2007

Molecular breeding of novel herbicide-tolerant rice by gene targeting

Masaki ENDO, Keishi OSAKABE, Kazuko ONO and Seiichi TOKI
Plant Genetic Engineering Research Unit

Exact modification of target genes is a prime goal in the precision engineering of crops. In this study, we created a novel herbicide-tolerant rice by gene targeting (GT)-mediated introduction of point mutations in the locus encoding the catalytic subunit of the enzyme acetolactate synthase (ALS). ALS catalyzes the initial step common to the biosynthesis of the branched-chain amino acids; leucine, isoleucine and valine. ALS is the primary target site of action for at least four structurally distinct classes of herbicides. In the previous study, Shimizu et al. (2005) reported the production of a rice cell line, which showed hyper tolerance to the ALS-inhibiting herbicide bispyribac (BS), and demonstrated that the BS-tolerant phenotype was due to a double mutation (W548L and S627I) in the rice *ALS* gene. Since the BS-tolerant cell line had lost the ability to regenerate during 2 years of tissue culture, we attempted to introduce these two point mutations into rice *ALS* gene via GT.

GT vector contained a partial *ALS* gene with W548L and S627I mutations so that random integrations of this GT vector did not confer BS tolerance. Using our highly efficient *Agrobacterium*-mediated transformation system, we obtained 66 independent GT rice plants from 1500 calli. Furthermore, 2/3 of these plants harbored the two point mutations exclusively, without any insertion of foreign DNA such as border sequences of T-DNA.

GT rice homozygous for the modified *ALS* locus showed hyper-tolerance to BS as compared to BS-tolerant plants produced by a conventional transgenic system. It was known that functional ALS complex consists of tetramers of ALS subunits. Exclusion of the BS-sensitive ALS allele is important to produce BS-hyper tolerant rice plants. Our results indicate that our GT method has successfully created novel herbicide-tolerant rice plants which are superior to those produced by conventional mutation breeding protocols or transgenic technology.

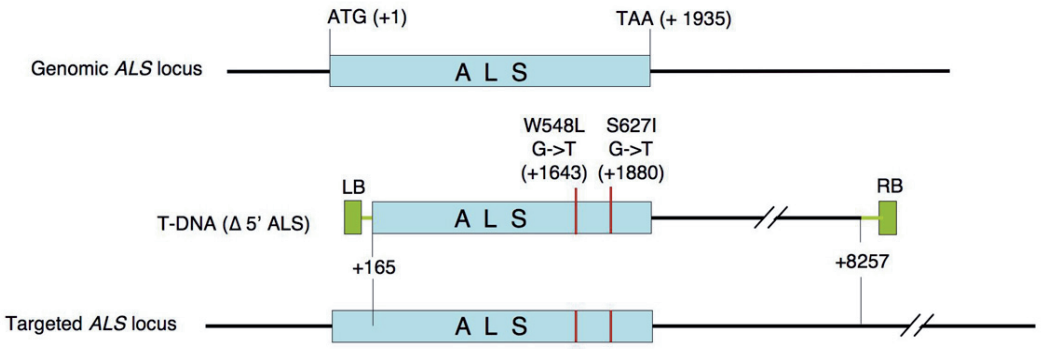

Fig. 1 Schematic representation of GT at ALS locus
A sequence encoding 55 amino acids, including the chloroplast-targeting signal, is deleted in the GT construct (△ 5' ALS), rendering the gene non-functional. Homologous recombination between genomic ALS locus and T-DNA confers BS-tolerant ALS.

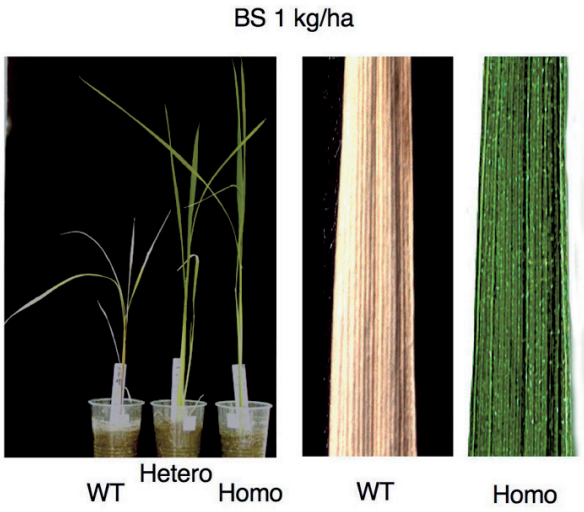

Fig. 2 BS sensitivity test
Heterozygote (Hetero) and homozygote (Homo) plant for the modified ALS exhibited tolerance to BS.

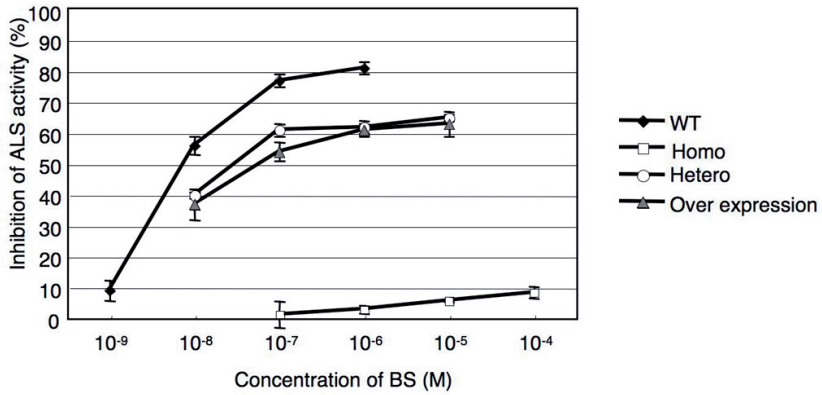

Fig. 3 Enzymatic activity of ALS
ALS proteins from wild-type (WT), homozygote (Homo) or heterozygote (Hetero) for GT plants and over-expressing plants (OE) of the *ALS* gene containing the same two mutations (W548L and S627I) were extracted and examined for their sensitivities to BS. In OE plant, transcription levels of ALS were extremely high (approximately 20-fold higher than wild-type). GT homozygote plants showed extreme tolerance to BS.