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Ac/Ds Mediated Gene Trapping System in Rice

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To establish gene tagging systems suitable for rice, we constructed Ac and gene trap Ds vectors and introduced them into the rice genome by <Agrobacterium/i>-mediated transformation. Rice plants that contained single and simple insertions of T-DNA were analyzed in order to evaluate the gene-tagging efficiency. Nearly 80% of Ds elements were excised from the original T-DNA sites, when Ac cDNA was expressed under a CaMV 35S promoter. 8% of transposed Ds elements expressed GUS in various tissues of rice panicles. Half of the Ds insertion sites showed simple hybridization patterns which could be easily utilized to locate the Ds.

Based on these data, we have established a genetic strategy that could be employed in a large scale mutagenesis using a heterologous Ac/Ds family in rice. Rice lines carrying either a single copy of Ac or Ds have been established. Both lines contained cytochrome P450 as a negative selection marker. A large number of transposant lines are being developed by massive pollinations between Ds and Ac separate lines to generate F1 seeds. In the F2 generation, the cytochrome P450 gene can be used to select unlinked transpositions of Ds and to eliminate Ac and Ds at original T-DNA loci. Successful application of a negative selection marker has been demonstrated in <Arabidopsis/i>. Overall, our preliminary data has provided molecular and genetic information that should be very informative for the establishment of effective gene tagging systems in rice.