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BLADE-ON-PETIOLE1 and *2* regulate Arabidopsis inflorescence architecture in conjunction with homeobox genes *KNAT6* and *ATH1*

Madiha Khan, Paul Tabb and Shelley R. Hepworth*

Department of Biology; Carleton University; Ottawa, ON Canada

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Inflorescence architecture varies widely among flowering plants, serving to optimize the display of flowers for reproductive success. In *Arabidopsis thaliana,* internode elongation begins at the floral transition, generating a regular spiral arrangement of upwardly-oriented flowers on the primary stem. Post-elongation, differentiation of lignified interfascicular fibers in the stem provides mechanical support. Correct inflorescence patterning requires two interacting homeodomain transcription factors: the KNOTTED1-like protein BREVIPEDICELLUS (BP) and its BEL1-like interaction partner PENNYWISE (PNY). Mutations in *BP* and *PNY* cause short internodes, irregular spacing and/or orientation of lateral organs, and altered lignin deposition in stems. Recently, we showed that these defects are caused by the misexpression of lateral organ boundary genes, *BLADE-ON-PETIOLE1* (*BOP1*) and *BOP2*, which function downstream of BP-PNY in an antagonistic fashion. BOP1/2 gain-of-function in stems promotes expression of the boundary gene *KNOTTED1-LIKE FROM ARABIDOPSIS THALIANA6* (*KNAT6*) and shown here, *ARABIDOPSIS THALIANA HOMEOBOX GENE1* (*ATH1*), providing KNAT6 with a BEL1-like co-factor. Our further analyses show that defects caused by BOP1/2 gain-of-function require both KNAT6 and ATH1. These data reveal how BOP1/2-dependent activation of a boundary module in stems exerts changes in inflorescence architecture.

Inflorescence architecture is remarkably diverse between plant species, serving to optimize the arrangement of flowers for successful pollination and seed set.[1] Timing, length, and pattern of internode elongation together with pedicel angle are key parameters in the organization of lateral branches and flowers on the primary stem. In the model plant, *Arabidopsis thaliana*, elongation of internodes begins at the transition to flowering and is associated with proliferation of cells in the rib meristem.[2,3] Post-elongation, internodes are fortified through the differentiation of interfascicular fibers with secondary cell walls.[4,5]

Two three-amino-acid loop-extension (TALE) homeodomain transcription factors: the class I KNOTTED1-like homeobox (KNOX) protein BREVIPEDICELLUS (BP) and its interaction partner, the BELL1-like (BELL) protein PENNYWISE (PNY) play significant roles in meristem maintenance and internode patterning.[6-11] Mutations in *BP* cause short internodes, downward-pointing pedicels, and reduced apical dominance whereas mutations in *PNY* cause altered phyllotaxy and irregular internode elongation leading to clusters of flowers on the primary stem, and reduced apical dominance.[6-9] Both mutants exhibit changes in vascular patterning, indicated by altered lignin deposition in stems.[6,7,12] Stem patterning defects in *bp pny* double mutants are enhanced, indicating that BP and PNY play related but distinct roles in internode development.[8] For example, whereas BP is a negative regulator of lignin biosynthetic genes required for differentiation of interfascicular fibers, PNY promotes internode elongation and lateral organ initiation by spatially regulating the expression of *PECTIN METHYLESTERASE5*, which is associated with loosening of the plant cell wall.[12-14]

In a recent paper, we showed that *bp* and *pny* inflorescence defects are caused by stem and pedicel misexpression of the lateral organ boundary genes *BLADE-ON-PETIOLE1* (*BOP1*) and *BOP2*.[13] *BOP1/2* encode BTB/POZ and ankyrin domain-containing transcriptional co-activators with redundant functions.[15,16] Loss-of-function *bop1 bop2* rescues *bp* and *pny* inflorescence defects and BOP1/2 gain-of-function mimics *bp* and *pny* inflorescence defects. We showed that BOP1/2 function downstream of BP-PNY in an antagonistic manner, acting as positive regulators of the KNOX boundary gene *KNAT6*, whose ectopic activity is required, but not sufficient, to induce changes in inflorescence architecture. We speculated that KNAT6 requires a co-factor, provided directly or indirectly by BOP1/2, to exert its activity.[13]

We show here that BOP1/2 activity induces expression of the *BELL* homeobox gene *ARABIDOPSIS THALIANA HOMEOBOX GENE1* (*ATH1*), whose transcripts are likewise upregulated in *bp* and *pny* internodes contributing to defects in pedicel orientation and internode patterning. KNAT6 forms a

*Correspondence to: Shelley R. Hepworth; Email: shelley_hepworth@carleton.ca
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heterodimer with ATH1.[11,17] Collectively, our work shows that both products are required by BOP1/2 to exert changes in inflorescence architecture. Thus, BOP1/2 gain-of-function in stems may promote the formation of a KNAT6-ATH1 complex that antagonizes BP-PNY activity. Our findings shed light on how interplay between KNOX-BELL complexes associated with meristem and boundary compartments governs inflorescence architecture in a model plant species.

BOP1/2 Promote *ATH1* Expression

The *BELL* homeobox gene *ATH1* is strongly expressed in vegetative apices prior to the floral transition where its product heterodimerizes with class I KNOX proteins (SHOOTMERISTEMLESS and BP) to promote maintenance of the vegetative meristem.[11,17,18] *ATH1* is also expressed in basal and lateral organ boundaries where its activity inhibits growth and controls patterning.[11,18] *35S:ATH1* plants have short internodes similar to *35S:BOP2* plants,[11,18] prompting us to test if BOP1/2 induce *ATH1* expression to exert changes in inflorescence architecture. Analysis of *ATH1* transcript using quantitative RT-PCR (qRT-PCR) in wild-type (WT) and mutant internodes showed a significant increase in *bp-2* (10.6-fold) and *pny-40126* (89.7-fold) internodes relative to WT control plants suggesting that BP and PNY repress *ATH1* expression (**Fig. 1**). Transcripts were also elevated in BOP1/2 gain-of-function lines, *35S:BOP2* and *bop1-6D* (20.1-\ and 24.3-fold, respectively), suggesting that BOP1/2 induce *ATH1* expression. Thus, BP and PNY are transcriptional repressors of several boundary genes, including *BOP1/2*,[13] *KNAT6*[19] and *ATH1*, whose products define a potentially linear genetic pathway.

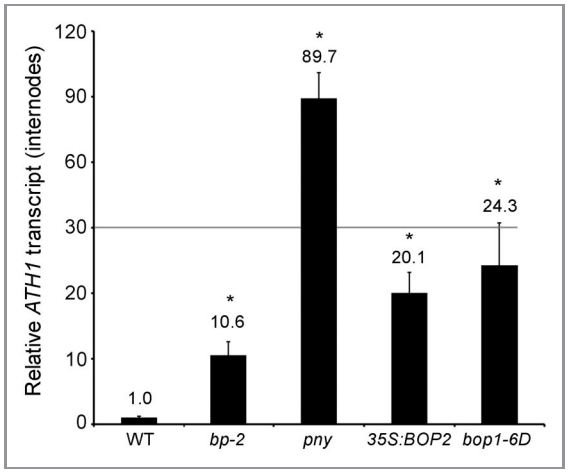

Figure 1. qRT-PCR analysis of relative *ATH1* transcript levels in internodes of WT, *bp-2*, *pny*, and *BOP1/2* gain-of-function lines: *35S:BOP2* and *bop1-6D*. Plant lines, growth conditions, and experimental conditions were as previously described.[13] WT was the Columbia-0 ecotype of Arabidopsis. Gene-specific primers for *ATH1* were: ATH1-qPCR-F1 (5′-ATACTCGCTCGA-TTATTCATCTCGA) and ATH-R1 (5′-ATCGATCATCCAACCATTTGAAGAAG). Asterisks, significantly different from WT (Student's t-tests, $p < 0.0001$ for all). Error bars, s.e.m.

Inactivation of *ATH1* Rescues *pny* and Partially Rescues *bp* Inflorescence Defects

To determine if *ATH1* misexpression in *bp* and *pny* internodes exerts changes in inflorescence patterning along with *BOP1/2* and *KNAT6,* we tested if inactivation of *ATH1* suppresses *bp* and/or *pny* mutant phenotypes.[13,19] Double mutants *bp-2 ath1-3* and *pny-40126 ath1-3* were generated by crossing and the resulting inflorescence phenotypes were examined (**Fig. 2**). Inactivation of *ATH1* dramatically rescued *pny* inflorescence defects but rescue of *bp* defects was less obvious (**Fig. 2A–F**; see also ref. 17). To better assess *ath1-3* rescue of *pny* inflorescence defects, we performed quantitative phenotypic analyses on 20 plants per genotype. Average plant height, rosette paraclade number, and internode lengths in *ath1-3 pny* double mutants were similar to WT control plants (**Fig. 2G–I**) as seen for *bop1 bop2* and *knat2 knat6* rescue of *pny* defects.[13,19] The *ath1-3* mutation also suppressed *pny-57747* (see ref. 8) silique clustering defects (data not shown similar to ref. 11). Measurement of pedicel angles showed that inactivation of *ATH1* partially rescues *bp* pedicel orientation, but less efficiently than inactivation of *KNAT6* (**Fig. 2J**; see ref. 19). Thus, misexpression of *ATH1* contributes unequally to *bp* and *pny* defects. This complexity is unsurprising. First, *ATH1* transcripts accumulate to higher levels in *pny* vs. *bp* internodes (**Fig. 1**). Second, ATH1 has the potential to form functionally distinct complexes with several KNOX proteins, including BP, KNAT2 and KNAT6, whose transcripts are differentially expressed in *bp* and *pny* stems.[11,17,19] Overall, the data show that inactivation of *ATH1* suppresses *bp-2* and *pny* inflorescence defects, similar to inactivation of BOP1/2[13] and KNAT6.[19] These data support the model that BOP1/2 function in conjunction with KNAT6 and ATH1 to antagonize BP and PNY activities.

BOP1/2 Require ATH1 Activity to Exert Changes in Inflorescence Architecture

To test if misexpression of *ATH1* contributes to restricted internode elongation in *35S:BOP2* transgenic plants, we examined the effect of *ath1-3* loss-of-function on the phenotype of a strong *35S:BOP2* line with short compact internodes.[13] Plants homozygous for a *35S:BOP2* transgene (see ref. 20) were crossed to WT control plants or *ath1-3* homozygous mutants. The phenotypes of F1 progeny were examined, revealing that partial *ath1-3* loss-of-function (*ath1-3*/+) was sufficient to restore internode elongation in *35S:BOP2* plants (**Fig. 3**). The average height of *35S:BOP2*/+ Col/+ control plants was 3.33 ± 0.23 cm vs. 19.14 ± 0.72 cm for *35S:BOP2*/+ *ath1-3*/+ plants (n = 24). Thus, BOP1/2 require both KNAT6[13] and ATH1 to exert changes in inflorescence architecture.

Roles for KNAT6 and ATH1 in Secondary Stem Development

Post-elongation, differentiation of vessel and fiber cells with secondary thickened cell walls strengthens the stem.[4,5] In *bp-2* mutants, the vascular ring contains gaps where lignin is abnormally deposited in the epidermal and cortical layers, and

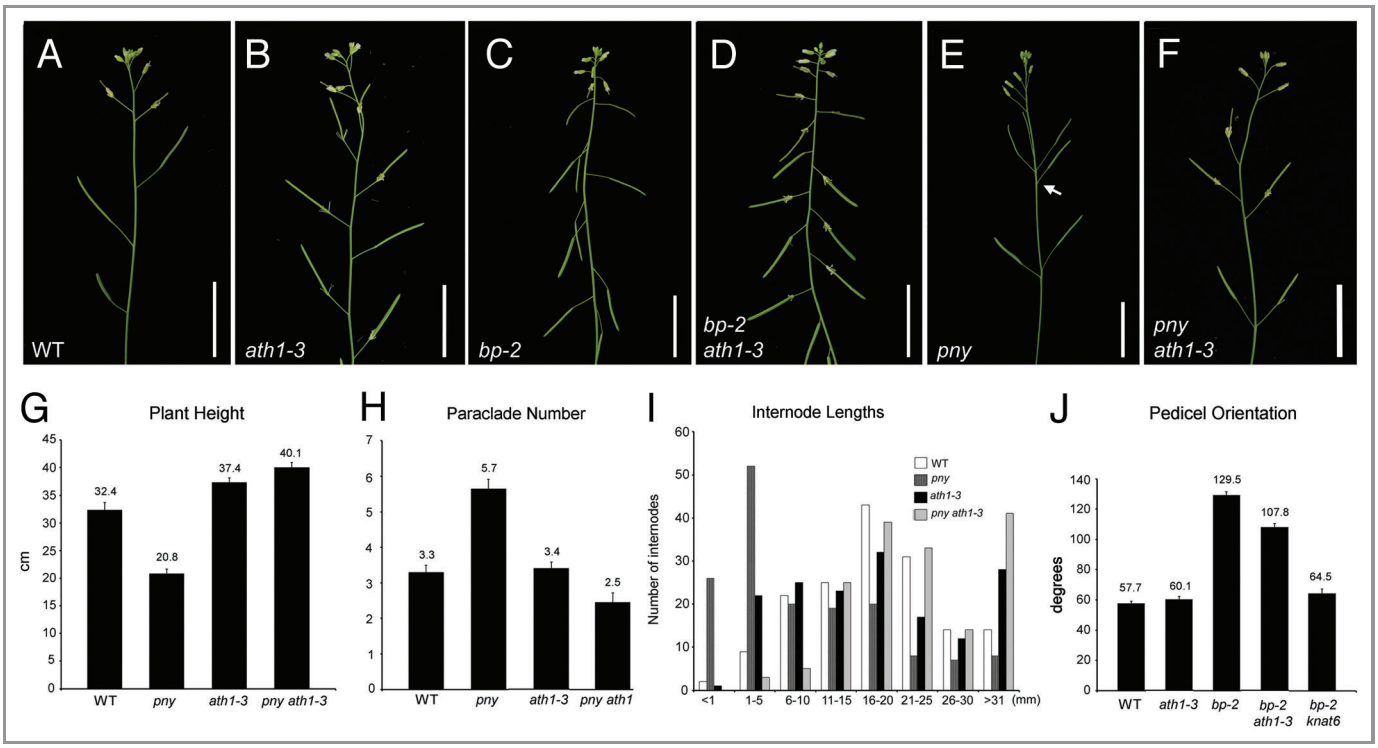

Figure 2. Phenotypic suppression of *pny-40126* and *bp-2* inflorescence defects by *ath1-3* loss-of-function. The *ath1-3* allele (see ref. 18) was obtained from the Arabidopsis Biological Resource Center. Mutant combinations were constructed by crossing and confirmed by PCR genotyping as described.[13,18] Inflorescences of five-week-old representative plants are shown for: (A) WT. (B) *ath1-3*. (C) *bp-2*. (D) *bp-2 ath1-3*. (E) *pny*; arrow denotes cluster of siliques. (F) *pny ath1-3*. (G–I) Quantitative analyses of *pny* phenotypic rescue by *ath1-3*. Seven-week-old plants were analyzed as previously described.[13] (G) Average plant height. (H) Average number of paraclades. (I) Distribution of internode lengths; internodes were measured between the first and 11th siliques on the primary stem (counting acropetally). (J) Pedicel orientation; angles were measured with a protractor (n = 55). Scale bars, 2 cm.

phloem fibers overlying the primary vascular bundles are prematurely lignified (**Fig. 4A and B**; see refs. 12 and 13). Loss-of-function *bop1 bop2* partially rescues *bp* lignin defects, resulting in a pattern more similar to WT.[13] To test if KNAT6 and ATH1 contribute to *bp-2* lignin defects caused by *BOP1/2* misexpression, cross-sections were cut from the base of fully elongated stems and stained with phloroglucinol-HCl (**Fig. 4**). While *knat2* mutation alone had no significant effect on *bp-2* stem patterning (**Fig. 4A–C**), *ath1-3* and *knat6* mutations partially rescued *bp-2* defects by closing the gaps in the vascular ring, but evidence of epidermal lignification remained (**Fig. 4A, B, D and E**). Loss-of-function *knat6* together with *knat2* significantly rescued *bp-2* stem patterning defects, similar to that seen in *bop1 bop2 bp-2* triple mutants (**Fig. 4A, B and F**; see ref. 13). These data confirm that both KNAT6 and ATH1 contribute to lignin defects in *bp-2* stems, which are caused by BOP1/2 gain-of-function.

Figure 3. Inactivation of *ATH1* rescues compact internodes in *BOP2* gain-of-function plants. Plants homozygous for a *35S:BOP2* transgene were crossed to WT control plants or *ath1-3* homozygous mutants. Representative F1 plants are shown. qRT-PCR analysis confirmed that BOP2 transcripts were expressed at similar levels in both genotypes. (A) *35S:BOP2*/+ Col/+. (B) *35S:BOP2*/+ *ath1-3*/+. Scale bars, 1 cm.

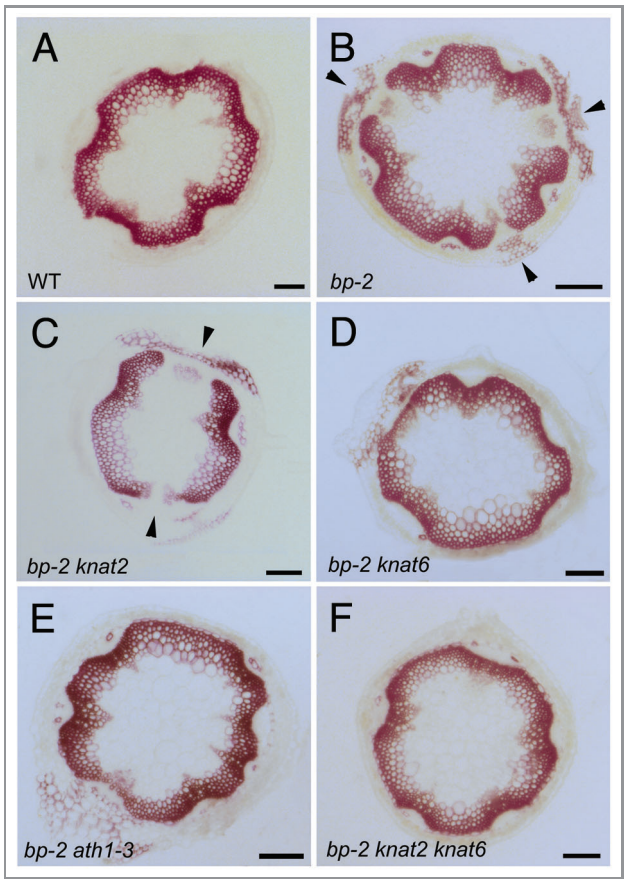

Figure 4. Effect of *KNAT6* and *ATH1* inactivation on vascular patterning in *bp-2* stems. Cross sections from the base of fully elongated primary stems were stained with phloroglucinol-HCl to detect lignin as described.[13] At least 50 sections from 4 or 5 plants per genotype[13] were examined. Representative sections are shown. (A) WT. (B) *bp-2*. (C) *bp-2 knat2*. (D) *bp-2 knat6*. (E) *bp-2 ath1-3*. (F) *bp-2 knat2 knat6*. Arrowheads denote gaps in the vascular ring. Scale bars, 100 μm.

BOP1/2 are Positive Regulators of a KNAT6-ATH1 Boundary Module

Internode elongation in many flowering plant species begins at the transition to flowering as a result of increased rib meristem activity in response to floral inductive signals.[2,3,21] Our data support the model that BP and PNY promote internode elongation by inhibiting expression of the lateral organ boundary genes, *BOP1/2*, *ATH1* and *KNAT6* in stems (see also ref. 19). BOP1/2 in stems induces the expression of *KNAT6*[13] and its potential co-factor *ATH1*, permitting the formation of a complex

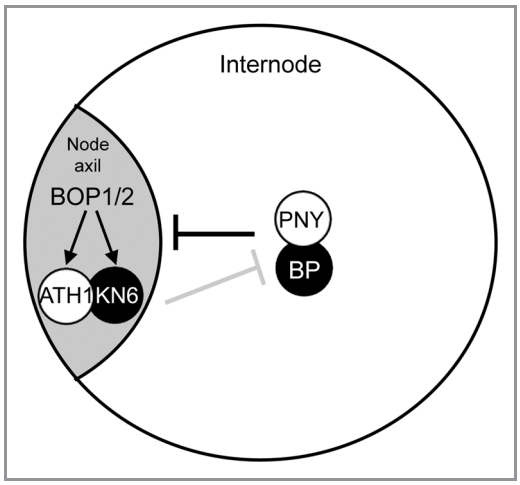

Figure 5. Summary of genetic interactions between BP-PNY, BOP1/2, and KNAT6-ATH1 in inflorescence patterning. BP and PNY restrict *BOP1/2* expression to the pedicel axil at nodes. BOP1/2 in stems induces *KNAT6* and *ATH1* expression, permitting the formation of a KNOX-BELL complex whose potential activity is antagonistic to BP and PNY. Arrows represent transcriptional activation. Black T-bar represents transcriptional repression. Grey T-bar represents an opposing activity.

that potentially antagonizes BP and PNY activities (**Fig. 5**; see also refs. 11 and 17). While it is yet unclear if BOP1/2 are direct transcriptional regulators of *KNAT6* or *ATH1*, these genes are likely to form a module whose functional interactions are conserved in development, at the meristem-leaf boundary during vegetative development, at floral abscission zones and at the valve margin-replum interface in developing fruits.[22-24] Future experiments will address these issues.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Supplementary Material

Supplementary materials may be found here:
www.landesbioscience.com/journals/psb/article/20599

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