Gene Identification Using Ac/Ds Mediated Enhancer/Gene Trap Systems in Rice

Chang-Deok Han

Plant Molecular Biology and Biotechnology Research Center (PMBBRC)
Gyeongsang National University

A. Introduction

Enhancer trap and gene trap are advanced versions of conventional transposon mediated insertional mutagenesis. An enhancer trap transposon harbors a weak minimal promoter fused with an open reading frame of a reporter gene and a genetic marker. Upon the element being inserted near or at a host gene, the weak or minimal promoter will be cis-activated by enhancer elements in the host gene. A P element promoter and 35S CaMV minimal promoter are commonly used for this system in Drosophila and plants, respectively. Reporter genes usually encode β-galactosidase (*lacZ*) in animals and β-glucuronidase (*gusA*) in plants. A gene trap transposon contains artificial splicing acceptor sites fused to the coding region of a reporter gene in three different frames. A fusion protein of the reporter gene with the N-terminal portion of a host gene will be produced if the element is inserted into either an exon or an intron of the host gene in the same orientation of transcription.

B. Objectives

The objectives of the project are 1) to establish gene tagging systems suitable for rice 2) to identify rice genes, based on phenotypes or expression patterns of genes.

During the last four years, we have constructed Ac/Ds-mediated gene/enhancer

trap systems and have evaluated the systems using transgenic plants.

1) Development of trap *Ds* and *Ac* lines
2) Transposon mutagenesis.
3) Isolation of phenotypic mutants generated by *Ds* insertions.
4) Identification of genes showing cell- or tissue-specific expression during plant development
5) Cloning of *Ds* insertion sites
6) Data management

C. Experimental Plans

In our systems, around 80% of trap *Ds* are mobilized in T1 plants carrying Ac. Therefore, it is very difficult to identify plants carrying *Ds* at original sites that could be used for next rounds of mutagenesis. A better way to mutagenize rice plants in a large scale might be to conduct massive pollinations between *Ds* and *Ac* plants. The following experiments will be conducted to generate large numbers of transposants by crossing and chemical selection.

1. Development of trap *Ds* and *Ac* lines

Ac or Trap *Ds* 'starter' lines will be developed and maintained by the following orders;

a. Via Agrobacterium mediated transformation technique, transgenic plants would be generated that carry either *Ds* or *Ac* T-DNA inserts. Transgenic plants that carry a single copy of T-DNA insert would be selected by scoring PPT- or Hygromycin resistant progeny and by Southern analysis.
b. To develop 'starter' lines that show full fertility and normal phenotypes, primary transformants would be back-crossed to normal rice plants.
c. 'Starter' lines would be propagated that are homozygous for single loci of *Ac* or trap *Ds*.

d. *Ds* donor sites would be cloned by TAIL-PCR or iPCR

Present status

More than 15 *Ds* starter lines have been selected that carry one copy of T-DNA insert. In the last summer, those *Ds* lines were backcrossed to *Ac* 'starter' lines have been propagated in greenhouse and were examined for the efficiency of mobility of original *Ds*.

In the meeting, the behaviour of Ds will be discussed.

2. Generation of transposants.

A genetic scheme would be employed to identify plants containing *Ds* insertions unlinked or loosely linked to the donor locus.

1) *Ac* and *Ds* lines are crossed each other.
2) PPT-resistant F1 plants are allowed to self-pollinate.
3) F2 seeds from each F1 plant are harvested
4) Around 1,000 F2 seeds are subjected to double selection (R7402 and PPT)
5) F2 plants that survive the double selection are propagated and maintained as transposant lines.

Expected performance

Each year, we will generate 15, 000 20,000 F1 seeds. Production of F1 seeds by hand-pollination will be achieved by collaboration with rice researchers at Yeong Nam Agricultural Experiment Station.

3. Isolation of *Ds*-tagged phenotypic mutants.

Trap systems have been implanted in Dong-jin, Japonica type rice varieties. The line is a top leading variety that is morphologically and genetically well studied and are most widely cultivated in Korea.

4. Identification of genes showing cell- or tissue-specific expression

Ultimate goals of this experiment are 1) to obtain molecular accesses to genes that determine pattern formation and 2) to make molecular and genetic dissection on stress-related phenomena. As described below, along with genetic analysis and morphological inspection, cell- or tissue-type specific GUS stains will be sought out to identify genes involved in organ formation and stress-response.

5. Cloning of *Ds* insertion sites

We have adapted TAIL-PCR and iPCR to isolate genomic regions flanking *Ds* insertions. We have designed two sets of three nested *Ds* primers to isolate either 5 or 3 *Ds* flanking regions. They are used in combination with one of four arbitrary degenerate primers to perform TAIL-PCR.

By combination of TAIL-PCR (thermal asymmetric interlaced PCR) and IPCR (inverse PCR), genomic DNA flanking with the following Ds loci will be cloned;

1) Original *Ds* insertion sites
2) *Ds* loci linked to phenotypic mutations of interest.
3) *Ds* loci responsible for GUS stains of interest.

6. Database management

We will develop a database of enhancer and gene trap lines, which includes pedigrees, segregation data, visible phenotypes, staining pattern, cloning information on *Ds* loci, and Southern data. This database could easily be adapted to include sequence and map data, and the project we are proposing is small enough to be managed without a specialized sequence database.