosevelt Rd., Taipei, Taiwan, Republic of China

(Received July 19, 2010; Accepted December 7, 2010)

ABSTRACT. Since the maize transposon *Ac* can relocate to a new location within the genome, it has been used in removing selectable markers in transgenic plants. We previously developed an inducible transposon system to truncate a selectable marker in transgenic plants by locating one end of the transposon in the intron of the marker gene (glyphosate tolerant *epsps* gene). We have now improved upon this system by locating the other end of the transposon in the intron of the transposase gene, which is controlled by the inducible promoter (PR-1a). Treatment with salicylic acid induced transposition of this transposon, *COKC*, which led to both marker gene and transposase gene breakages in exons. The behavior of *COKC* was analyzed in single-copy transgenic rice plants. We determined the expression of the modified transposase and *epsps* genes and the transposition events in transgenic plants. The *COKC* element thus exhibits potential as a tool to create "marker-off" transgenic plants for woody or vegetatively propagated plants species.

Keywords: *Ac* transposase; *epsps*; Inducible transposon; Selectable marker; Transgenic plants.

Abbreviations: HPT, hygromycin phosphotransferase; ***epsps*,** 5-enolpyruvylshikimate-3-phosphate synthase gene; **GOI,** gene of interest; ***TPase*,** transposase gene; **SA,** salicylic acid.

INTRODUCTION

Genetic modification of plants offers improved agricultural practices, food safety, and human health. In current plant transformation systems, a selectable marker gene is co-delivered with the gene of interest (GOI) to identify and separate rare transgenic cells from non-transgenic cells. Marker genes, however, are not usually needed once transgenic plants have been identified. Transgenic crop plants carrying antibiotic and herbicide resistance markers in particular, may elicit environmental and consumer concerns. The development of marker-free transgenic plants is thus desirable in agricultural biotechnology. Many strategies to produce marker-free transgenic plants have been described, including co-transformation, *cre/lox* and transposon systems (reviewed by Ebinuma et al., 2001; Hare and Chua, 2002; Hohn et al., 2002; Miki and McHugh, 2004). Among these, the transposon system (e.g. *Ac*/*Ds*) offers the advantage of providing information about the new location of the removed marker's DNA for commercial regulatory approval.

The maize transposon *Ac* is an autonomous transposable element of 4,565 bp and is active in a wide range of plant species. It codes for a single gene product, *Ac* transposase, which, together with the inverted repeats and about 250 bp of both ends (terminal regions) of the transposon and putative host factors, is the only prerequisite for transposition of the *Ac* element in plants (Haring et al., 1991). In the transformation vector, the marker gene is inserted into the *Ds* element. The expression of the *Ac* transposase excises both ends of the transposon and usually re-integrates into other locations on the chromosome. When the transposon transposes within the same chromosome (linked transposition), both insertion sites of the T-DNA (harboring the marker gene) and the transposon (harboring the GOI) need regulatory approval for commercialization. With unlinked transposition, the marker gene can be removed by out-crossing. Although the work is time consuming, all removed information remains clear for regulatory approval. Furthermore, with the transposon system, one successful transformation can create more independent transgenic lines because of the re-integrated loci. This feature is especially valuable in creating transgenic plants from species low in transformation efficiency. Out-crossing with this system, however, cannot be used with vegetatively propagated plants and woody tree species. We have thus designed a system, called "marker-off," that truncates a marker gene after transposition,respects the marker-free system, and does not require segregating the marker away (Charng et al., 2008). This system involves introducing an intron-containing marker gene (glyphosate tolerant *epsps* gene), accompanied by a transposon

*Corresponding author: E-mail: bocharng@ntu.edu.tw; Tel: +886-2-33664774; Fax: +886-2-23620879.

whose one end is located in the intron of the marker gene, into a transformation vector. The new construct, termed as KCEH, contains the *Ac*-based inducible transposon *KCEH* and the glyphosate-tolerant marker gene containing one end of *KCEH* in its first intron. When KCEH was transformed into rice calli, the marker gene acted glyphosate resistant to yield transgenic rice. Subsequently, the *KCEH* in the transgenic rice was induced to transpose, which truncated the marker gene, and consequently the transgenic plants became marker-off. However, it remains the possibility that the *KCEH* continues to transpose and makes the transgenic plants unstable. In this report, we improve the system by locating the other end of the transposon in the intron of the transposase gene itself, which termed as COKC system. Our results show that the transposition of the new transposon truncates both the marker gene as well as the transposase gene and that the expression of these two genes terminates. In addition, the transposon stabilizes and the marker gene shuts off.

MATERIALS AND METHODS

DNA manipulation and plant transformation

The construction procedures for the COKC system are depicted in supplementary material. All cloning and DNA manipulations followed standard procedures (Sambrook and Russel, 2001) with use of chemicals from Roche (Basel, Switzerland). All transformations involved use of rice (*Oryza sativa* L. cv TNG67) as described (Toki, 1997).

Induction of *COKC* transposition

For experiments inducing *COKC*, the T1 rice seeds of each transformed line were incubated on callus induction medium containing hygromycin for 4 weeks to yield enough calli. Hyg^R calli were incubated with 5 mM salicylic acid (SA) for 7 days, then transferred to callus induction medium without SA for 4-8 weeks before PCR analysis.

RT-PCR analysis for *epsps* and transposase gene expression

RNA extracted from transgenic rice was reverse transcribed with use of the SuperScript™ First Strand Synthesis System (Invitrogen). Primers specific to each of the target sequences were used in subsequent PCR amplification: CF (5'-CGTTCAGTGCTGGTGGTCGT-3') and ER (5'-ACAGGGCCCTCATGGAGAGGAGCC-3') for *Ac* transposase; R-E1FC (5'-ATCGTGCTCCAGCCCATCAG -3') and mKRT2R (5'-GGAAACAGTCGACATCCGCGT -3') for *epsps* gene.

PCR analysis of COKC excision events

Transposition of *COKC* in transgenic plants was analyzed by PCR with oligonucleotide primers: primer CF (5'-CGTTCAGTGCTGGTGGTCGT-3') and primer JR (5'-CTACAGCTCTTTTTGCAACTTTATC-3').

The flanking sequences of the T-DNA or *COKC* integration sites in transgenic plants were determined by use of arbitrary degenerate (AD) primers and TAIL-PCR as described previously (Liu et al., 1995; Sha et al., 2004), with modification: the primary TAIL-PCR involved approximately 150 ng of rice genomic DNA. The flanking sequences were amplified with the following oligonucleotide primers: TLnew4 (5'-GGTCAAGACCAATGTGGAGC -3'), TLnew3 (5'-GATTGTGTACGCCCGACAG-3') and TLnew2 (5'-GGATTTTAGTACTGGATTTTGG-3') for T-DNA and 3-1 (5'-GTGTGCTCCAGATTTATATGG-3'), 3-2 (5'-GATTTCGACTTTAACCCGACCGGA-3') and 3-3 (5'-CGTTTTCGTTACCGGTATATCCCG-3') for the 3' end of *COKC*.

Isolation of genomic DNA and Southern blot analysis

Genomic DNA was isolated from transformed plants by use of a kit (Genemark, Tainan, Taiwan). In brief, fresh leaves (2 g) or callus tissue (0.1 g) was frozen in liquid nitrogen and ground with use of a mortar and pestle. Nuclei were isolated and lysed by protease treatment, and genomic DNA was precipitated with ethanol and dissolved in TE buffer (10 mM Tris-HCl, 1 mM EDTA; pH 8.0). About 10 μg of each DNA was digested with the appropriate restriction enzyme under the conditions specified by the suppliers and fractionated on 0.8% agarose gels (in 1×TAE) overnight at 1 V/cm. Southern blot analysis was performed as described (Charng and Pfitzner, 1994).

Assay of T1 progeny resistant to glyphosate

Successful transgenic rice containing *COKC* (transposed or non-transposed) were self-pollinated to obtain T1 seeds. The seeds were imbibed in flowing water for two days, then transferred to an iron grid and kept in water for two weeks. The rice seedlings were sprayed once with 5000 ppm Roundup®. The effect could be observed after one week.

RESULTS

Construction of the one-time transposon system COKC

The construction of the new "marker-off" system COKC is described in Figure 1A and supplementary data. First, the 5' end of the *Ac* element was inserted into the third intron of the transposase gene, driven by a PR-1a promoter from tobacco. This inducible transposase gene was flanked with the hygromycin phosphotransferase (HPT) gene by inserting into the binary vector pCAMBIA 1300, yielding the plasmid pPRA5EA. Then, the 3' end of the *Ac* element was constructed in the first intron of the glyphosate tolerant epsps gene as the selectable marker (Charng et al., 2008), which subsequently fused with a nos promoter upstreamly and a polyA signal downstreamly. This new marker gene was inserted into the pPRA5EA, yielding the plasmid pCOKC, which was then introduced

into rice plants by use of transfection with *Agrobacterium tumefaciens* strain LBA4404. We used 21 single-copy T-DNA integration transgenic plants for the induction and detection of transposition events.

Normal expression of transposase and marker genes with COKC

Previously, we created a construct by inserting the *Ac* 5' end into the first intron of a glyphosate-tolerate rice *epsps* gene to create a "marker-off" transgenic system (Charng et al., 2008). The expression of this modified *epsps* gene showed normal and additional transcripts. Besides the first intron of the modified *epsps* gene, transcription proceeded to the partial 5' end of the transposon, which resulted in an alternative splicing RNA fragment (Charng et al., unpublished results). In construct COKC, the 3' end of *Ac* was inserted in the first intron of *epsps* gene while 5' end was in the third intron of tansposase gene (Figure 1A). We therefore determine whether the transposase and *epsps* genes were expressed normally in the transgenic plants by RT-PCR. Since, the modified *epsps* in the COKC construct and endogenous rice *epsps* differ in 4 bp, primer mKRT2R was designed to rule out the the possibility of amplifying the endogenous rice *epsps*. This primer is mismatched at the two 3' terminal bases for endogenous *epsps* and has more power to identify transgenes. The total RNA of each transgenic line was extracted for RT-PCR. By using primers R-E1FC and mKRT2R, PCR reactions yielded single 439-bp DNA fragment (Figure 1B). These results indicated the normal expression of *epsps* gene. Additionally, since the transposase gene was triggered with PR-1a inducible promoter, the induction was carried out by applying 5 mM SA in the CIM medium for 7 days for single-copy transgenic rice calli. By using primers CF and ER to determine the transposase transcripts, a 263-bp DNA fragment was obtained (Figure 1C). Sequencing analysis confirmed the normal transcripts of the *epsps* and transposase genes in *COKC* (data not shown). All these results indicate that the transposase and marker genes expressed normally in the transgenic plants.

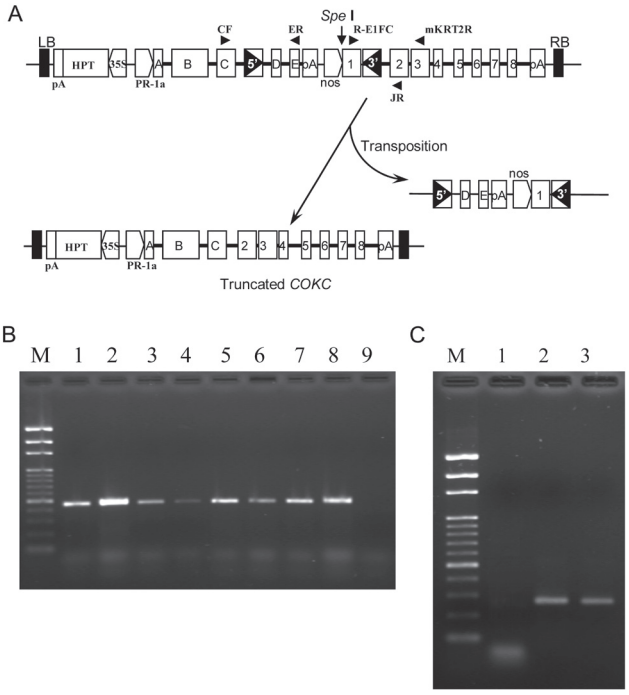

Figure 1. (A) Schematic diagram of the one-time transposon system COKC and location of primers (shown as solid triangle). LB, left border; RB, right border; 5' and 3', *Ac* left and right terminal-inverted repeat; PR-1a, PR-1a inducible promoter; HPT, hygromycin phosphotransferase gene; pA, poly(A) fragment; NOS, nopaline synthase promoter; A~E, transposase gene exon 1~exon 5; 1~8, epsps gene exons; (B) RT-PCR analysis of modified *epsps* expression in transgenic rice lines (1-8) and TNG67 (9); (C) RT-PCR analysis of induced transposase gene expression in transgenic rice calli treated with water (1) or 5 mM SA (2 and 3). M, 100 bp marker.

Transposition events of *COKC* and termination of the glyphosate-tolerant *epsps* in transgenic rice

Previously, we introduced an *Ac*-based inducible transposon, *INAc*, into rice and found the highest transposition efficiency induced with 5 mM SA (Charng et al. 2007). Thus, to remove the functional glyphosate-tolerant *epsps*, we applied the same method trigger the *COKC* transposon. Calli regenerated from the T1 rice seeds of each transformed line harboring a single copy of *COKC* were incubated on callus induction medium containing 5 mM SA for 7 days then incubated on normal CIM for further regeneration. The excision events were determined by Southern blot and PCR analysis. Genomic DNA from 50 transformed lines was subjected to Southern blot analyses. As probes, the 1.4-kb *Bam* HI/*Eco* RV fragment comprising the HPT gene, and the PCR amplified fragment (comprising the 5' end, exon D and E of the transposase gene) were used. The DNA samples were digested with *Spe* I and hybridized with HPT probe. As an example, Figure 2 shows that the transformed lines 17, 19, 20, 21 and 24 yielded single hybridizing fragments demonstrating that these transformed line harboring a single copy of T-DNA. Single copy transgenic plants were confirmed together with further analysis, for example, progeny analysis (PCR based genotyping or glyphosate tolerance) and regular PCR to determine excision events and TAIL PCR to determine T-DNA/transposon flanking sequences (data not shown). Transformed lines 18 and 23 contain more than one T-DNA copies. After removal of the HPT probe, the same filter was hybridized with the TPase probe. The transformed lines 17, 18, 19, and 23 yielded the same hybridizing patterns, indicating the primary donor sites of the un-transposed *COKC* element. For transformed line 20, 21 and 24, in addition to the same hybridizing patterns with HPT probe (un-transposed *COKC*), several new bands were yielded indicating the transposition of *COKC* (Figure 2). By Southern blot analysis, 34 out of 50 transformed lines showed single copy of T-DNA integration (with HPT probe) and 4 out of these lines yielded new

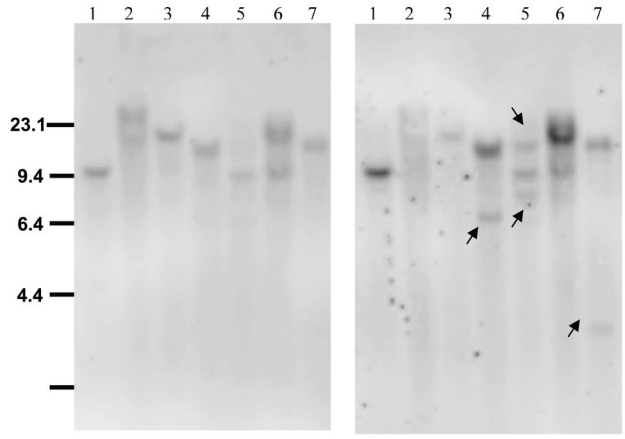

Figure 2. Southern blot hybridization of *Spe* I digested genomic DNAs isolated from different transgenic rice lines with the HYG probe or with the TPase probe (for construction see Figure 1). The HYG probe (*left*) revealed the T-DNA copy and together with the TPase probe (*right*) revealed the un-transposed or transposed *INAc* (indicated by the *arrow*). 1-7 represent the selected plants from transgenic line 17, 18, 19, 20, 21, 23 and 24, respectively.

bands with TPase probe. The experiments were expanded by PCR analysis, in order to determine the transposition event which yielded weak signal by Southern blot analysis. The same DNA samples were used for PCR analysis with the primers CF and JR. To determine the empty donor site, a 450-bp DNA fragment was expected (Figure 3A and B). Of 34 single-copy lines, 7 transgenic lines yielded the expected 450-bp DNA products, including those lines determined by Southern analysis. Sequencing analysis confirmed the residual DNA after the excision of the transposon (Figure 3C). These results indicated somatic transposition efficiency of *COKC* is more than 20%.

The results described above and our previous studies indicated that *Ac*-based inducible transposons are very active in induced rice calli, but sometimes only a portion of cells contain the empty donor site (partial transposition pattern; Charng et al., 2007). We therefore determined whether the transposition events passed through the germ line and were inherited in the progeny (germinal transposition) or not (somatic transposition). The remaining calli of the transposed lines, as well as non-SA treated calli (controls), were cultured to set shoots then transplanted to soil for self-pollination. The seedlings of the progeny underwent PCR to determine the inheritance of the transposition events. Of 7 lines showing transposition, 5 showed the transposition events inherited in the progeny, which yielded 450 bp product with CF and JR primers (data not shown).

Since *COKC* was constructed for one-time transposition, the stability of the transposed *COKC* was determined with progeny of plants harboring single transposed *COKC*. The patterns of *COKC* transposition in transgenic rice were detected by Southern blot analysis, with a DNA fragment (containing the 5' end, exon D and E of the transposase gene) used as a probe. *Spe* I digestion of the genomic DNA resulted in a unique hybridizing band, depending on the line (e.g., 7 kb for line K-20 Starter line), which corresponds to the presence of a transposed *COKC* element (Figure 4). As an example, shown in Figure 4, 8 progeny yielded the unique hybridizing band of *COKC* revealed all assayed progeny with the same hybridization patterns as their control samples. Furthermore, since the transposition of *COKC* resulted in the termination of the marker gene, the progeny of transposed lines were determined for glyphosate-tolerance. The seedlings of the progeny were treated with Roundup®. All seedlings were glyphosate sensitive, which indicates the loss of the glyphosate-tolerant function (Figure 5A). As a control, the same transgenic line which had not been induced with SA for transposition showed glyphosate-tolerance as a single Mendelian locus pattern (Figure 5B). Taken together, the results indicate

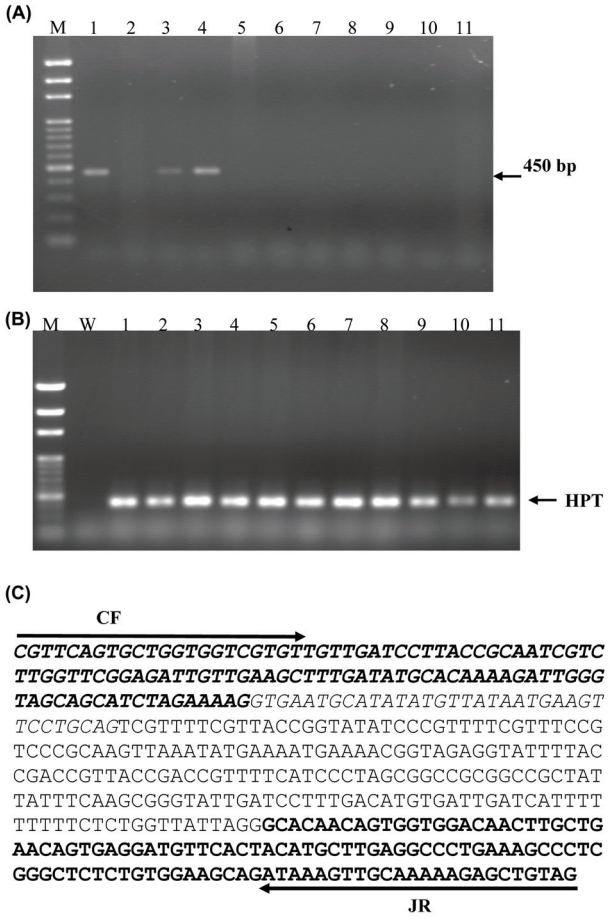

Figure 3. Analysis of transposition events of SA-induced transgenic rice harboring COKC system. (A) PCR analysis of *COKC* transposition with the primers CF and JR and the expected fragments; (B) The control HPT specific products which were amplified with each sample; (C) Sequence of the empty donor site of the transposition events, leaving the sequences from the truncated transposase gene (italics) and the truncated *epsps*, in which exons are shown in bolds. The primers CF and JR are indicated as arrows. M, 100 bp marker. W, PCR negative control which contains no genomic DNA template. 1-11 represent the transgenic line 17, 18, 19, 20, 1, 2, 3, 4, 5, 6, 7, respectively.

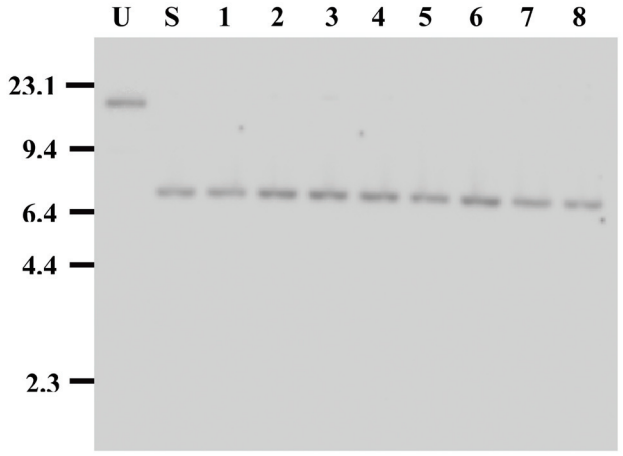

Figure 4. Southern-blot reveals stable transposition of *COKC* from transgenic line K-20. U, progeny line of non-SA induced K-20; S, Starter line harboring the transposed *COKC* after SA treatment; numbers represent 8 selected progeny from K-20-S families after primary PCR analysis. Size markers after ethidium bromide staining are indicated on the left.

that the COKC system offers a desirable selectable marker for rice transformation and the ability to remove the marker thereafter.

DISCUSSION

Many marker-free systems have been estimated in transgenic plants including co-transformed genes, Site-specific recombination system and transposon mediated system. Site-specific recombination system removes the marker gene by using single enzymes (e.g. Cre, FLP, R) acting on specific target sequences. Each of the target sites is similar in that short oligonucleotides surrounded by short inverted repeats determine the orientation of the target site. The expression of the recombinase causes recombination between the target sites and results in lost of the marker gene flanked by the sites. A concern is that high levels of recombinase expression may result in genome rearrangements at cryptic-target sites in plants (Hajdukiewicz, 2001). In contrast to the short target recognition sequences (e.g. 34 bp for *lox*P sites), the existence of pseudo-target sites in plant genome for transposon (e.g. about 250 bp for *Ac*/*Ds*) is believed rare. Transposon mediated system offers the possibility that the GOI and the marker integrate into different loci in the plant genome. Unlinked marker genes can then be segregated away from the GOI and allow the production of marker-free transgenic plants (reviewed by Ebinuma et al., 2001). This technology is not useful for woody plants or plants that reproduce vegetatively. We therefore have applied an inducible transposon technology to develop a marker-off system without the need for out-crossing (Charng et al., 2008). After obtaining stable transgenic plants and inducing transposition, the transposon excised, the marker gene became truncated, and its expression was terminated. This strategy was demonstrated successfully in rice and tobacco plants. However, it remains the possibility that the excised transposon continues to transpose, which results in the transgenic plants being unstable. Therefore, in this report, we constructed the two ends of transposon, one end located in transposase gene and the other in marker, yielding the new COKC system for stable transgenic plants lacking marker and transposon functions.

To study the COKC system, we first determined whether the modified *epsps* was a functional marker, because its first intron was inserted by the 3' end of the transposon. Figure 1B and sequence analysis (data not shown) indicated that the splice junction of the first intron is identical to the junction of the native *epsps*. Figure 2 domonstrated the glyphosate-tolerance function of the modified *epsps*

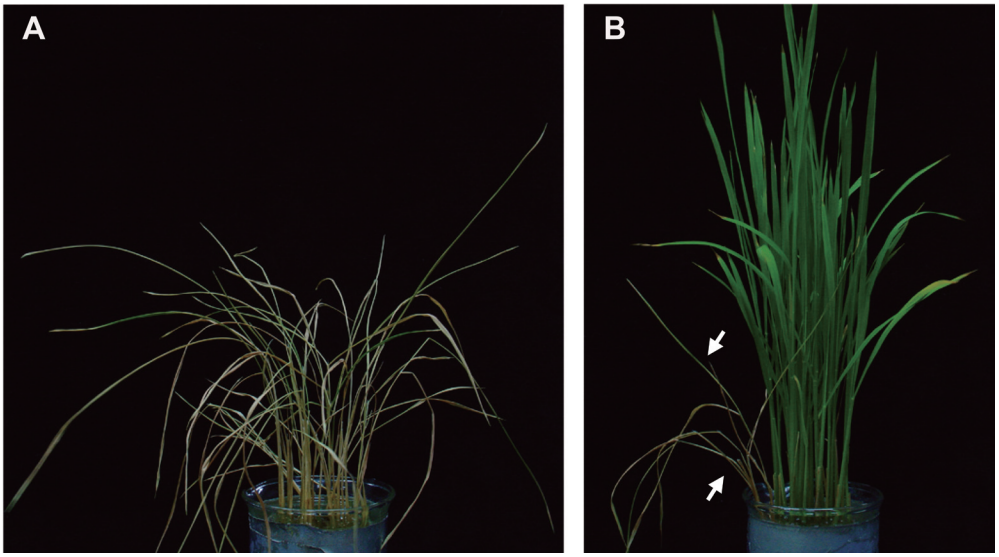

Figure 5. Glyphosate-tolerance analysis of the self-pollinated progeny of *COKC* transposed (A) and untransposed line (B). Arrows indicate the null *COKC* progeny, which are not resistant to glyphosate.

in transgenic rice. Subsequently, we induced the modified transposase gene to determine the transposition, since the third intron of *TPase* was inserted by the 5' end of the transposon itself. Our results shown that *COKC* harbors similar transposition efficiency as previous PR-1a::*TPase*-based inducible transposon in rice (Charng et al., 2007; Li et al., 2008).

In our previous and current works, we located the end of the inducible transposon in an intron of a target gene for subsequent removing its function in transgenic plants (Charng et al., 2008; Li et al., 2008). All these works are based on the fact that, before transposition, the insertion of the end of transposon in an intron did not obviously affect the normal splicing process of the target gene, e.g. the glyphosate-tolerant marker gene (*epsps*). However, the application of our invention is limited to those widely used selectable marker gene without native introns, e.g. Hygromycin- or Kanamycin resistant genes. This fact encourages us to create an artificial intron containing one end of the transposon to expand the application of the "marker-off" transgenic system. Indeed, by inserting the intron harboring the *Ac*-end into the hygromycin phosphotransferase gene as the selectable marker, we obtained successful transgenic plants after screening with the selection agent. Induction of the transposition resulted in the "marker-off" transgenic progenies (Li and Charng, 2011). All these features indicate the application of an inducible transposon for plant biotechnology.

Acknowledgments. This project was supported by the National Science Council (Grant No. NSC98-2324-B-002-003-) of Taiwan.

LITERATURE CITED

Charng, Y.C., K.T. Li, H.K. Tai, N.S. Lin, and J. Tu. 2008. An inducible transposon system to terminate the function of a selectable marker in transgenic plants. Mol. Breed. **21:** 359-368.

Charng, Y.C. and A.J.P. Pfitzner. 1994. The firefly luciferase gene as a reporter for *in vivo* detection of *Ac* transposition in tomato plants. Plant Sci. **98:** 175-183.

Charng, Y.C., G. Wu, C.S. Hsieh, H.N. Chuan, J.Y. Huang, L.C. Yeh, Y.H. Shieh, and J. Tu. 2007. The inducible transposon system for rice functional genomics. Bot. Stud. **48:** 1-11.

Ebinuma, H., K. Sugita, E. Matsunaga, S. Endo, K. Yamada, and A. Komamine. 2001. Systems for the removal of a selection marker and their combination with a positive marker. Plant Cell Rep. **20:** 383-392.

Hajdukiewicz, P.T. 2001. Multiple pathways for Cre/*lox*-mediated recombination in plastids. Plant J. **27:** 161-170.

Hare, P.D. and N.H. Chua. 2002. Excision of selectable marker genes from transgenic plants. Nat. Biotechnol. **20(6):** 575-80.

Haring, M.A., C.M. Rommens, H.J. Nijkamp, and J. Hille. 1991. The use of transgenic plants to understand transposition mechanisms and to develop transposon tagging strategies. Plant Mol. Biol. **16:** 449-461.

Hohn, B., A.A. Levy, and H. Puchta. 2001. Elimination of selection markers from transgenic plants. Curr. Opin. Biotechnol. **12:** 139-143.

Li, K.T. and Y.C. Charng. 2012. The use of hpt gene with an artificial intron in order to obtain marker-off transgenic plants. Afr. J. Biotechnol (in press).

Li, K.T., Y.L. Lin, J.Y. Huang, W.Y. Li, and Y.C. Charng. 2008. A one-time inducible transposon for creating knockout mutants. Mol. Breed. **22:** 85-94.

Liu, Y.G., N. Mitsukawa, T. Oosumi, and R.F. Whittier. 1995. Efficient isolation and mapping of *Arabidopsis thaliana* T-DNA insert junctions by thermal asymmetric interlaced PCR. Plant J. **8:** 457-463.

Miki, B. and S. McHugh. 2004. Selectable marker genes in transgenic plants: applications, alternatives and biosafety. J. Biotechnol. **107:** 193-232.

Sambrook, J. and D.W. Russel. 2001. Molecular Cloning: a Laboratory Manual, 3rd edn. Cold Spring Harbor, NY.

Sha, Y., S. Li, Z. Pei, L. Luo, Y. Tian, and C. He. 2004. Generation and flanking sequence analysis of a rice T-DNA tagged population. Theor. Appl. Genet. **108:** 306-314.

Toki, S. 1997. Rapid and efficient Agrobacterium-mediated transformation in rice. Plant Mol. Biol. Rep. **15:** 16-21.

單次轉位之可誘導轉位子終止轉基因篩選標記

戴宏光　李冠德　常玉強

國立台灣大學 農藝學系

使用篩選標記基因 (selectable marker genes) 可以快速、有效篩選轉殖株，然而在獲得轉殖株後，篩選標記基因就無特殊用途，並有生物安全疑慮，本研究建構單次可誘導轉位子以終止篩選標記之功能。將轉位酶基因上游建構可誘導啓動子，且轉位子之 5 端構築於轉位酶顯子 (exon) C 和 D 間，而將轉位子之 3 端建構於抗嘉磷賽之 *epsps* (5-enolpyruvylshikimate-3-phosphate synthase；篩選標記基因) 顯子 1 和 2 之間，完成之構築命名為 *COKC*。透過誘導劑水楊酸處理，企圖使轉位子轉位 (transposition) 將兩端及其內部分轉位酶基因和部分 *epsps* 基因片段切離，達到終止轉位酶基因和修飾之 *epsps* 基因之功能。

關鍵詞：轉位酶；*epsps*；可誘導轉位子；篩選標記基因；轉基因植物。