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Ac/Ds-induced chromosomal rearrangements in rice genomes

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Abbreviations: SCT, sister chromatid transposition; SLCT, single chromatid transposition; HR, homologous recombination; *Ac*, activator; *Ds*, dissociation

A closely-linked pair of *Ac/Ds* elements induces chromosomal rearrangements in Arabidopsis and maize. This report summarizes the *Ac/Ds* systems that generate an exceptionally high frequency of chromosomal rearrangements in rice genomes. From a line containing a single *Ds* element inserted at the *OsRLG5* locus, plants containing a closely-linked pair of inversely-oriented *Ds* elements were obtained at 1% frequency among the population regenerated from tissue culture. Subsequent regeneration of the lines containing *cis*-paired *Ds* elements via tissue culture led to a high frequency (35.6%) of plants containing chromosomal rearrangements at the *OsRLG5* locus. Thirty-four rearrangement events were characterized, revealing diverse chromosomal aberrations including deletions, inversions and duplications. Many rearrangements could be explained by sister chromatid transposition (SCT) and homologous recombination (HR), events previously demonstrated in Arabidopsis and maize. In addition, novel events were detected and presumably generated via a new alternative transposition mechanism. This mechanism, termed single chromatid transposition (SLCT), resulted in juxtaposed inversions and deletions on the same chromosome. This study demonstrated that the *Ac/Ds* system coupled with tissue culture-mediated plant regeneration could induce higher frequencies and a greater diversity of chromosomal rearrangements than previously reported.

Understanding transposon-induced chromosomal rearrangements can provide new insights into the relationship between transposable elements and genome evolution, as well as a means to perform chromosomal engineering for crop improvement. Rice is a staple cereal crop worldwide. Complete genome sequencing and rich genetic resources are great advantages for the study of the genomic complexity induced by transposable elements.[1–2] The combination of tissue culture with genetic lines carrying a pair of closely located *Ac/Ds* elements greatly increases the frequency and diversity of rearrangements in rice genomes. The methodology and its efficiency and significance are briefly summarized.

Regenerated Plants Exhibit a High Frequency of Transposon-Induced Chromosomal Rearrangements

Reports have described the effects of tissue culture regeneration systems on the activation of transposable elements in maize[1] and rice.[2-4] In maize, transposition frequencies were at least three times higher in regenerated populations compared with natural populations. In a regenerated rice population harboring an *Ac/Ds* system, approximately 70% of the population carried independent *Ds* insertions. This represents a more than 7-fold increase, as compared with the transposition frequency of a population propagated by crossing or selfing (**Fig. 1**).[5] Epigenetic aspects of the *Ac* and *Ds* elements were characterized in this system. During plant regeneration from rice calli, the reactivation of *Ac/Ds* elements was attributed to both alterations in the steady-state *Ac* mRNA levels as well as changes in the *Ds* end-specific methylation patterns.[4] These observations expand previous reports of *Ac* reactivation during tissue culture of maize. Among 270 plants that were regenerated from *OsRLG5:Ds* seeds, two lines contained a pair of *Ds* elements in *cis*-configuration at the *OsRLG5* locus. In a recently published study, the regeneration of lines carrying a pair of closely linked *Ds* elements resulted in high frequencies of both standard (approximately 70%) and alternative (36%) transposition events involving the 5' and 3' termini of different transposable elements.[6] Some lines contained both transposition types. These high activities may result from alleviation of the epigenetic silencing of *Ac/Ds* elements that are typically observed during tissue culture. In addition to transposition events, 11% of the rearrangements were derived from homologous recombination (HR) events that resulted in inter-transposon segment (ITS) inversions. The frequency of HR between two direct repeats was previously

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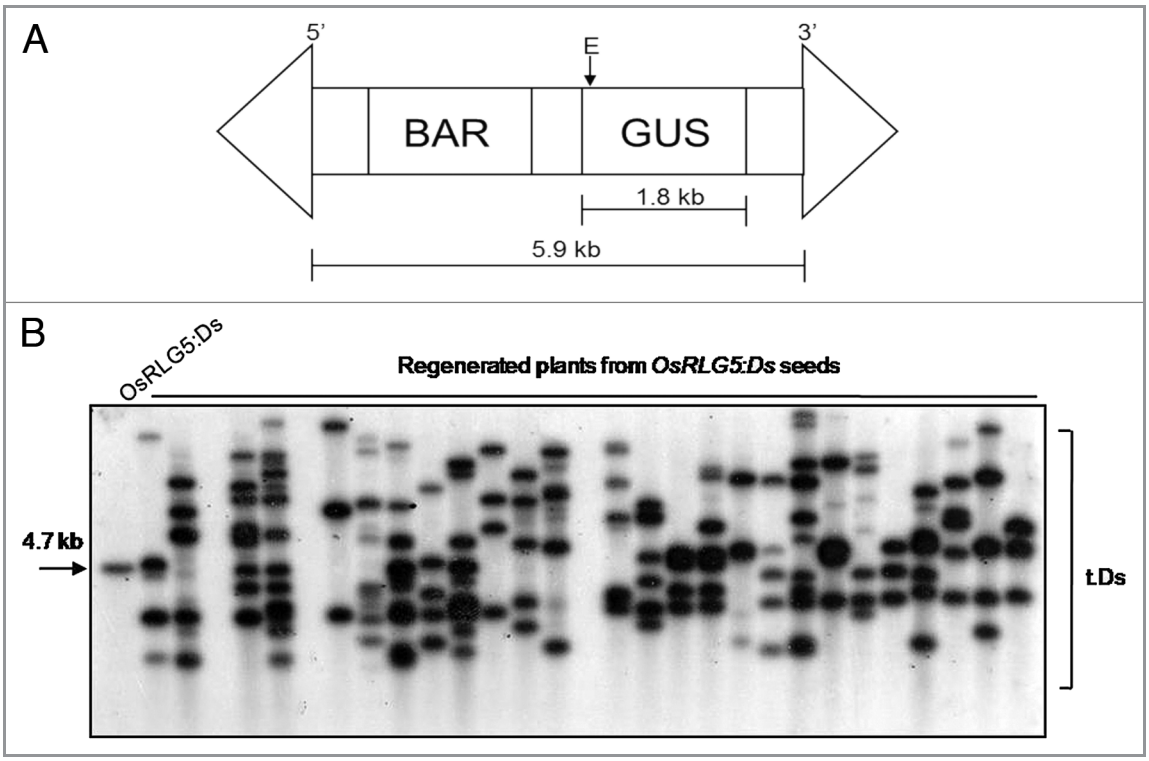

Figure 1. Structure of the *Ds* T-DNA vector and the polymorphic display of *Ds* elements in regenerated plants. (A) A BAR selection marker and GUS reporter gene were contained within the *Ds* T-DNA vector. The short vertical arrow indicated by 'E' is an *EcoRI* restriction site inside the GUS coding region. The horizontal lines below the map indicate the sizes of the *Ds* vector (5.9 kb) and GUS coding region (1.8 kb). The numbers "5" and "3" above the *Ds* T-DNA vector indicate the 5′ and 3′ *Ds* termini, respectively. (B) Southern blot hybridization was performed to identify *Ds* transpositions in plants regenerated from *OsRLG5::Ds* seeds. *EcoRI*-digested genomic DNA was hybridized with a 1.8 kb DNA fragment from the GUS coding region. The 4.7 kb arrow indicates the location of the original *Ds* element. Transposed *Ds* elements of regenerated plants were collectively named t.Ds.

reported to be enhanced by *Ac/Ds* transposition in maize and Arabidopsis.[7,8] Because HR between the two linked *Ds* elements was not detected in the population propagated by crossing and selfing, the exceptionally high *Ds* element activity induced by the tissue culture regeneration system may be responsible for the high HR rates observed in the regenerated plants.

Identification of a Novel Transposition Mechanism: Single Chromatid Transposition (SLCT)

Like standard transpositions, alternative transpositions that involve the 5' and 3' termini of different transposable elements often take place during or shortly after DNA replication. Only one of the two daughter elements is known to be competent for transposition following DNA replication.[9-12] Transposition competence depends on strand-specific methylation patterns, with hemi-methylated strands being preferred substrates for *Ac* transposase compared with fully-methylated strands.[13,14] However, *Ac* transposase also binds to unmethylated strands even though its binding affinity for unmethylated strands is less than that of hemi-methylated strands.[15] Accordingly, when two *Ac/Ds* elements are situated in the same orientation, the transposition-competent 5' and 3' *Ac/Ds* termini are all located within the same chromatid. The possible resulting transpositional modes include standard transpositions of the two individual elements, transposition of a macrotransposon formed by the outermost termini of the two paired elements, and reversed-ends transposition involving the inward-facing termini of the two elements.[16-18] In contrast, when two *Ac/Ds* elements are inversely-oriented, SCT events involving transposition-competent termini on different sister chromatids can occur.[19,20] In this report, a new class of rearrangements was detected. This new class includes inversions and deletions juxtaposed on the same chromosome. This configuration was designated single chromatid transposition (SLCT). In SLCT, alternative transposition reactions involve the 5' and 3' termini of two inversely-oriented *Ds* elements located on the same chromatid (**Fig. 2**). This generates novel genomic rearrangements that have not previously been demonstrated in any other system. Such novel events might be explained by the previous finding that the regeneration process can modify the methylation of *Ac/Ds* termini,[3] which may lead to unusual alternative transpositions. Frequent SLCT events in the regenerated population imply the possibility that SLCT might contribute to the genomic complexity of higher organisms.

A Novel Genetic Approach to Induce Chromosomal Rearrangements

The heritability of various genomic rearrangements in and around the *OsRLG5* locus was examined. Among the

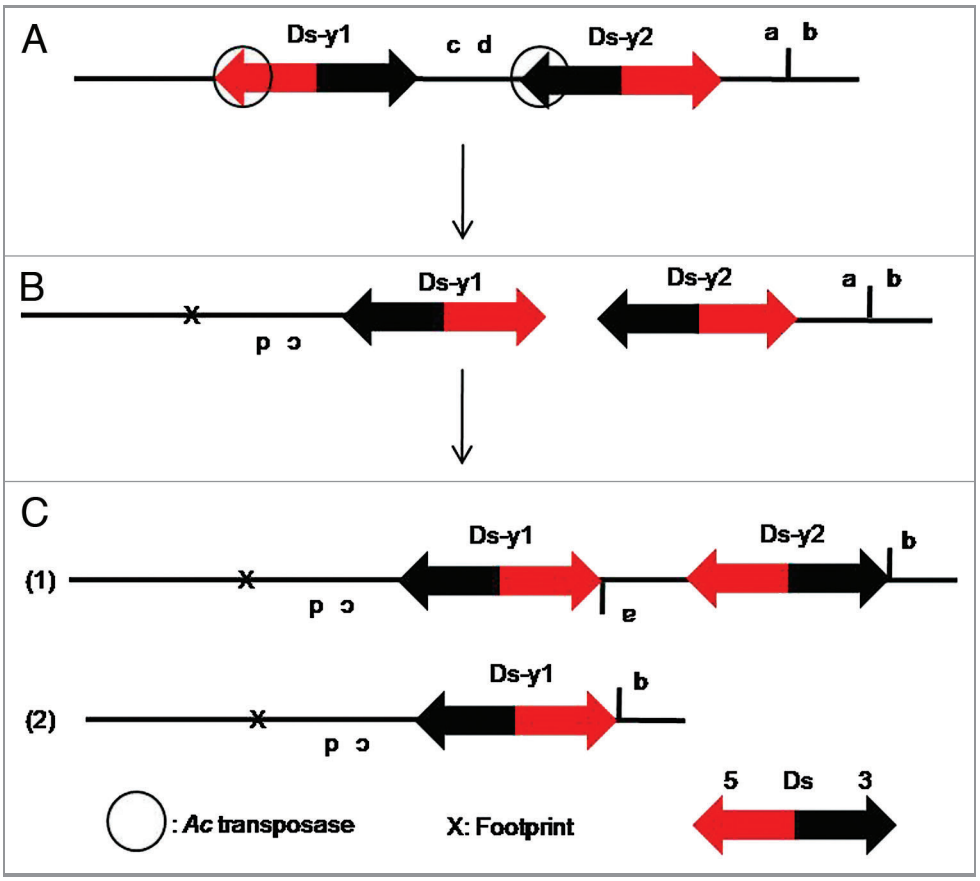

Figure 2. Models for single chromatid transposition (SLCT) with a distal target site. The inversion/deletion process derived from single chromatid transposition is depicted in three steps (A–C). (A) *Ac* transposase (circles) cuts at the 5′ end of *Ds-y1* and the 3′ end of *Ds-y2*. (B) Ligation of the host sequences flanking the excised *Ds* termini leads to generation of a footprint ('x') and inversion of the inter-transposon segment *c–d*. (C) Reinsertion of the 5′ and 3′ termini of *Ds-y1* and *Ds-y2* into distal target site *a–b* (black arrow). Reinsertion in either of two possible orientations results in products shown in parts (C1) and (C2). Part (C1) shows the inversion of fragment *a*, and inversion of both *Ds-y1* and *Ds-y2*. Part (C2) shows the deletion of fragment *a*, deletion of *Ds-y2*, and inversion of *Ds-y1*.

34 rearrangement events characterized, 22 events were heritable, while 12 events were restricted to somatic tissues. Among the 22 heritable events, 15 contained deletions. Therefore, deletion was the most common heritable genomic modification induced by the transpositional activities of the two inversely-oriented *Ds* elements at the *OsRLG5* locus. The deletions ranged from 184 bp to 520 kb in size. The endpoints of nine deletions were identified; collectively these deletions encompassed approximately 133.3 kb of genomic DNA around the *OsRLG5* locus (**Fig. 3A**). Among 15 deletions, 10 could be maintained as homozygotes. Two lines homozygous for deletions of approximately 85 kb (line E106) and 124 kb (line 25) proximal to the *OsRLG5* locus showed necrotic symptoms on their leaves (**Fig. 3B**). *OsRLG5* belongs to a cluster of 36 *RLK* genes localized on rice chromosome 1. These *RLK* genes were also predicted as putative rust resistance kinase *Lr10 (Leaf rust resistance 10)* (NCBI).[21] Induction of lesion-mimic necrosis by deletions spanning multiple *RLK* genes suggests the possibility that the *RLK* cluster might perform the similar pathogen-related function as the wheat homolog *Lr10*. This observation demonstrates how a series of overlapping transposon-induced deletions may be effective for analysis of clusters of functionally similar genes. In the rice genome, 29% of the predicted genes are reported to be organized in clustered gene families (International Rice Genome Sequencing Project, 2005). Technical limitations are well recognized in exploring the biological meaning of these gene clusters due to their functional redundancy. Multiple point mutations, insertions and/or deletions need to be generated and analyzed in order to determine the biological functions of clustered genes. Given the high frequency of transposition events and the resulting deletions that arise during plant regeneration, the activation of *Ac/Ds* systems by calli-mediated tissue culture provides a powerful genetic tool for functional genomics. In addition, these results suggest a possible mechanism for the changes in karyotype that are frequently associated with speciation. At this time it is difficult to estimate the contribution of transposon-induced rearrangements to chromosome evolution based on the dramatic changes found in tissue-culture regenerated plants. However, elucidation of the underlying mechanisms will likely facilitate the development of testable hypotheses and thereby further our understanding of transposon-mediated chromosomal evolution.

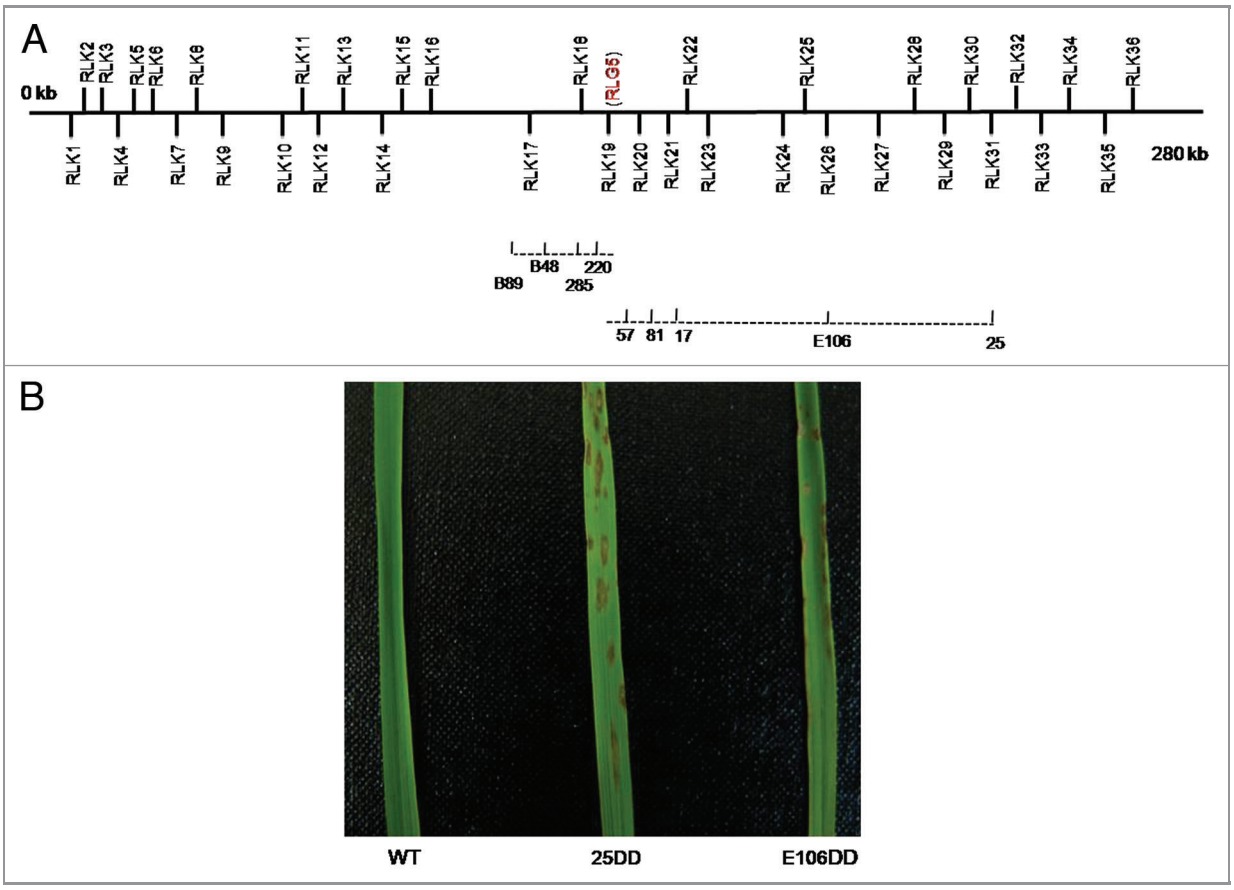

Figure 3. Phenotypic expression of plants homozygous for deletions. (A) The 280 kb genomic block of rice chromosome 1 contains 36 *receptor-like kinase* genes (*RLKs*), as shown in the upper diagram. *RLK 19* and *RLG 5* represent the same gene. Below the map of the RLK cluster, deleted regions and the names of deletion lines are shown as dotted lines and numbers, respectively. Vertical lines indicate the deletion endpoints in each deletion lines. All deletions begin at *RLG5* and extend to either the distal or proximal region. (B) Plants homozygous for deletions 25 and E106 (25DD and E106DD) show a necrotic phenotype. Leaves of 2-week-old plants are shown.

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