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SHORT COMMUNICATION

Isolation and functional analysis of *LiYAB1*, a *YABBY* family gene, from lily (*Lilium longiflorum*)

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Summary

YABBY family proteins are plant-specific transcriptional factors. *YABBY* genes can be divided into three subfamilies. Within the *CRC*/*DL* subfamily, the *Arabidopsis CRC* (*CRABS CLAW*) and the rice *DL* (*DROOPING LEAF*) have functionally diverged in the control of leaf development. *CRC* has no function in leaf development, while *DL* plays an important role in the formation of leaf midribs. In this study *LiYAB1*, an ortholog of *CRC*/*DL* genes from lily (*Lilium longiflorum*), was isolated by screening a cDNA library derived from young lily flower buds. Subcellular localization analysis indicated that LiYAB1 is nuclear localized. *LiYAB1* is expressed strongly in the carpels of the lily flower and weakly in the leaves, which is similar to *DL* in rice. Ectopic expression of *LiYAB1* in the rice *dl* mutant could rescue the drooping leaf phenotype of *dl* in some of the transgenic rice plants and cause abnormal leaves in the other transgenic plants. The overexpression of *LiYAB1* in the wild-type *Arabidopsis* caused leaf abnormality. The results suggest that *LiYAB1*, a member of the *CRC*/*DL* subfamily genes, might have an important function in regulating the leaf development in lilies, as *DL* does in rice.

Introduction

YABBY proteins are plant-specific transcriptional factors (Siegfried et al., 1999; Kumaran et al., 2002). There are six *YABBY* genes in *Arabidopsis* and eight in rice. According to the sequence similarity, *YABBY* genes can be divided into three subfamilies: the *CRC*/*DL* subfamily, the *FIL* subfamily, and the

Abbreviations: Bp, base pair; GFP, green fluorescent protein; *LiYAB1*, Lily YABBY gene 1; PCR, polymerase chain reaction; RT-PCR, reverse transcriptional polymerase chain reaction; SAM, shoot apical meristem; SEM, scanning gel electron microscope.

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INO subfamily (Bowman, 2000). In *Arabidopsis*, *CRC* regulates carpel and nectary development, the *FIL* subfamily genes regulate the dorsoventral (adaxial/abaxial) polarity of the leaves and floral organs, and *INO* regulates the adaxial/abaxial polarity of the floral ovules (Villanueva et al., 1999).

The three genes of the *CRC*/*DL* subfamily that have been studied are *AmbCRC* in *Amborella trichopoda*, *CRC* in *Arabidopsis thaliana*, and *DL* in *Oryza sativa*. *A. trichopoda* is commonly regarded as the most basal angiosperm plant. *AmbCRC* is expressed in the flower ovaries (Fourquin et al., 2005), while *CRC* is expressed specifically in the flower carpels and nectaries. Neither *Amb*RC nor *CRC* is expressed in the leaves. However, *DL* is expressed in both flower gynoecia and leaves in rice, playing an important role in the formation of leaf midribs in addition to the specification of carpels (Nagasawa et al., 2003; Yamaguchi et al., 2004). In all *crc* mutants, the mutations affect the development of flower gynoecia, but not the leaves (Bowman and Smyth, 1999). However, all *dl* alleles cause defects in the midrib formation in rice leaves, resulting in a drooping leaf phenotype. Moreover, the transgenic rice plants with over-expressed *DL* showed an aberrant leaf phenotype with midrib-like structure in the lateral regions as well as in the central region (Yamaguchi et al., 2004).

Functional comparisons of *CRC* and *DL* indicate an essential difference in their functions in leaf development, as described above, although they function similarly in flower gynoecia (Fourquin et al., 2007). *CRC* is not expressed in the leaves or involved in leaf development in *Arabidopsis*, whereas *DL* is expressed in leaf primordia and plays an important role in the leaf development, especially in the formation of the leaf midribs in rice. Considering the significant differences in the leaf structures between monocot and eudicot species, a question may be raised as to whether the difference between the *Arabidopsis CRC* and the rice *DL* in specifying leaf development reflects the functional divergence of the *CRC*/*DL* subfamily genes between the monocot and eudicot species. In order to address this question, more genes of the *CRC*/*DL* subfamily need to be functionally identified.

Lilies are monocot plants that belong to Liliaceae. The leaf morphology of lily is similar to that of rice in that they both have parallel venation instead of reticulate venation. The floral structure of lily is more similar to that of *Arabidopsis* in that they both have sepals and tepals rather than lemma, palea, and lodicules in their whorls 1 and 2. This combination makes the lily ideal for studying the functions of *CRC*/*DL* subfamily genes in regulating the development of leaves and flowers. In this study, we isolated a *CRC*/*DL* subfamily gene, *LiYAB1*, from a cDNA library of lily (*Lilium longiflorum* cv. Snow Queen) floral buds. Analyses indicated that *LiYAB1* was expressed in both carpels and leaves in lily, which is similar to the *DL* expression pattern in rice. Ectopic expression of *LiYAB1* in *Arabidopsis* caused abnormal leaves and arrested the shoot apical meristem (SAM), whereas overexpression of *LiYAB1* in a rice weak *dl* mutant could rescue the drooping leaf phenotype in some of the transgenic rice plants and cause abnormal leaves in the other transgenic plants, suggesting that *LiYAB1* may regulate the leaf development in lily as *DL* does in rice.

Materials and methods

Plant materials

The lily (*Lilium longiflorum* Thunb. cv. Snow Queen) plants used in this study were grown in the lily nursery greenhouse in Beijing. The *Arabidopsis thaliana* (ecotype: Columbia-3) plants were grown on solid MS medium for about 15 d before being transferred to soil. All plants were grown under 16 h of light. The transgenic *Arabidopsis* plants were generated by the infiltration method (Bechtold et al., 1993).

The rice *dl-1* mutant was kindly provided by Prof. Nagato. The naturally occurring *dl-le* mutant was identified as an allele of *dl-1* in our lab (data not shown). The mutant plants were grown in the field on the farm of the Institute of Genetics and Developmental Biology in Beijing. The procedures for rice tissue culture and transformation with *Agrobacterium tumefaciens* were as described previously (Hiei et al., 1994).

cDNA library constructing and screening

RNA was isolated with a Qiagen RNeasy Mini Kit (Qiagen). The cDNA library was constructed using the SMART^{TM} cDNA Library Construction Kit (CLONTECH, USA) with about 5 μg of RNA isolated from lily floral buds of approximately 10 mm in size. To screen the *YABBY* orthologous gene, a rice *DL* fragment of 550 bp was amplified from the rice young panicle cDNA by RT-PCR and used as probe, using the primer set DL-F and DL-R (Table 1). A total of 42,000 plaque forming units were screened with the *DL* fragment as the probe. The hybridization procedures were carried out according to the