

(05-1-22)

## Comparative Analysis of AN and a Member of the AN Subfamily, IAN

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### **Objectives**

Understanding the evolution of AN, plant-type CtBP, requires an analysis of AN homologues from different plants. As a first step in such comparative studies, we report the AN homolog from *Ipomoea nil* (L.) Roth., IAN.

### **Materials and Methods**

1. Material: *Ipomoea nil* (L.) Roth (Japanese morning glory), *Arabidopsis thaliana* (L.) Heynh.
2. Methods: Semi-quantitative RT-PCR was performed using a Superscript<sup>TM</sup> One-Step RT-PCR with Platinum Taq Kit. RNA was prepared by using RNeasy<sup>®</sup> Mini Kit. The GeneAmp<sup>TM</sup> PCR system (model9700) was used in each case.

### **Results and Discussion**

*ANGUSTIFOLIA* (*AN*) gene regulates the width of leaves of *Arabidopsis thaliana*, by controlling the polar elongation of leaf cells. We found that the abnormal arrangement of cortical microtubules (MTs) in *an* leaf cells appeared to account entirely for the abnormal shape of the cells. It suggested that the *AN* gene might regulate the polarity of cell growth by controlling the arrangement of cortical MTs. We characterized an ortholog of *AN* from *Ipomoea nil* (L.) Roth (Japanese morning glory) and designated it *Ipomoea nil's AN* (*IAN*). *IAN* is a single-copy gene in the genome and is expressed ubiquitously in various organs of *I. nil*. *IAN* contains not only a D2-HDH motif, which is highly conserved within the CtBP family, but also LXCXE, NLS and PEST motifs, which are specific to the AN subfamily. The expression of *IAN* cDNA driven by the cauliflower mosaic virus 35S promoter restored a defect in leaf expansion in the leaf width direction in the *angustifolia-1* (*an-1*) mutant of *Arabidopsis*, suggesting that *IAN* retains a common function with AN. In contrast, the complementation by *IAN* of a defect in the trichome branching pattern on the leaf surface of the *an-1* mutant was less effective than that observed for leaf shape. These results suggest that the mechanisms by which *AN* regulates leaf width and trichome branching are separable.