Development of stress-tolerant rice plants without growth defect using Oshox24 promoter

Rice production is largely inhibited by environmental stresses such as drought and high salinity. Developing transgenic rice plants with enhanced stress tolerance is therefore necessary. Stress-responsive promoters with low expression under normal growth conditions are needed to minimize the adverse effects of stress-tolerance genes on rice growth. We aim to find stress-inducible promoters with low expression levels under normal growth conditions, and develop the technology to produce rice plants showing enhanced stress tolerance without growth inhibition.

We conducted expression analyses of drought-responsive genes in rice plants using a microarray, and selected *Oshox24* for promoter analysis. Transient assays using the rice promoters indicated that AREB/ABF (abscisic acid (ABA)-responsive element-binding protein/ABA-binding factor) transcription factors enhanced expressions of the gene. We generated transgenic rice plants containing the *Oshox24* promoter and the β-glucuronidase (GUS) reporter gene. GUS assays revealed that the *LIP9* and *OsNAC6* promoters that have been used were induced by drought, high salinity, and ABA treatment, and both promoters showed strong activity under normal growth conditions in the root (Fig. 1A). The *Oshox24* promoter was strongly induced by stresses and ABA, but showed low activity under normal growth conditions (Fig. 1A). In seeds, GUS staining showed that *Oshox24* expression was low and expressions of the other genes were high (Fig. 1B). Transgenic rice plants overexpressing a stress-tolerant gene under the control of the *Oshox24* promoter showed increased tolerance to drought and high salinity, and no growth defects (Fig. 2).

These data suggest that the *Oshox24* promoter is useful for overexpressing stress-tolerance genes without adversely affecting growth. Verification of the transgenic plants expressing stress-tolerant genes and the *Oshox24* promoter in fields is required.

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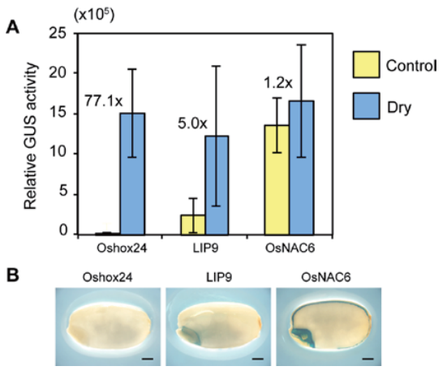

Fig. 1. Expression analysis of the newly isolated rice *Oshox24* promoter and rice *LIP9* and *OsNAC6* promoters that have been used.
We generated transgenic rice plants containing each promoter and the β-glucuronidase (GUS) reporter gene.
(A) GUS activity at 0h (Control) and 5h drought (dry) condition in the shoot. Error bars: SD.
(B) GUS staining in seeds of transgenic plants. Bars: 1mm.
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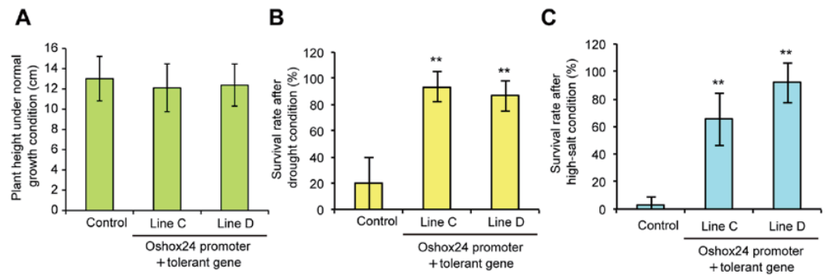

Fig. 2. Phenotype of transgenic plants expressing a stress-tolerant gene using the *Oshox24* promoter.

(A) Plant heights of 14-day-old plants under normal growth condition.
(B) Drought tolerance of 14-day-old plants.
(C) High-salinity tolerance of 14-day-old plants.
Error bars: SD. Asterisks indicate significant increase compared with the control (P<0.01).
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1) K. Nakashima, A. Jan, D, Todaka, K. Maruyama, S. Goto, K. Shinozaki, K. Yamaguchi-Shinozaki. (2014) Comparative functional analysis of six drought-responsive promoters in transgenic rice. Planta. 239:47-60.