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Induction of chromosomal inversion by integration of T-DNA in the rice genome

Chuanfeng Zhu, Jiahe Wu, Chaozu He*

State Key Laboratory of Plant Genomics, *Institute of Microbiology*, *Chinese Academy of Sciences*, *Beijing 100101*, *China*

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Abstract

Transfer DNA (T-DNA) of *Agrobacterium tumefaciens* integration in the plant genome may lead to rearrangements of host plant chromosomal fragments, including inversions. However, there is very little information concerning the inversion. The present study reports a transgenic rice line selected from a T-DNA tagged population, which displays a semi-dwarf phenotype. Molecular analysis of this mutant indicated an insertion of two tandem copies of T-DNA into a locus on the rice genome in a head to tail mode. This insertion of T-DNA resulted in the inversion of a 4.9 Mb chromosomal segment. Results of sequence analysis suggest that the chromosomal inversion resulted from the insertion of T-DNA with the help of sequence microhomology between insertion region of T-DNA and target sequence of the host plant.

Keywords: *Agrobacterium tumefaciens*-mediated transformation (ATMT); T-DNA; chromosomal inversion

Introduction

Agrobacterium tumefaciens is well known for tumor-induction in host wound sites and for transferring an oncogenic segment of DNA from the bacterium into the plant cell. *Agrobacterium tumefaciens* possesses a tumor-inducing (Ti) plasmid that encodes the major proteins required for processing and transferring of transfer DNA (T-DNA). Ti plasmid has now been modified to serve as a vector for introducing foreign DNA into plant chromosomes (Hoekema et al., 1983; Zambryski et al., 1983; Bevan, 1984; An et al., 1985). Such vectors have been used successfully for DNA transfer in a large number of dicotyledonous species and some cereal crops, such as rice, maize and barley. In addition, *Agrobacterium tumefaciens*-mediated transformation (ATMT) is used extensively for the study of functional genomics, because T-DNA insertion has an advantage over other methods in its stable inheritance, low copy number, disruption of the expression of the gene into which it is inserted, and the use of the inserted fragment as a marker for subsequent analysis of the mutants. Some approaches, such as inversed PCR and thermal asymmetric interlaced-PCR (TAIL-PCR), have been used to recover the sequences flanking the T-DNA insertion site in the plant genome.

T-DNA appears to be integrated randomly into chromosomal sites by non-homologous recombination in plant, fungal and mammalian cells. However, accumulated evidence suggests that T-DNA integration often induces base substitutions, insertions and rearrangements at the insertion site. Major rearrangements of the plant DNA associated with T-DNA transformation have been observed in *Arabidopsis* and rice (Castle et al., 1993; Takano et al.,

* Corresponding author. Tel: +86-10-6480 7397.
E-mail address: hecz@im.ac.cn

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1997; Nacry et al., 1998; Laufs et al., 1999). Chromosomal rearrangement including translocation and inversion is thought to be a common feature of T-DNA transformed plants (Nacry et al., 1998). Inversion can be divided into paracentric and pericentric inversion. Breakpoints of inversion were found to lie in the transposon, retrotransposon and gene families arranged in tandem (Caceres et al., 1999; Dehal et al., 2001).

Although an illegitimate recombination model was proposed for T-DNA integration in plant cells, the role of host DNA sequences and topological genome structure at the integration site has not been elucidated. Major chromosomal rearrangements are fewer, and their origins are unclear. It is not clear if they are directly associated with the integration of the T-DNA(s) or if they occur after the integration of the T-DNA(s). In the present study, a rice transgenic line was obtained from a T-DNA tagged population. Molecular and sequence analyses suggest that an inversion of the chromosomal fragment occurred and that this chromosomal inversion may result from T-DNA integration.

Materials and methods

Rice growth conditions

The rice cultivar, *Oryza sativa* L. cv. Nipponbare, was used as wild type in this study. A rice transgenic line was obtained from a screening of a T-DNA tagged population (Sha et al., 2004). Rice plants were grown in a greenhouse. Leaves were sampled and ground to fine powder in liquid nitrogen.

DNA extraction and DNA gel blot analysis

Genomic DNA was extracted as previously described (Sha et al., 2004). For DNA gel blot analysis, about 10 μg of genomic DNA was digested with *Hin*d III or *Apa* I, fractioned in 1% agarose gel, blotted onto hybond-N^+ membranes and hybridized with α-^{32}P-dCTP-labeled probes. PCR products amplified from rice genomic DNA using corresponding primers (Table 1) was used as probes. Hybridization and washing were conducted using standard protocols (Clark, 1997). Hybridized membranes were exposed to a phosphor screen and the signal was detected by scanning the phosphor screen with a STORM 840 phosphorimager and ImageQuant software (Amersham Biosciences, USA).

TAIL-PCR and sequence analysis

TAIL-PCR was conducted according to a previous method (Sha et al., 2004). The flanking sequences at the right and left border of the insertional T-DNA fragment in line A1473 was isolated by TAIL-PCR (Liu et al., 1995)

Table 1
Primers used in the study

Name	Forward primer	Name	Reverse primer	Remarks
H_f	5′-TGCGCCCAAGCTGCATCAT-3′	H_r	5′-TGAACTCACCGCGACGTCTGT-3′	Probe h
d_f	5′-CATTCCAGACAAAGGAATGG-3′	d_r	5′-CTGTTACAGTGGGATGCACA-3′	Probe d
e_f	5′-GAGTCGTGCAACAGAAGGTT-3′	e_r	5′-GCCACAAAAGCTGAAGTGAT-3′	Probe e
f_f	5′-TACAACGGGTTTCGCTCTAC-3′	f_r	5′-ACCAGACCTGAAGTGAGCAG-3′	Probe f
g_f	5′-CCCTTGTTCTCCAAATGATG-3′	g_r	5′-ACTGCAAACACCTCGTTCTC-3′	Probe g
C1_f	5′-GGAATAGTTAATGGCGTCAGG-3′	C1_r	5′-AACAACCGCCGCTTCGTTCAGGTAT-3′	Probe c
LT1_f	5′-CGTCTGGACCGATGGCTGTG-3′	LT1_r	5′-CCATCTAATCGCCTTGGGTA-3′	Probe b
SP1_f	5′-GGTGACCAGCTCGAATTTCCC-3′	R2_r	5′-TCGTGCAGGAAAGTAGCCGT-3′	Probe a
SP1	5′- GGTGACCAGCTCGAATTTCCC-3′	LT1_f	5′-CGTCTGGACCGATGGCTGTG-3′	For TAIL-PCR analysis
SP2	5′ –TGAATCCTGTTGCCGGTCTTG-3′	LT2_f	5′-TACTCGCCGATAGTGGAAACCG-3′	
SP3	5′ GCGCGCGGTGTCATCTATGT-3′	LT3_f	5′-TCGTCCGAGGGCAAAGAAATA-3′	
AD4	5′-TG(A/T)GNAG(A/T)ANCA(G/C)AGA-3′			
AD8	5′-(G/C)TTGNTA(G/C)TNCTNTGC-3′			
AD9	5′-(A/T)CAGNTG(A/T)TNGTNCTG-3′			
C1_f	5′-GGAATAGTTAATGGCGTCAGG-3′	C2_r	5′-GTTTAGCAGTTTGAAAAGCGTG-3′	For PCR analysis
C1_f	5′-GGAATAGTTAATGGCGTCAGG-3′	C1_r	5′-AACAACCGCCGCTTCGTTCAGGTAT-3′	
R1_f	5′-TAATGGCGTCAGGTGGCTG-3′	R2_r	5′-TCGTGCAGGAAAGTAGCCGTA-3′	For co-segregation analysis
Rb_f	5′-GCGCGCGGTGTCATCTATGT-3′			