

Leafy head is a unique type of plant architecture found in some vegetable crops, with leaves bending inwards to form a compact head. Heading (development of a leafy head) in lettuce is a quantitative trait, controlled by multiple genes. We genetically dissect the heading trait in an F₂ segregating population derived from the cross between a crisphead (heading) lettuce and a romaine (nonheading) lettuce. Three QTLs for heading were identified in the segregating population, and two of them have been genetically cloned and verified. The major QTL (*LsKN1*) encodes a protein with homology to the KN1 gene in maize. The *LsKN1* gene in the heading parent has a CACTA-like transposon inserted into its first exon. The transposon sequences act as a promoter rather than an enhancer and drive high expression of *LsKN1*. The LsKN1 protein binds the promoter of *LsASI* and downregulates its expression to alter leaf dorsoventrality.

The second QTL (*LHL1*) had a 1-bp deletion in its first exon, knocking out its function. CHIP-seq showed that LHL1 binds to the promoters of several genes, including both adaxial and abaxial genes. The loss-of-function of *LHL1* upregulated adaxial genes but downregulated abaxial genes. Y1H, EMSA, and dual luciferase assays suggested that LHL1 binds the promoter and upregulates the expression of *LsASI*, antagonistic to LsKN1. Furthermore, LHL1 directly interacts with LsKN1, and the interaction compromised the suppressing effects of LsKN1 on *LsASI*. LHL1 controls heading through its collective effects on multiple genes in the adaxial and abaxial domains of leaves.

This study provides insight into the differentiation of adaxial-abaxial axis of plant leaves and shed lights on the molecular mechanism of heading. The results will facilitate the breeding of heading lettuce cultivars, and provide excellent reference for studies on other heading vegetables.