2. Identification of mutants with impaired regulation of *KNOX* genes expression

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Knotted1-like homeobox (*KNOX*) genes are expressed in the shoot apical meristem (SAM) to maintain cells in a meristematic state, and are repressed in leaf founder cells on the flanks of the SAM (Jackson et al. 1994). Previous studies revealed that *KNOX* gene repression in leaf primordia is required to control hormonal levels and cell differentiation in leaf development (Sakamoto et al. 2001, Mele et al. 2003). Since expression patterns of *KNOX* genes in the shoot apex are correlated with phyllotaxy and plastochron, elucidation of a regulatory mechanism of *KNOX* gene expression would bring progress in understanding the mechanisms of early leaf development, phyllotaxy and plastochron. Although some genes have been reported to be necessary to repress *KNOX* genes in leaf primordia, a regulatory system of *KNOX* gene expression is still unclear.

In rice, it was reported that constitutive expression of *KNOX* genes caused typical defects on leaf morphogenesis like bladeless leaves and invasion of sheath region into blade region (Sentoku et al. 2000, Ito et al. 2001, Fig.1A, B). These characteristics could be useful criteria for screening of mutants misexpressing *KNOX* genes in leaves.

To understand a regulatory system of *KNOX* gene expression, we screened a rice *Tos17* mutant population for mutants that misexpress *KNOX* genes in leaves. In the primary screening, we selected 33 candidate recessive mutant lines that showed phenotypes similar to those of the constitutive expressor of *KNOX* genes (Fig. 1A, B). Secondly, we examined the expression of *OSH1*, one of *KNOX* genes, in leaves of candidate lines by RT-PCR. Finally, we identified 11 lines that ectopically expressed *OSH1* in leaves. All of these mutants were segregated as single recessive mutations in their M2 generation, and showed similar phenotype including bladeless leaves, severe dwarfism, dark green leaves, and seedling lethality (Fig. 1D-F). These mutants also showed ectopic expression of other members of rice *KNOX* genes, including *OSH6*, *OSH15* and *OSH71*, except *OSH43*, in leaves (Fig. 1F). Cloning of causal genes of these mutants is expected to reveal key factors in the repression machinery.

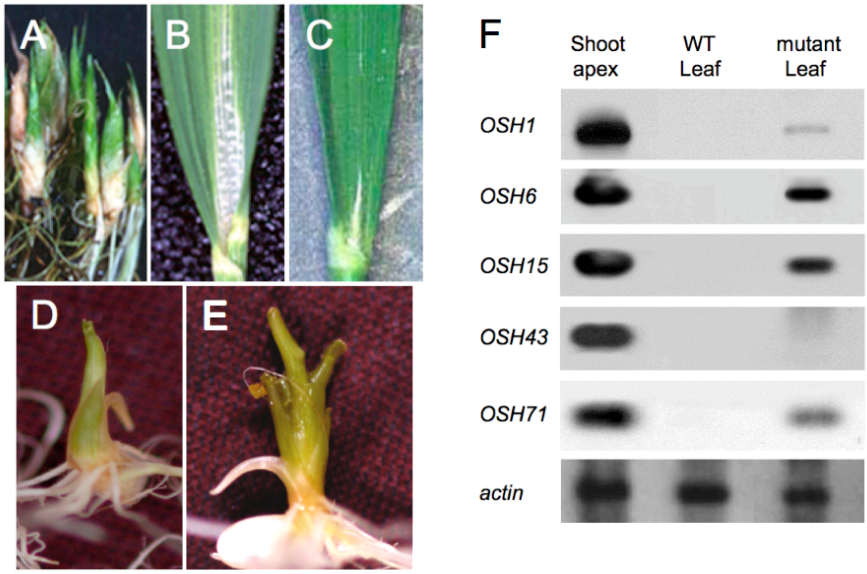

Fig. 1. Phenotypes of *KNOX* overexpressors, wild-type, and mutant seedlings. A: Severe phenotype of a *KNOX* overexpressor. B: Mild phenotype of a *KNOX* overexpressor. C: Wild-type leaf. D: A seedling of line #1, one of 11 mutant lines, showing severe phenotype. E: A seedling of line #17 showing mild phenotype. F: Expression analysis of *KNOX* genes in wild-type shoot apex, wild-type leaf and mutant leaf. The mutant line used here was #17. For clear detection, we conducted Southern hybridization after RT-PCR.

References

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