Establishment of rice transformation systems to study gene functions

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We have screened a large number of rice mutants induced by heavy-ion beam. To identify the causal gene for each mutant, a complementation test is needed. The complementation test is the experiment used to determine whether a gene is a causal gene for a mutant. The Recovery of a specific phenotype of a mutant is observed when an intact causal gene is introduced to a mutant (Fig. 1).[1)] To clarify the causal gene for the rice mutant of interest, we used a reliable Agrobacterium-mediated transformation system.[2, 3)] In the Agrobacterium-mediated method, a DNA region termed "T-DNA" transfers from a circular DNA in Agrobacterium into plant chromosomes (Fig. 2). We introduce both the gene of interest and the marker gene for a selection reagent, such as antibiotics or herbicides, into the T-DNA region. Plant cells that have accepted T-DNA will only survive on a medium containing a selection reagent. The Agrobacterium-mediated method is a good means to obtain a transgenic plant with high efficiency and a low copy number.

For a pilot experiment, we introduced the hygromycin phosphotransferase (HPT) gene in rice. The HPT gene confers hygromycin-resistance to rice cells and is one of the most frequently used marker gene in transformation experiments of rice. We obtained more than 20 plantlets from the calluses induced from 100 rice seeds (Fig. 3).

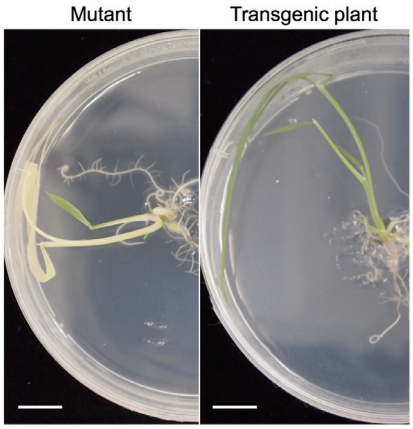

Fig. 1. Complementation test performed in the study of a rice virescent mutant. The virescent mutant generates white leaves when they are grown at 20 °C (left). Mutant plants that contain T-DNA including the intact causal gene show green leaves under the same condition (right). Rice plants are grown in the Murashige and Skoog medium. Bar = 1 cm.

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The transformation efficiency of our experiment (over 20%) was equivalent to the efficiency reported by Toki et al. (2006). Using this technique, we will determine the causal gene for a rice mutant exhibiting an interesting phenotype.

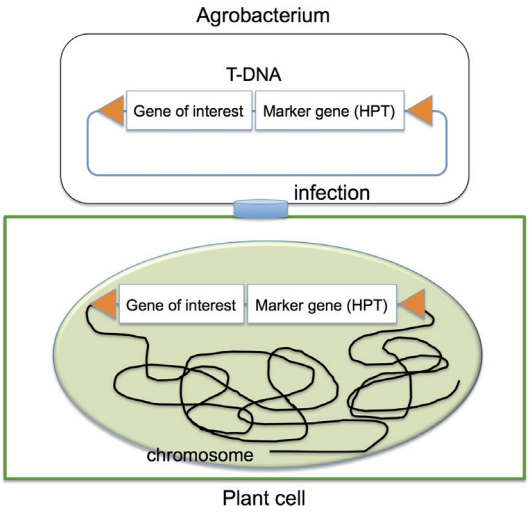

Fig. 2. Schematic representation of the Agrobacterium-mediated plant transformation system.

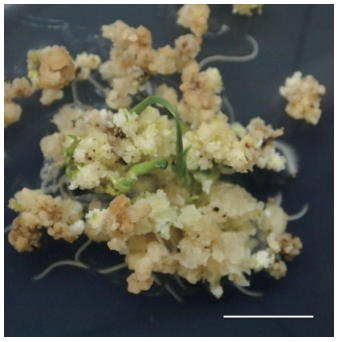

Fig. 3. Regenerated plant from the hygromycin-resistant callus. Bar = 1 cm.

References

1) R. Morita et al.: RIKEN Accel. Prog. Rep. **46**, 259 (2013)
2) S. Toki et al.: Plant J. **47**, 969 (2006)
3) H. Saika et al.: Transgenic Plants: Methods and Protocols **3**, 67 (2012)