30. Identification of the conserved region in intron 1 of the *DROOPING LEAF* genes among the species in the grass family

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The *DROOPING LEAF* (*DL*) gene in *Oryza sativa* has two distinct roles in rice development; midrib formation in the leaf and carpel specification in the flower. (Nagasawa et al., 2003, Yamauchi et al., 2004). Loss-of-function mutant of *DL* fails to form midrib and shows a floral homeotic change of the carpel to the stamens.

One of the *dl* alleles, *dl-sup3*, which has the retrotransposon *Tos17* insertion in intron 1, shows the same morphological defects as the loss-of-function mutation allele *dl-sup1*, suggesting that intron 1 contains regulatory regions responsible for *DL* expression in both the leaf and the flower. We hypothesized that sequences in the putative regulatory regions should be conserved in species related to rice.

To identify the conserved regions in intron 1 of *DL*, we performed phylogenetic footprinting analysis using six species, which cover three subfamily in the family Poaceae: *O. sativa*, *O. punctata*, *O. officinalis*, and *O. brachyantha* from the *Oryza* genus in the Ehrhartoideae; *Sorghum bicolor* from the Panicoideae; and *Triticum aestivum* from the Pooideae. Genomic DNA were isolated from young leaves of six species and subjected to PCR amplification of the sequences in intron 1 and intron 5 of *DL* for each species. Nucleotide sequences of the PCR products were determined by direct sequencing. Identification and visualization of conserved regions was carried out using the program mVISTA (http://genome.lbl.gov/vista/index.shtml).

The results showed that sequences are conserved much higher in intron 1 than in intron 5 among species in the *Oryza* genus. No similarities, however, were detected in intron 5 among species in different subfamilies, suggesting that intron 5, like general introns, may have no function (Fig. 1B). By contrast, we detected several highly conserved regions in intron 1 among species in different subfamilies in Paceae (Fig. 1A). Alignment of the conserved regions in six species is shown in Fig. 1C. It is likely that these conserved sequences in intron 1 are associated with some function, probably with the regulation of *DL* expression. *Tos17* was inserted in the region between region a and region b in intron 1 of *dl-sup3* (Fig. 1A). This suggests that separation of these conserved regions by a long extra DNA segment also causes serious defects in the expression of *DL*.

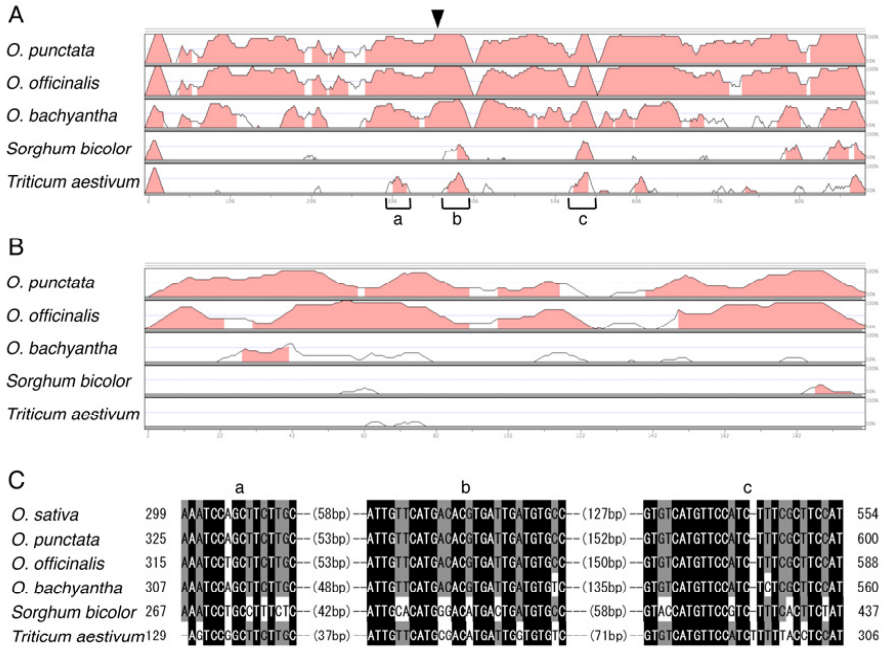

Fig. 1 Comparison of sequences of intron 1 and intron 5 of *DL* from six species. (A) and (B) VISTA analysis of intron 1 (A) and intron 5 (B) by the 25 base sliding window. Sequences *O. sativa* (Nipponbare) is used as a reference. Regions with >75% identity shared by *O. sativa* and the species indicated are shaded in pink. An arrowhead at the top indicates the position of *TOS17* insertion in *dl-sup3*. (C) Sequence alignment of three conserved region (indicated in panel A) from six species examined.

References

Nagasawa N., M. Miyoshi, Y. Sano, H. Satoh, H.-Y. Hirano, H. Sakai and Y. Nagato, 2003. *SUPERWOMAN 1* and *DROOPING LEAF* genes control floral organ identity in rice. Development 130: 705-718.

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