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Arabidopsis BLADE-ON-PETIOLE1 and 2 promote floral meristem fate and determinacy in a previously undefined pathway targeting *APETALA1* and *AGAMOUS-LIKE24*

Mingli Xu[1], Tieqiang Hu[1], Sarah M. McKim[2], Jhadeswar Murmu[1], George W. Haughn[3] and Shelley R. Hepworth[1,*]

[1]*Department of Biology, Carleton University, Ottawa, Ontario, K1S 5B6, Canada,*

[2]*Department of Plant Sciences, University of Oxford, Oxford, OX1 3RB, UK, and*

[3]*Department of Botany, University of British Columbia, Vancouver, British Columbia, V6T 1Z4, Canada*

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*For correspondence (fax +613 520 3539; e-mail shelley_hepworth@carleton.ca).

SUMMARY

The transition to flowering is a tightly controlled developmental decision in plants. In Arabidopsis, *LEAFY (LFY)* and *APETALA1 (AP1)* are key regulators of this transition and expression of these genes in primordia produced by the inflorescence meristem confers floral fate. Here, we examine the role of architectural regulators *BLADE-ON-PETIOLE1 (BOP1)* and *BOP2* in promotion of floral meristem identity. Loss-of-function *bop1 bop2* mutants show subtle defects in inflorescence and floral architecture but in combination with *lfy* or *ap1*, synergistic defects in floral meristem fate and determinacy are revealed. The most dramatic changes occur in *bop1 bop2 ap1-1* triple mutants where flowers are converted into highly branched inflorescence-like shoots. Our data show that BOP1/2 function distinctly from LFY to upregulate *AP1* in floral primordia and that all three activities converge to down-regulate flowering-time regulators including *AGAMOUS-LIKE24* in stage 2 floral meristems. Subsequently, BOP1/2 promote A-class floral-organ patterning in parallel with LFY and AP1. Genetic and biochemical evidence support the model that BOP1/2 are recruited to the promoter of *AP1* through direct interactions with TGA bZIP transcription factors, including PERIANTHIA. These data reveal an important supporting role for BOP1/2 in remodeling shoot architecture during the floral transition.

Keywords: ***AGAMOUS-LIKE24 (AGL24), APETALA1, BLADE-ON-PETIOLE,* floral meristem identity, *LEAFY*.**

INTRODUCTION

The switch from vegetative to reproductive development in Arabidopsis is a tightly controlled process mediated by multiple genetic pathways in response to developmental cues and environmental signals (Kobayashi and Weigel, 2007; Turck *et al.*, 2008). Inputs from flowering-time pathways converge to regulate the expression of a small number of genes with floral integrator activity including *LEAFY* (*LFY*), *FLOWERING-TIME LOCUS T* (*FT*) and *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1* (*SOC1*) whose upregulation in shoot apices promotes the production of an inflorescence (Parcy, 2005). The MADS-box flowering-time genes *AGAMOUS-LIKE24* (*AGL24*), *SOC1,* and *SHORT VEGETATIVE PHASE* (*SVP*) encode key determinants of inflorescence meristem identity in Arabidopsis (Hartmann *et al.*, 2000; Michaels *et al.*, 2003; Yu *et al.*, 2004; Liu *et al.*, 2007). Early in the transition to flowering, the inflorescence meristem produces cauline leaves, which generate secondary inflorescences in their axils. Subsequently, leaf development is fully repressed and lateral shoots acquire floral fate (Sablowski, 2007).

Two key regulators of floral meristem identity in Arabidopsis are *LFY* and *APETALA1* (*AP1*). Expression of these genes in lateral organ primordia confers floral fate (Blázquez *et al.*, 2006). The initial upregulation of LFY specifies floral meristems by activating floral meristem identity genes including the MADS-box transcription factors encoded by *AP1* and *CAULIFLOWER* (*CAL*) and the homeodomain leucine-zipper (HD-Zip) transcription factor encoded by *LATE MERISTEM-IDENTITY1* (*LMI1*) (Kempin *et al.*, 1995; Liljegren *et al.*, 1999; Saddic *et al.*, 2006). LFY is a direct regulator of *AP1* (Parcy *et al.*, 1998; Wagner *et al.*, 1999) but activation is also directed by a complex of FT/FD (Abe *et al.*, 2005; Wigge *et al.*, 2005) and by SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL) proteins that control age-related flowering time (Wang *et al.*, 2009; Yamaguchi *et al.*, 2009).

Prior to their specification of floral organ identity, AP1 and LFY activities block the continued expression of inflorescence meristem identity genes in the floral meristem so that these shoots become determinate and develop as flowers (Yu *et al.*, 2004; Liu *et al.*, 2007, 2009a). Over-expression of *AGL24, SOC1,* or *SVP* partially transforms floral meristems into inflorescence meristems leading to branched flowers and floral bracts. These phenotypes are thereby suppressed in *lfy* and *ap1* by loss-of-function mutations in *agl24, soc1,* or *svp* (Yu *et al.*, 2004; Liu *et al.*, 2007). After commitment to flowering, activation of *AGAMOUS* (*AG*) in the dome of the floral meristem leads to repression of the stem-cell organizer *WUSCHEL* (*WUS*) ensuring that shoot determinacy is complete (Lenhard *et al.*, 2001; Lohmann *et al.*, 2001).

BLADE-ON-PETIOLE1 (*BOP1*) and *BOP2* encode BTB-ankyrin transcriptional co-regulators that are expressed in lateral organ boundaries and that control the architecture of leaves, fruits, and flowers. *bop1 bop2* mutants develop leafy petioles and receptacle defects in flowers. Flowers have bracts and two petaloid structures typically replace the abaxial sepal (Hepworth *et al.*, 2005; Norberg *et al.*, 2005; McKim *et al.*, 2008). The role of BOP1/2 is best understood in leaves where they are direct activators of the lateral-organ boundary marker *ASYMMETRIC LEAVES2* (Jun *et al.*, 2010). Co-misexpression of meristematic genes such as *BREVI-PEDICELLUS* (*BP*) and the blade-promoting transcription-factor-encoding *JAGGED* (*JAG*) in *bop1 bop2* mutants promotes indeterminacy in leaf petioles leading to ectopic leaflet formation (Ha *et al.*, 2003, 2004, 2007; Norberg *et al.*, 2005).

Two previous reports have provided preliminary evidence that the *BOP* genes also promote floral meristem identity. *In situ* experiments by Karim *et al.* (2009) have suggested that the redundant activities of BOP1/2 and PUCHI, an EREBP transcription factor, promote *LFY* expression in lateral meristems. In *bop1 bop2 puchi* triple mutants, extra inflorescence-like shoots arise with the eventual production of *bop1 bop2*-like flowers. Norberg *et al.* (2005) also showed that *bop1 bop2* enhances *lfy-26* in bract formation and floral meristem identity defects but the mechanism was not addressed.

Here, we use a genetics approach to examine the role of BOP1/2 in the floral transition. Loss-of-function *bop1 bop2* mutants show minor defects in inflorescence and floral architecture but in combination with *lfy* or *ap1,* synergistic defects in floral fate and shoot architecture are revealed. We show that BOP1/2 function in parallel with LFY to control determinacy in floral shoots through activation of *AP1* and repression of *AGL24* in developing flowers. To establish mechanism, we provide evidence that BOP1/2 are recruited to the promoter of *AP1* in part through direct interactions with the TGA bZIP factor PERIANTHIA (PAN). These data reveal an important supporting role for BOP1/2 in remodeling shoot architecture during the floral transition.

RESULTS

Dynamic pattern of *BOP* expression in the inflorescence apex

To clarify the dynamics of *BOP* expression during floral development, we examined the expression pattern of a *GUS* reporter gene driven by the *BOP2* promoter. *BOP2* expression quickly cleared from lateral inflorescence meristems and localized to the axil of cauline leaves (Figure 1a). Expression was detected in floral anlagen (stage 0) and in the adaxial portion of stage 1 floral primordia (Figure 1c,d). At late stage 2, expression shifted to the boundary between the floral primordium and the cryptic bract (Figure 1a,b,d,e, asterisks). *BOP2* expression was focused in the dome of the floral meristem at early stage 3 but shifted to the sepal axils and was maintained (Figure 1c,f,g, arrows). *BOP1::GUS* was expressed in a similar pattern (not shown). These data are in agreement with previous *in situ* data (Hepworth *et al.*, 2005; Norberg *et al.*, 2005; Karim *et al.*, 2009) and are consistent with a role for BOP1/2 in promotion of floral meristem identity.

Weak floral meristem identity defects in *bop1 bop2* mutants

Close inspection of *bop1 bop2* plants grown in inductive photoperiods revealed phenotypes consistent with mild defects in floral meristem identity. Some of these defects were reported previously but here they are considered collectively. First, visible bracts subtended about 20% of flowers grown in continuous light (Table 1 and Figure 2a). Hepworth *et al.* (2005) showed that bracts are initiated on most flowers at stage 1–2 but that their further development is variable. Second, *bop1 bop2* mutants displayed a small but reproducible increase in secondary inflorescences compared with wild-type (Figure 2b); Norberg *et al.*, 2005). Cauline leaves were sometimes absent from the base of shoots preceding the node of first flower (Figure 2c). Sometimes this was due to ectopic pedicel elongation causing displacement of the cauline leaf but other times the cauline leaf was lacking indicating that its development was repressed. Third, branched flowers arose at a low frequency (Figure 2d); Table 1; Ha *et al.*, 2007). This phenotype is characteristic of *ap1* mutants, caused by the ectopic initiation of floral meristems in the axils of sepal-whorl organs, but occurs less commonly in *lfy* mutants (Irish and Sussex, 1990; Schultz and Haughn, 1991, 1993; Weigel *et al.*, 1992). Overall, the range of floral meristem identity defects in *bop1 bop2* closely resembles those in *lfy* mutants consistent with the notion that like LFY, BOP1/2 promote floral fate.

Interactions with LEAFY and LATE-MERISTEM-IDENTITY1

LFY is the central floral meristem identity regulator in Arabidopsis and its loss-of-function generates a large increase in secondary inflorescences, floral bracts, and some

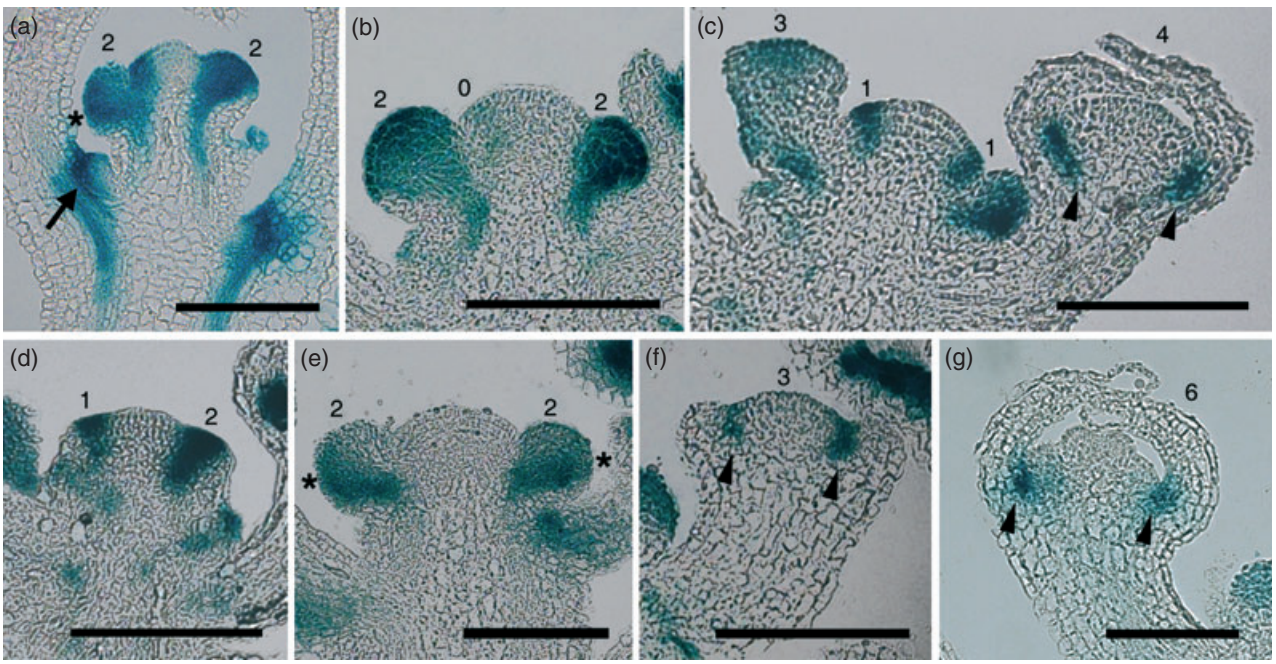

Figure 1. *BOP2::GUS* expression in inflorescence apices.
(a) 14-day-old seedling; expression at the boundary of a lateral shoot meristem (arrow).
(b–e) Expression begins in floral anlagen (0) and associates with the adaxial part of floral meristems until late stage 2, when expression shifts to the floral meristem/bract boundary (asterisks).
(c, f, g) Expression is in the dome of early stage 3 flowers but shifts to sepal axils (arrowheads). Numbers in panels indicate floral stage. Scale bars, 100 μm.

Table 1 Quantitative analysis of floral meristem identity phenotypes in wild-type and mutants

Genotype	No. secondary inflorescences (*n*)	Flowers with bracts % (*n*)	Plants with branched flowers % (*n*)	Branched flowers %
WT (Col)	1.94 ± 0.32 (36)	0.0 (360)	0.0 (24)	0.0
bop1 bop2	2.83 ± 0.14* (36)	22.3 (235)	14.9 (22)	0.3
lfy-2	5.74 ± 0.23 (35)	4.2 (407)	54.5 (22)	5.6
bop1 bop2 lfy-2	5.51 ± 0.28 (29)	100 (281)	100 (32)	60.1
WT (Col)	n.d.	0.0 (333)	0.0 (12)	0.0
bop1 bop2	n.d.	28.6 (297)	25.0 (12)	1.0
lfy-1	n.d.	51.5 (94)	100 (12)	18.1
bop1 bop2 lfy-1	n.d.	100 (89)	100 (6)	29.2
WT (Col)	1.69 ± 0.11 (36)	0.0 (360)	0.0 (24)	n.d.
bop1 bop2	3.17 ± 0.12* (36)	11.2 (420)	0.0 (24)	n.d.
lmi1-1	1.78 ± 0.11 (36)	0.0 (360)	0.0 (24)	n.d.
bop1 bop2 lmi1-1	2.54 ± 0.11 (36)	53.7 (808)	0.0 (24)	n.d.
WT (Col)	1.65 ± 0.11 (35)	0.0 (525)	0.0 (35)	0
bop1 bop2	2.67 ± 0.14* (36)	21.6 (540)	0.0 (36)	0
ap1-12	2.04 ± 0.16 (26)	0.0 (390)	100 (26)	27.7
ap1-1	2.7 ± 0.11 (27)	0.0 (525)	100 (35)	35.8
bop1 bop2 ap1-1	2.94 ± 0.11 (32)	31.7 (480)	100 (32)	81.0
bop1 bop2 ap1-1	5.4 ± 0.34 (17)	69.2 (240)	100 (19)	89.5
WT (Col)	1.83 ± 0.17 (12)	0 (180)	0 (12)	0
agl24–3	2.33 ± 0.26 (12)	0 (180)	0 (12)	0
lfy-2	9.71 ± 0.60 (17)	3.9 (255)	70.6 (6)	14.9
lfy-1	11.50 ± 0.50 (6)	32.8 (171)	83.0 (6)	9.9
agl24–3 lfy-2	11.30 ± 0.50 (20)	75.4 (285)	80.0 (20)	15.4
agl24–3 lfy-1	12.90 ± 0.76 (13)	95.3 (358)	53.9 (13)	3.6

*Significantly different from WT as determined by Student's *t*-test. n.d. not determined.

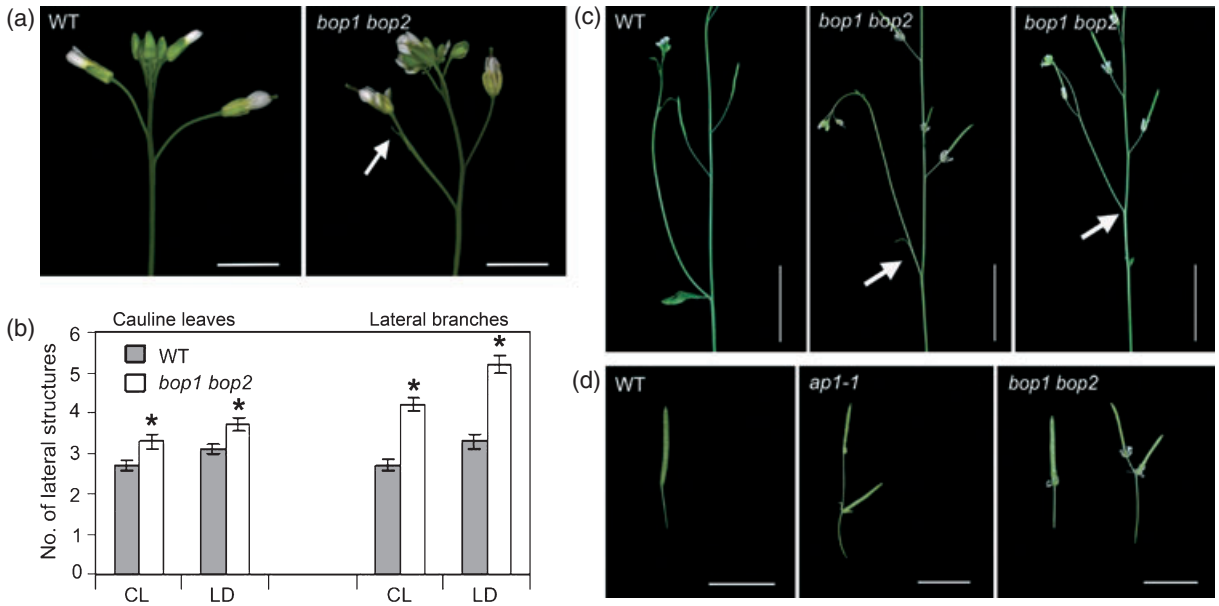

Figure 2. Analysis of floral meristem identity defects in *bop1 bop2* mutants.
(a) Flowers in *bop1 bop2* plants often have a bract (arrow). Scale bars, 2 cm.
(b) Graph showing that lateral branches are more numerous in *bop1 bop2* mutants relative to WT in continuous light (CL) or long-day (LD) photoperiods and that not all branches have a cauline leaf. Asterisks indicate a significant difference from WT (Student's *t*-test).
(c) Cauline leaves may be absent or displaced at transitional nodes in *bop1 bop2* plants (arrows). Scale bars, 0.5 cm.
(d) Siliques of WT, *ap1-1,* and *bop1 bop2* mutants. Scale bars, 1 cm.

branched flowers (Schultz and Haughn, 1991; Weigel *et al.*, 1992). Enlarged floral bracts and nodes without flowers were reported for *bop1 bop2* crossed to the strong *lfy-26* mutant suggesting that BOP1/2 and LFY contribute independently to floral meristem identity (Norberg *et al.*, 2005). To examine this further, we crossed *bop1 bop2* mutants to strong (*lfy-1*) and weak (*lfy-2*) alleles and assayed the triple mutants for defects in floral meristem identity (Tables 1 and 2; Figure 3). In continuous light, *lfy-2* plants generated about 3.8 more secondary inflorescences than wild-type but no further increase occurred in triple mutants with *bop1 bop2* suggesting that BOP1/2 do not function redundantly with LFY to control the number of secondary inflorescences (Table 1). Comparison of *lfy-2* mutants to *bop1 bop2 lfy-2* triple mutants showed a significant increase in plants with branched flowers (54.4% versus 100%) and floral bracts (4.2% versus 100%). Branching patterns in *bop1 bop2 lfy-2* flowers were more complex than in *lfy-2* with enhanced internode elongation between successive floral organs (Figure 3a–c). Bracts in *bop1 bop2 lfy-2* were enlarged and late in the primary inflorescence, nodes containing a bract but no flower developed (Figure 3d,g). Bracts and floral branching were also elaborated in *bop1 bop2 lfy-1* mutants (Figure 3e,f) confirming separate roles for BOP1/2 and LFY in promotion or maintenance of floral meristem identity.

LFY promotes flowering by activating a suite of downstream floral meristem identity regulators. *LMI1,* which encodes an HD-Zip transcription factor, is upregulated by LFY in stage 1 flowers before localizing to the cryptic bract (Saddic *et al.*, 2006). Loss-of-function *lmi1* enhances the number of secondary inflorescences in weak *lfy* mutants, showing that LMI1 functions in part as a meristem-identity factor downstream of LFY. LMI1 has a second LFY-independent role in leaf and bract repression: in short-day photoperiods *lmi1* mutants develop petiole leaflets similar to *bop1 bop2* mutants (Hepworth *et al.*, 2005; Saddic *et al.*, 2006). No increase in the number of secondary inflorescences or floral branching occurred in *bop1 bop2 lmi1* triple mutants (Table 1) nor were petiole leaflets in *bop1 bop2* mutants enhanced (data not shown). However, the frequency of flowers with bracts was much higher the triple mutant relative to *bop1 bop2* (53.7% versus 11.2%; Figure 3o–r) indicating that BOP1/2 and LMI1 contribute separately to bract repression and have little impact on the number of secondary inflorescences when LFY is functional.

Interactions with APETALA1

The primary target of LFY in promotion of floral fate is *AP1.* Activation of *AP1* together with *CAL* and *LMI1* feed-forward to reinforce *LFY* expression so that floral induction is sharp and unidirectional (Saddic *et al.*, 2006). The strong *ap1-1* (Col) mutant shows elaborate floral branching only in the first few nodes after the switch to flowering (Table 1; Irish and Sussex, 1990; Schultz and Haughn, 1993). In *bop1 bop2 ap1-1* triple mutants, floral meristems were dramatically converted to partial inflorescences, with curd-like apices

Table 2 Quantitative analysis of floral-organ identity phenotypes in wild-type and mutants

Genotype	No[a] of flowers scored	Sepal whorl		Petal whorl			Stamen whorl		Carpel whorl	
		Sepal	Carpelloid attributes	Petal	Sepaloid attributes	Carpelloid attributes	Stamen	Sepaloid or petaloid attributes	Fused	Unfused
WT (Col)	20	4.00 ± 0.00	–	4.00 ± 0.00	–	–	5.90 ± 0.07	–	2.00 ± 0.00	–
bop1 bop2	23	4.78 ± 0.09	–	4.04 ± 0.00	–	–	6.74 ± 0.09	–	–	–
lfy-2	90	3.97 ± 0.02	–	3.11 ± 0.09	0.10 ± 0.05	–	–	2.04 ± 0.09	1.78 ± 0.08	0.34 ± 0.09
bop1 bop2 lfy-2	34	1.03 ± 0.23	1.97 ± 0.67*	0.03 ± 0.03	0.53 ± 0.19	0.56 ± 0.18*	0.68 ± 0.16	0.32 ± 0.12	0.85 ± 0.18	1.21 ± 0.18*
lfy-1	51	3.90 ± 0.04	0.04 ± 0.03	0.02 ± 0.02	2.57 ± 0.19	0.78 ± 0.14	0.08 ± 0.04	0.25 ± 0.09	0.68 ± 0.15	1.56 ± 0.16
bop1 bop2 lfy-1	21	1.76 ± 0.30	2.05 ± 0.33*	0.00 ± 0.00	0.10 ± 0.10	3.05 ± 0.30*	0.00 ± 0.00	0.00 ± 0.00	0.10 ± 0.10	2.67 ± 0.22*
WT (Col)	25	4.00 ± 0.00	–	4.00 ± 0.00	–	–	5.75 ± 0.09	–	2.00 ± 0.00	–
bop1 bop2	25	4.88 ± 0.07	–	4.42 ± 0.07	–	–	6.70 ± 0.09	–	2.00 ± 0.00	–
ap1-12	25	3.24 ± 0.16	–	1.36 ± 0.27	–	–	5.68 ± 0.10	–	2.00 ± 0.00	–
bop1 bop2 ap1-12	25	4.20 ± 0.12	0.04 ± 0.04	0.44 ± 0.16	0.08 ± 0.05	–	5.80 ± 0.18	0.52 ± 0.15	2.00 ± 0.00	–
ap1-1	25	3.16 ± 0.19	–	0.08 ± 0.05	–	–	5.68 ± 0.13	0.44 ± 0.17	2.00 ± 0.00	–
bop1 bop2 ap1-1	29	3.38 ± 0.17	0.72 ± 0.16*	0.38 ± 0.14	–	–	6.00 ± 0.11	0.10 ± 0.06	2.00 ± 0.00	–

*Significantly different from the *lfy* parental control as determined using Student's *t*-test.
[a]Flowers from nodes 1–15 were scored.

similar to *ap1 cal* mutants (Figure 4a–e); Bowman *et al.*, 1993). Highly branched shoots, often indeterminate, developed in place of all floral nodes. Single peduncles showed enhanced floral branching and internode elongation between successive floral organs (Figure 4f–j); Tables 1 and 3). Floral bracts in stage 1–2 flowers of the triple mutant were highly developed relative to parental controls and first-whorl organs showed spiral instead of whorled phyllotaxy (Figure S1). Branching was enhanced in *bop1 bop2 ap1-12* mutants but all shoots remained determinate, likely due to residual AP1 function in *ap1-12* (Table 1 and Figure 4l–m). These interactions reveal that BOP1/2 and AP1 redundantly suppress inflorescence characteristics in floral shoots.

BOP activity has A-class function in flowers

After flowers are initiated, LFY and AP1 promote floral patterning. Floral organ identity is determined by the overlapping activities of three classes of homeotic genes termed A, B, and C that specify sepals, petals, stamens and carpels according to the ABC model (Haughn and Somerville, 1988; Coen and Meyerowitz, 1991). Strong *lfy* mutants lack petals and stamens due lack of B-class gene activation and sepal-whorl organs become carpelloid as a result of *AG* misexpression (Drews *et al.*, 1991; Weigel and Meyerowitz, 1993; Liu and Meyerowitz, 1995). Comparison of *lfy-2* and *bop1 bop2 lfy-2* triple mutants showed enhancement of *lfy* floral-organ identity defects (Table 2 and Figure 3c,j–m). Whereas weak *lfy-2* mutants develop some petals and stamens, sepal-like or mosaic sepal/carpel organs formed in all whorls of *bop1 bop2 lfy-2* flowers, with *AG* misexpression in the outer whorls (Figure 3i). Mutation of *bop1 bop2* similarly enhanced the carpelloid character of outer-whorl organs in strong *lfy-1* mutants (Table 2 and Figure 3h,n) similar to *lfy ap1* mutants (Schultz and Haughn, 1993). AP1 also contributes to A-function by specifying sepal and petal identity (Irish and Sussex, 1990; Schultz and Haughn, 1991; Mandel *et al.*, 1992). Flowers in *bop1 bop2 ap1-12* triple mutants closely resembled those in the strong *ap1-1* mutant, lacking petals (Figure 4g,l,m); Table 2). In *bop1 bop2 ap1-1* triple mutants, sepal-whorl organs gained carpelloid features (Figure 4k and Table 2) consistent with misexpression of *AG* in sepal margins (data not shown). Overall, these data reveal that BOP1/2 promote A-class floral patterning.

BOP1/2 and LFY are distinct regulators of *AP1* in floral meristems

Given that BOP activity promotes floral meristem identity, we reasoned that *LFY* and/or *AP1* might be targets of regulation. *In situ* hybridization by Karim *et al.* (2009) showed a dramatic lack of *LFY* expression in *bop1 bop2 puchi* apices, providing one explanation for the severe flower-to-shoot reversions observed in *bop1 bop2 ap1-1* triple mutants. We therefore monitored *LFY* expression in the inflorescence apices of wild-type, *bop1 bop2, ap1-1,* and *bop1 bop2 ap1-1*

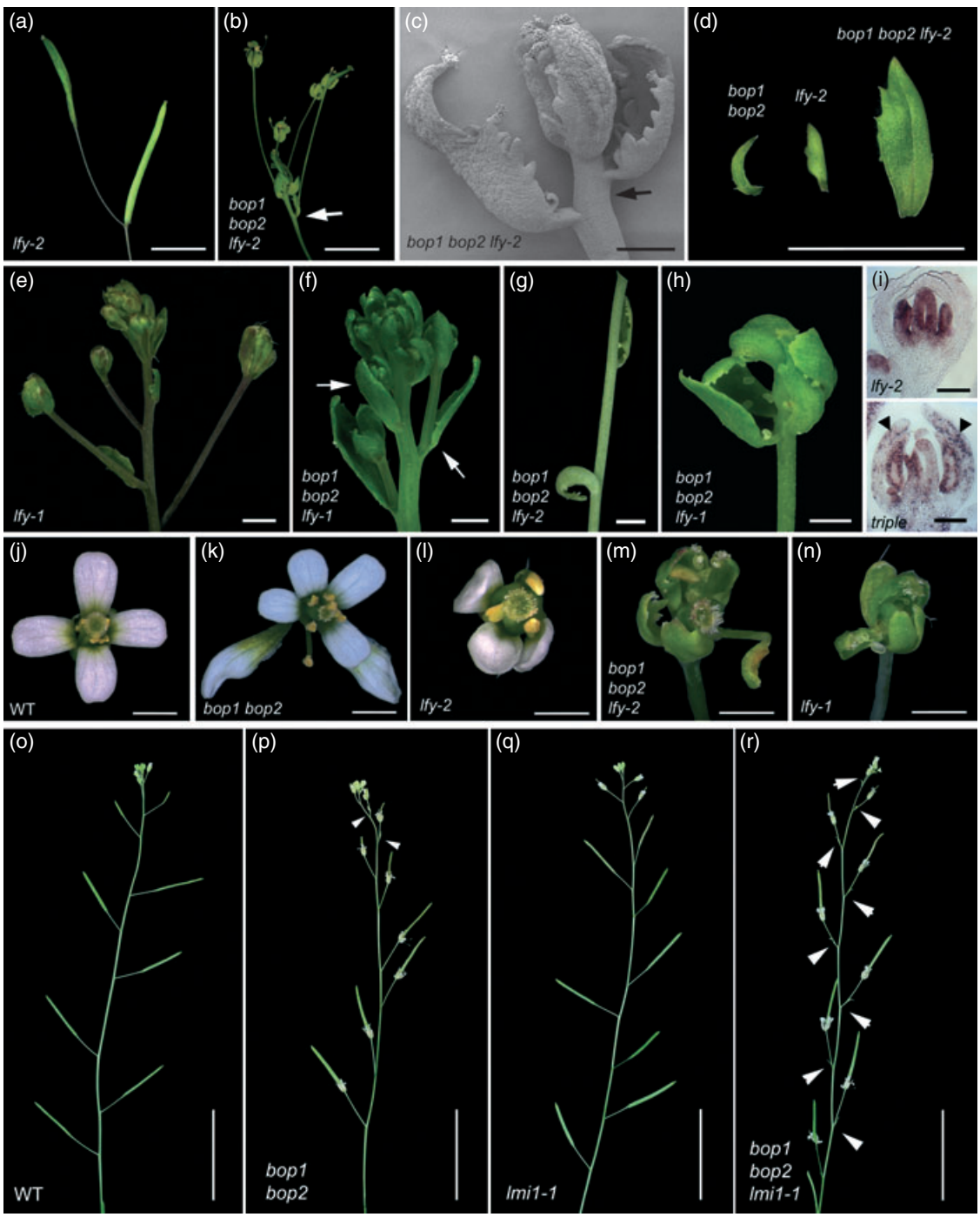

Figure 3. Mutation of *bop1 bop2* enhances *lfy* and *lmi1-1* floral defects.
(a) *lfy-2.*
(b) *bop1 bop2 lfy-2;* enhanced branching complexity (arrow).
(c) SEM of *bop1 bop2 lfy-2* flower; carpelloid sepals and internode elongation between floral organs (arrow).
(d) Comparison of floral bracts in *bop1 bop2, lfy-2,* and *bop1 bop2 lfy-2* mutants.
(e, f) Comparison of *lfy-1* and *bop1 bop2 lfy-1* inflorescences; bracts are larger and more numerous (arrows).
(g) *bop1 bop2 lfy-2* bract-only nodes.
(h, j–n) Representative flowers of the indicated genotypes. Ectopic stigmatic papillae and ovules and unfused carpels are more prevalent in *bop1 bop2 lfy-2* and *bop1 bop2 lfy-1* flowers compared with *lfy-2* or *lfy-1* respectively.
(i) Misexpression of *AG* in the perianth whorls of *bop1 bop2 lfy-2* triple mutant flowers.
(o–r) Representative inflorescences of the indicated genotypes; bract formation in *bop1 bop2* is enhanced by *lmi1* (arrows). Scale bars: 1 mm, except (a, b, d), 5 mm; (c), 0.5 mm; (i), 100 μm; (o–r), 2 cm.

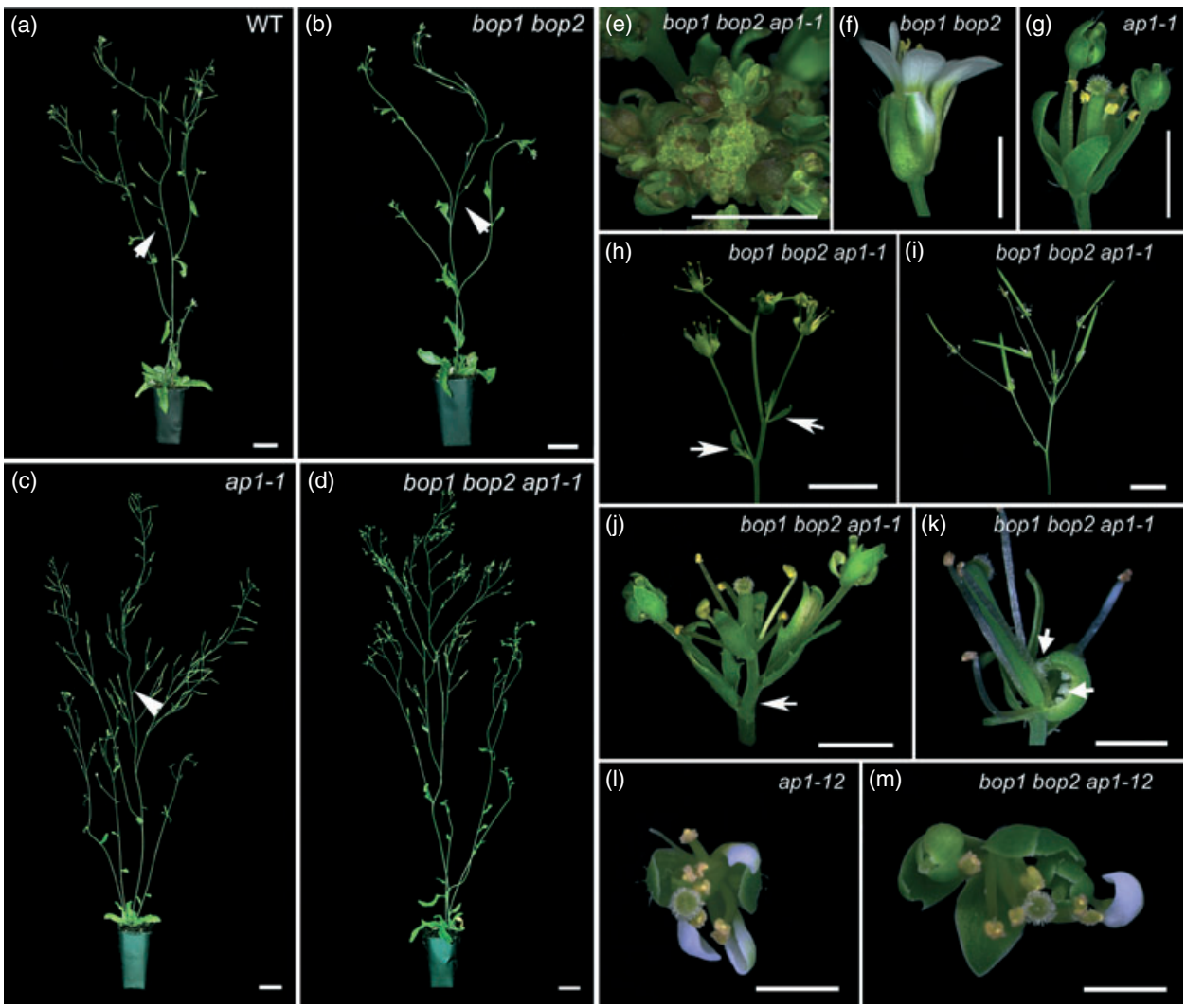

Figure 4. Mutation of *bop1 bop2* enhances *ap1* floral defects.
(a–d) Representative inflorescences for the genotypes indicated. Arrows denote node of first flower. Floral nodes in *bop1 bop2 ap1-1* are highly branched and often indeterminate.
(e) Curd-like *bop1 bop2 ap1-1* inflorescence apex. Comparison of floral architectures:
(f) *bop1 bop2;* most flowers are unbranched, abaxial sepals are petaloid.
(g) *ap1-1;* secondary flowers in the axils of first-whorl floral organs.
(h) *bop1 bop2 ap1-1* floral shoot with determinate architecture. Large leafy sepal/bracts subtend flowers (arrows).
(i) Late stage *bop1 bop2 ap1-1* floral node showing complex branching architecture.
(j) Early stage *bop1 bop2 ap1-1* floral node; internode elongation between first-whorl organs and floral bracts (arrow).
(k) *bop1 bop2 ap1-1* flower; arrows indicate ovules on the margin of carpelloid sepals.
(l) *ap1-12* flower; weak allele.
(m) *bop1 bop2 ap1-12* flower; similar to *ap1-1*. Scale bars: (a–d), 2.5 cm; (e–g), 2 mm; (h), 0.5 cm; (i–m), 1 mm.

plants grown in continuous light, using *bop1 bop2 puchi* triple mutants as a control. Apices were examined at three time-points: 14-day-old seedlings committed to flowering, 1-cm bolts, and 5-cm bolts. However, *LFY* transcript accumulation in both triple mutants was similar to *ap1-1* control apices in which *LFY* accumulates to WT levels (Figure S2; Weigel *et al.*, 1992) indicating that the dramatic flower-to-shoot phenotypes in *bop1 bop2 ap1-1* mutants cannot easily be attributed to lack of *LFY* expression.

Rather, *in situ* hybridization revealed that in combination with all mutant genotypes examined, loss of BOP activity had a significant impact on *AP1* transcript levels. *AP1* is independently activated by LFY and FD/FT in stage 1 flowers and represents the earliest known marker of commitment to floral fate (Hempel *et al.*, 1997; Wagner *et al.*, 1999; Abe *et al.*, 2005; Wigge *et al.*, 2005). As seen previously, *AP1* transcript in *lfy-1* and *lfy-2* apices was reduced (Figure 5; Liljegren *et al.*, 1999; Mandel and Yanofsky, 1995a;

Table 3 Quantitative analysis of floral branching and bract suppression by loss-of-function *agl24*

Genotype	No. of secondary inflorescences	No. of flowers per pedicel/peduncle			No. of plants scored	Flowers with bracts % (*n*)
		Floral node 1–5	Floral node 6–10	Floral node 11–15		
WT (Col)	n.d.	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	24	0.0 (360)
bop1 bop2	n.d.	1.04 ± 0.02	1.05 ± 0.03	1.00 ± 0.00	22	26.5 (287)
ap1-1	n.d.	3.84 ± 0.23	1.50 ± 0.14	1.03 ± 0.01	25	0.0 (375)
agl24–3	n.d.	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	24	0.0 (360)
ap1-1 agl24–3	n.d.	2.58 ± 0.20	1.20 ± 0.04	1.01 ± 0.01	30	0.0 (360)
bop1 bop2 agl24–3	n.d.	1.01 ± 0.01	1.00 ± 0.00	1.00 ± 0.00	24	56.8 (572)
bop1 bop2 ap1-1	n.d.	9.95 ± 0.87	6.42 ± 0.42	3.56 ± 0.33	22	73.6 (336)
bop1 bop2 ap1-1 agl24–3	n.d.	3.96 ± 0.30*	2.72 ± 0.28*	1.45 ± 0.15*	20	79.0 (238)
WT (Col)	2.28 ± 0.18	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	18	0.0 (270)
bop1 bop2	3.43 ± 0.25	1.01 ± 0.01	1.00 ± 0.00	1.00 ± 0.00	23	10.1 (345)
lfy-2	9.71 ± 0.60	1.23 ± 0.07	1.08 ± 0.04	1.20 ± 0.06	17	3.9 (255)
agl24–3	4.90 ± 0.35	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	24	0.0 (360)
lfy-2 agl24–3	11.2 ± 0.52	1.33 ± 0.04	1.18 ± 0.05	1.24 ± 0.07	19	75.4 (285)
bop1 bop2 agl24–3	4.45 ± 0.29	1.01 ± 0.01	1.00 ± 0.00	1.00 ± 0.00	22	51.2 (330)
bop1 bop2 lfy-2	9.18 ± 0.83	2.33 ± 0.17	n.d.[a]	n.d.[a]	11	100 (165)
bop1 bop2 lfy-2 agl24–3	9.35 ± 0.35	1.03 ± 0.06*	n.d.[a]	n.d.[a]	23	100 (345)

*Significantly different from the triple mutant control as determined using Student's *t*-test.
[a]Floral branching could not be accurately scored due to presence of nodes without a flower (bract only): *bop1 bop2 lfy-2* (21.4%, *n* = 121), *bop1 bop2 lfy-2 agl24–3* (49.36%, *n* = 235).

Ruiz-Garcia *et al.*, 1997). In *lfy-1* apices, *AP1* first accumulated in the sepal whorl of stage 3 flowers and slightly earlier in *lfy-2* apices (Figure 5g–i,m–o). In contrast, little or no *AP1* accumulated in *bop1 bop2 lfy-1* or *bop1 bop2 lfy-2* flowers at stage 1–2, nor was there expression at stage 3 in *bop1 bop2 lfy-1* flowers (Figure 5j–l,p–r). Reduced *AP1* expression was also apparent in *bop1 bop2 puchi* control apices (Figure 5s–j). These data identify *AP1* as a major target of BOP regulation.

To examine this further, we used qPCR to monitor the expression of *LFY* direct targets *AP1* and *CAL* in the inflorescence apices of *lfy-1* versus *bop1 bop2 lfy-1* triple mutants with 5-cm bolts. Both genes are expressed specifically in floral primordia (Wagner *et al.*, 1999; Ferrándiz *et al.*, 2000; William *et al.*, 2004). A dramatic reduction in *AP1* transcript was observed in *bop1 bop2 lfy-1* triple mutants; *CAL* levels were also slightly reduced (Figure 6a). We further monitored *AP1, CAL,* and *LMI1* transcript levels: (i) in apices of *lfy-1* versus *bop1 bop2 lfy-1*; and (ii) in *fd-2* versus *fd-2 lfy-12* control apices; using the experimental design of Abe *et al.* (2005) (1-cm bolts; Figure 6b). Dramatically lower levels of *AP1* were observed in *bop1 bop2 lfy-1* mutants relative to *lfy-1* mutants [comparable with *fd-2* versus *fd-2 lfy-12* apices (Abe *et al.*, 2005; Wigge *et al.*, 2005)] and in *bop1 bop2 puchi* control apices. *CAL* and *LMI1* transcripts were also slightly reduced in *bop1 bop2 lfy-1* triple mutants compared with *lfy-1*, suggesting that BOP1/2 also have some effect on genes other than *AP1.* This finding promoted us to test if BOP activity resides in the FT pathway. However, levels of *AP1* and *CAL* in *bop1 bop2 ft-1* apices were much lower than in *ft-1* single mutants (Figure 6b) and bolting was greatly delayed in *bop1 bop2 ft-1* triple mutants (apex first visible at 54.2 ± 0.66 days, *n* = 33) compared with *ft-1* mutants (44.4 ± 0.55 days, *n* = 34) and *ft-10* null mutants (47.8 ± 0.55 days; *n* = 24). These genetic data indicate that BOP1/2 promote floral meristem identity independently of LFY and FT/FD.

BOP activity contributes to down-regulation of inflorescence identity genes

During the transition to flowering, *AGL24, SOC1,* and *FUL* are up-regulated in shoot apices to drive the production of primary and secondary inflorescences (Mandel and Yanofsky, 1995b; Hempel *et al.*, 1997; Ferrándiz *et al.*, 2000; Yu *et al.*, 2002; Michaels *et al.*, 2003). Together with AP1, these factors initially promote floral meristem fate but are subsequently down-regulated to permit development of the determinate floral shoot (Yu *et al.*, 2004; Liu *et al.*, 2007, 2009b; Gregis *et al.*, 2008). The architecture of floral nodes in *bop1 bop2 ap1* and *bop1 bop2 lfy-2* triple mutants suggests that BOP activity contributes to this down-regulation. Supporting this, qPCR analysis showed elevated *AGL24, SOC1,* and *FUL* transcript in triple mutant apices relative to control apices (Figure 6c). *In situ* hybridization was used to monitor the expression patterns of these genes in more detail.

In wild-type and *bop1 bop2* apices, *AGL24* was expressed in the inflorescence meristem and floral primordia until late stage 2 when expression became restricted to the cryptic bract (Figure 7a,b); Michaels *et al.*, 2003). At stage 3, when floral organs begin to differentiate, *AGL24* expression sometimes occurred in the dome of *bop1 bop2* flowers

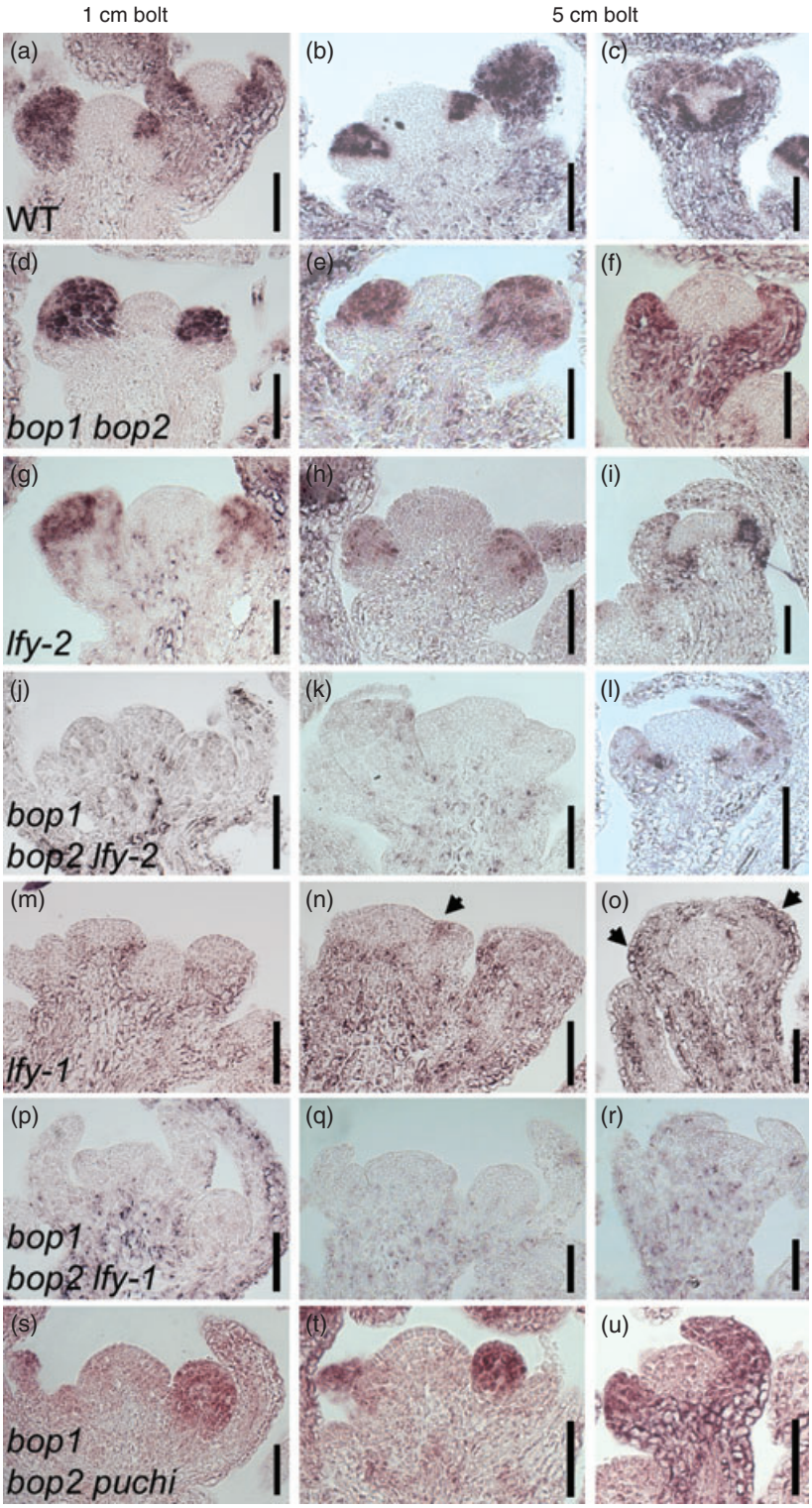

Figure 5. *In situ* analysis of *AP1* expression in WT and mutant apices. Inflorescence apices from 1-cm or 5-cm bolts were examined.
(a–c) WT.
(d–f) *bop1 bop2.*
(g–i) *lfy-2.*
(j–l) *bop1 bop2 lfy-2; AP1* transcript reduced relative to *lfy-2.*
(m–o) *lfy-1.* Arrows indicate expression in stage 2–3 flowers.
(p–r) *bop1 bop2 lfy-1;* no detectable *AP1* transcript.
(s–u) *bop1 bop2 puchi* control. Scale bars, 50 μm.

(Figure 7d). In *ap1-1* and *lfy-2* control apices, ectopic expression of *AGL24* was consistently detected in the dome and/or sepal whorl of stage 2 and 3 flowers (Figure 7f,g,h,i). Misexpression was dramatically enhanced in *bop1 bop2 ap1-1* and *bop1 bop2 lfy-2* triple mutants, detected throughout stage 1–3 floral primordia, consistent with their partial conversion into inflorescences (Figure 7j,k). Misexpression of *SOC1* and *FUL* in triple mutant apices was similar to

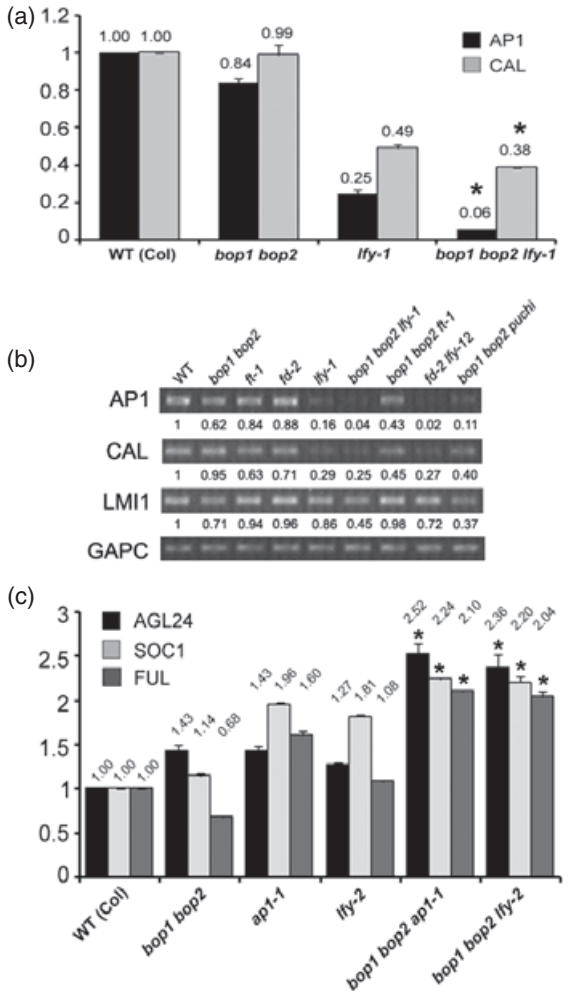

Figure 6. Quantitative analysis of *AP1, CAL,* and flowering-time gene expression in WT and mutant apices.
(a) Relative *AP1* and *CAL* transcript levels in apices of 5-cm bolts. *AP1* transcript is significantly lower in plants lacking both BOP and LFY activities.
(b) *AP1* transcript levels in the apices of 1-cm bolts for genotypes as indicated. Loss-of-function *bop1 bop2* lowers *AP1* transcript accumulation in both *lfy* and *ft* mutants.
(c) Relative *AGL24, SOC1,* and *FUL* transcript levels in apices of 5-cm bolts for the indicated genotypes. Transcript levels are highest in triple mutant apices. Asterisks in (a) and (c) indicate that values are significantly different from parental controls (Student's *t*-test).

AGL24 (Figures S3 and S4). These data indicate that BOP1/2, AP1, and LFY activities converge at stage 2 to down-regulate genes that confer inflorescence identity.

Rescue of floral branching but not bract formation by loss-of-function *agl24*

Loss-of-function *agl24* rescues floral branching in *ap1-1* and bract formation in *lfy-6* (Liu *et al.*, 2007; Yu *et al.*, 2004; L*er* ecotype) identifying misexpression of *AGL24* as a leading cause flower-to-shoot reversion in these mutants. Whilst loss-of-function *agl24* did not significantly reduce floral branching in *bop1 bop2, lfy-2,* or *ap1-1* plants (Col ecotype), branching in the triple mutants *bop1 bop2 ap1-1* and *bop1 bop2 lfy-2* was dramatically rescued as evidenced by steep reductions in the average number of flowers per peduncle and reestablishment of determinacy at all floral nodes in *bop1 bop2 ap1-1* plants (Table 3; Figures 8 and S5). These data confirm that shoot architectural defects in *bop1 bop2 lfy-2* and *bop1 bop2 ap1-1* triple mutants are due in part to continued expression of *AGL24* in floral meristems.

Evidence that BOP1/2 are recruited to the *AP1* promoter by direct interaction with PAN

BOP1/2 are transcriptional regulators of *AP1* and *AGL24* in floral meristems raising the possibility that this regulation is direct. BOP1/2 are BTB-ankyrin proteins similar to the pathogen defense regulator NPR1, a transcriptional co-activator that exerts most or all of its function via TGA bZIP transcription factors (Boyle *et al.*, 2009; Després *et al.*, 2000; Rochon *et al.*, 2006; Zhang *et al.*, 1999). BOP similarly interacts with a subset of TGA factors including PAN and BOP1/2-PAN function in the same genetic pathway to control perianth floral organ number (Hepworth *et al.*, 2005). Several other TGA factors are broadly expressed in inflorescence apices (e.g. Li *et al.*, 2009; Maier *et al.*, 2009) suggesting that BOP1/2 may function through one or more of these factors to promote floral meristem identity. Supporting this, *pan-1* mutants display floral meristem identity defects similar to *bop1 bop2* mutants, albeit at a lower frequency: floral bracts (2.8% of plants, $n = 71$), cauline leaves absent from the base of shoots preceding node of first flower (5.6% of plants, $n = 71$), and branched flowers (2.68% of node 1–5 flowers, $n = 149$) (Figure 9a–c). Using bimolecular fluorescence complementation (BiFC) assays, BOP–PAN interaction was confirmed in the nucleus of Arabidopsis mesophyll cells (Figure S6b–j; Data S1). Conversely, no interaction in yeast was detected between BOP proteins paired with LFY, FD, AGL24, SPL3, SPL9, or SEPALATTA1-4 (Figure S6a; Data S1; data not shown) representing other direct regulators of *AP1* and *AGL24* (Wagner *et al.*, 1999; Wigge *et al.*, 2005; Liu *et al.*, 2007, 2008; Gregis *et al.*, 2008; Wang *et al.*, 2009; Yamaguchi *et al.*, 2009). Using the AthaMap tool (Galuschka *et al.*, 2007) we identified potential TGA binding sites in the promoter of *AP1* (Figure 9d) and tested for BOP1 occupancy at these sites using chromatin immunoprecipitation (ChIP) assays. These assays used *bop1 bop2* plants complemented by a BOP1p:BOP1–GFP fusion protein. Strong and selective occupancy of BOP1–GFP was reproducibly detected at sites 1 and 3 in the *AP1* promoter (Figure 9f). Site 3 maps close to binding sites for FD, LFY, and SPL3/9 in the main control region for *AP1* (Parcy *et al.*, 1998; Wigge *et al.*, 2005; Wang *et al.*, 2009; Yamaguchi *et al.*, 2009). Enrichment of GFP–SPL9 at site 1 in the *AP1* promoter served as a positive control (Figure 9f); Wang *et al.*, 2009). These results collectively provide strong evidence that BOP1/2 are recruited to sites in the *AP1* promoter via TGA

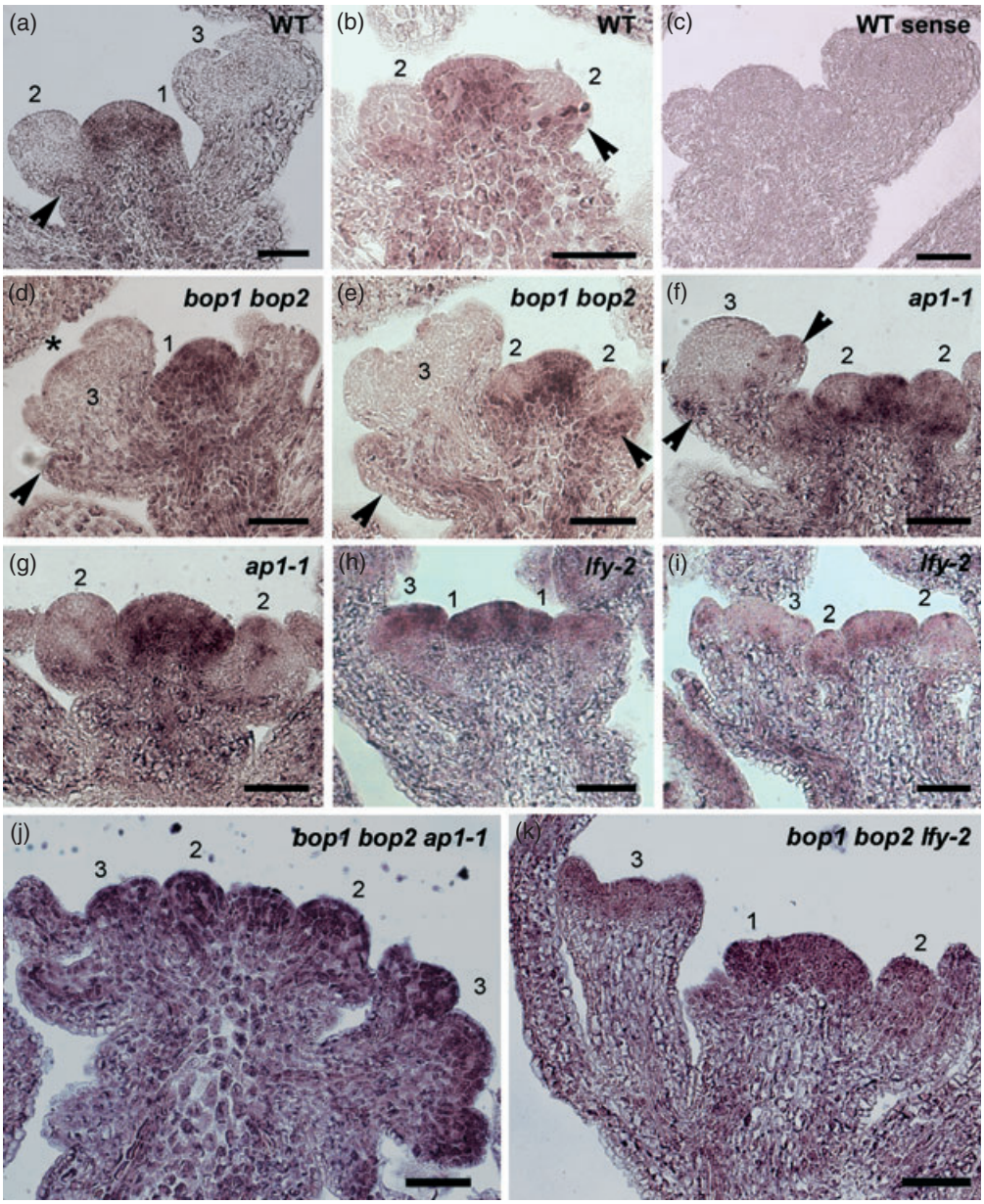

Figure 7. *In situ* analysis of *AGL24* expression in WT and mutant inflorescence apices.
(a–c) WT control; transcript localizes to the cryptic bract (arrows) at late stage 2.
(d, e) *bop1 bop2;* enlarged bracts (arrows) and misexpression in the dome of stage 3 flowers (asterisk).
(f, g) *ap1-1;* ectopic expression in the dome of stage 2 flowers and in stage 3 sepals (arrows).
(h, i) *lfy-2;* ectopic expression in the dome of stage 3 flowers.
(j, k) *bop1 bop2 ap1-1;* enhanced misexpression.
(l, m) *bop1 bop2 lfy-2;* enhanced misexpression. Numbers in panels indicate floral stage. Scale bars, 50 μm.

binding factors where they function as transcriptional co-regulators thereby expanding the role of TGA transcription factors in development and revealing a previously undefined pathway for promotion of floral fate.

DISCUSSION

In this study, we use a genetics approach to examine the role of architectural regulators BOP1 and BOP2 in promotion of floral fate. These genes are expressed in lateral organ primordia, including floral meristems, together with regulators of floral meristem identity including LFY, LMI1, and AP1. Loss-of-function *bop1 bop2* show only subtle defects in floral fate making their function more easily analyzed in sensitized genetic backgrounds. Double mutants with *lfy, ap1,* and *lmi1* mutants define in detail how BOP activity promotes floral meristem identity (Figure 10). We show that

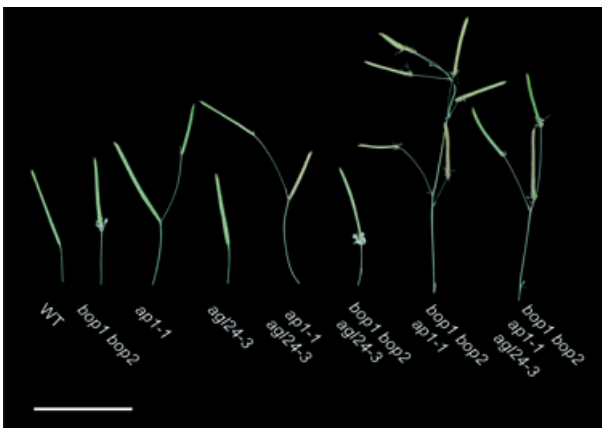

Figure 8. Floral branching in *bop1 bop2 ap1-1* triple mutants is suppressed by *agl24*.
Representative shoots (nodes 6–10) are shown for the indicated genotypes. Scale bar, 2 cm.

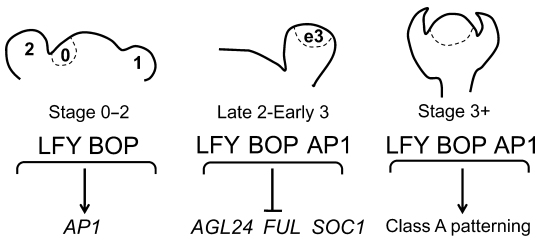

Figure 10. Model for BOP promotion of floral meristem identity.
The *BOP* genes are co-expressed with *LFY* in floral anlagen and function distinctly from LFY to upregulate *AP1* in stage 1 flowers. During stage 2, the overlapping activities of BOP1/2, LFY, and AP1 down-regulate *AGL24, SOC1,* and *FUL* in floral meristems to maintain floral fate. After stage 3, the continued activities of BOP1/2, LFY, and AP1 promote A-class floral patterning.

BOP1/2 promote flowering distinctly from LFY, contributing to activation of *AP1* and repression of inflorescence identity genes including *AGL24, SOC1,* and *FUL,* crucial for generating a determinate floral shoot. At stage 3, BOP1/2 exerts A-class floral patterning activity in parallel with LFY and AP1. Several lines of evidence support the model that BOP1/2 bind *in vivo* to regulatory sequences in the *AP1* promoter through direct interaction with TGA transcription factors, including PAN.

BOP activity is required for maintaining floral fate

Mutations that impair floral meristem identity cause the full or partial reversion of flowers into lateral branches. Delayed commitment to floral fate is associated with extra secondary inflorescences and floral bract outgrowth corresponding to defects in LFY expression or activity (Liu *et al.*, 2009a). In *ap1-1* mutants, *LFY* transcript accumulation is normal but maintenance of floral fate is compromised leading to branched flowers (Weigel *et al.*, 1992). The combination of *bop1 bop2* with *ap1-1* also shows robust expression of *LFY* throughout the floral transition and normal levels of *CAL* (data not shown) but extreme floral branching and indeterminate shoot growth, indicating that BOP1/2 plays a strong

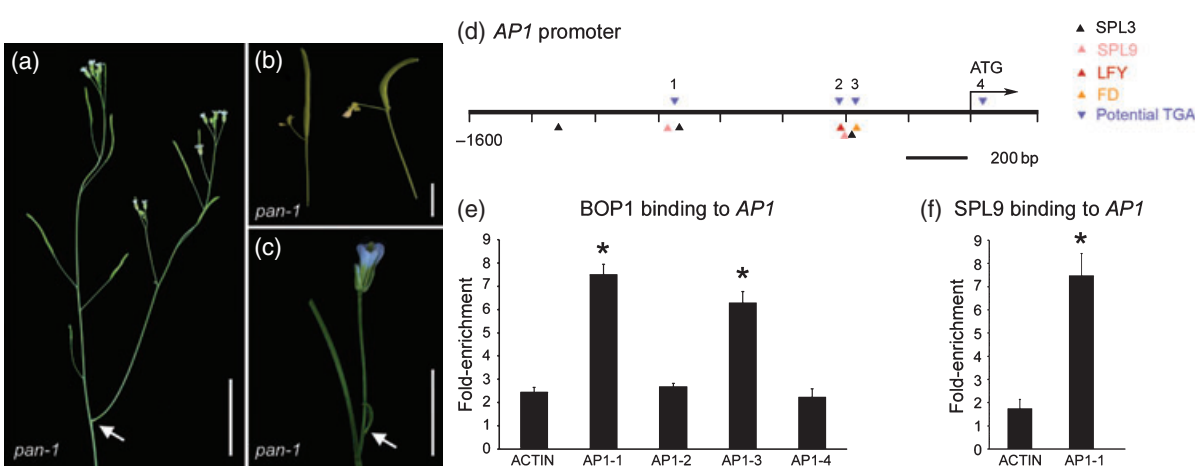

Figure 9. BOP1 binds *in vivo* to sites containing TGA binding motifs in the *AP1* promoter. Floral meristem identity defects in *pan-1* mutants:
(a) Cauline leaf absent at transitional node (arrow). Scale bar, 2 cm.
(b) Branched flowers.
(c) Flower with bract. Scale bars in (b) and (c), 5 mm.
(d) Scale diagram of the *AP1* promoter indicating the binding sites of direct regulators and potential TGA binding motifs. Numbers indicate regions tested for BOP1–GFP occupancy in ChIP assays (e, f). Binding site locations derived from: Wang *et al.*, 2009; Wigge *et al.*, 2005; and Yamaguchi *et al.*, 2009. See text for identification of potential TGA binding sites.
(e) BOP1–GFP occupancy at *AP1* promoter sites.
(f) GFP–SPL9 occupancy at *AP1* promoter site 1. Asterisks, significantly different from *ACTIN* control (Student's *t*-test).

role in commitment to floral fate. The situation is similar in *bop1 bop2 puchi* plants (see also Karim *et al.*, 2009). Moreover, *bop1 bop2* mutations fail to increase the formation of secondary inflorescences in weak *lfy* and *lmi1* mutants but rather increase bract formation and floral branching. These data indicate that BOP1/2 function in parallel with LFY and that the redundant activities of BOP1/2 and AP1 enforce floral fate.

Down-regulation of flowering-time genes

In stage 2 floral meristems, down-regulation of inflorescence identity genes prevents continuation of the shoot developmental program and permits LFY and AP1 to initiate differentiation of floral organs (Liu *et al.*, 2009a). *AGL24, SOC1,* and *SVP* are directly repressed by AP1, whereas repression by LFY is indirect (Yu *et al.*, 2004; Liu *et al.*, 2007; Gregis *et al.*, 2008). BOP1/2 contributes to this down-regulation as evidenced by dramatic misexpression of *AGL24, FUL,* and *SOC1* in *bop1 bop2 ap1-1* and *bop1 bop2 lfy-2* apices. *SVP* was not a target of BOP repression (data not shown). Loss-of-function *agl24* strongly suppressed floral branching in triple mutants but rescue was incomplete, likely due to continued misexpression of *SOC1* and *FUL*. It remains unclear if BOP1/2 directly repress *AGL24,* but the promoter contains two TGA binding motifs (not shown). In petioles, BOP1/2 promotes determinacy by repression of *BP* (Ha *et al.*, 2003, 2007) but the mechanism appears to differ in flowers as *BP* is not misexpressed in triple mutant apices nor do *bp* mutations rescue floral branching (data not shown).

Bract formation

Arabidopsis flowers develop in the absence of a visible bract, which is specified but repressed in its development (Hepworth *et al.*, 2006). The number and/or size of bracts was enhanced in all double mutant combinations tested: *lfy lmi1, bop1 bop2 lfy, bop1 bop2 lmi1, bop1 bop2 ap1,* and *bop1 bop2 agl24–3,* indicating that several inter-related pathways contribute to bract repression. Of these, *LMI1* and *AGL24* localize to the cryptic bract in late stage 2 whereas *BOP1/2* localize to the boundary between the floral meristem and the cryptic bract (this study; Karim *et al.*, 2009; Saddic *et al.*, 2006). *LFY* and *AP1* are expressed in the floral meristem contributing non-cell autonomously to bract repression (Sessions *et al.*, 2000; Hepworth *et al.*, 2006). One mutual target of repression is *JAG,* whose mutation alleviates bract formation in *lfy* and strong *ap1* mutants (Dinneny *et al.*, 2004; Ohno *et al.*, 2004). BOP1/2 also represses *JAG* in the cryptic bract but *jag* mutations fail to rescue bract formation in *bop1 bop2* mutants supporting the involvement of additional factors (Norberg *et al.*, 2005).

BOP1/2 and LFY are independent regulators of *AP1*

BOP1/2 and LFY are co-expressed in floral anlagen and activate *AP1,* a key marker of commitment to floral fate. Apical expression of *AP1* is strongly delayed in *lfy* mutants and essentially abolished in *lfy ft* and *lfy fd* double mutants indicating that FT/FD and LFY are the major direct regulators of *AP1* expression (Parcy *et al.*, 1998; Abe *et al.*, 2005; Wigge *et al.*, 2005). SPL transcription factors provide additional positive input (Wang *et al.*, 2009; Yamaguchi *et al.*, 2009). In *lfy ft* double mutants, leaves are generated in place of flowers (e.g. Ruiz-Garcia *et al.*, 1997). Similar nodes containing only a bract occurred late in the primary inflorescence of *bop1 bop2 lfy* plants providing evidence that BOP and LFY are independent regulators of floral meristem fate. ChIP analysis showed enrichment of BOP1–GFP at two potential TGA binding sites in the *AP1* promoter close to binding sites for LFY, FD, and SPL3/9 (Parcy *et al.*, 1998; Wigge *et al.*, 2005; Wang *et al.*, 2009; Yamaguchi *et al.*, 2009). Consistent with this, we found weak floral meristem identity defects in *pan-1* mutants similar to *bop1 bop2* and show that BOP2 interacts selectively with PAN over known direct regulators of *AP1* expression. BOP1/2 interact in yeast with several TGAs, including PAN, which functions in the same genetic pathway as BOP1/2 to control sepal number in flowers (Hepworth *et al.*, 2005). Several other TGAs are expressed in inflorescence apices including TGA2, TGA3, and TGA7, which interact with the floral glutaredoxin ROXY1 (Xing *et al.*, 2005; Li *et al.*, 2009) and TGA4, which binds *in vitro* to the *FT* promoter and with CONSTANS, a direct regulator of *FT* (Samach *et al.*, 2000; Song *et al.*, 2008). One of these may preferentially contribute to repression of *AGL24* since no enhancement of floral branching occurs in *pan-1 ap1-1* mutants (data not shown). Thus far, genetic redundancy has hampered our attempts to identify which of these might function with PAN in the floral transition.

BOP activity regulates shoot architecture by controlling determinacy

The transition to flowering involves dramatic changes in shoot architecture, beginning with the formation of primary and secondary inflorescences and ending with the production of flowers. How changes in shoot architecture are coordinated with the decision to flower remains unclear. In leaves, BOP1/2 control the determinacy and hence architecture of petioles through co-repression of class I KNOX homeobox genes and blade-growth regulators such as *JAG* (Ha *et al.*, 2004, 2007; Jun *et al.*, 2010). This study shows that BOP1/2 likewise control determinacy and architecture of floral shoots through activation of *AP1* and repression of *AGL24.* Shoot determinacy further depends on the activation of *AG* at the dome of the floral meristem causing termination of the stem cell population. LFY and WUS are the main activators of *AG* (Busch *et al.*, 1999; Lenhard *et al.*, 2001; Lohmann *et al.*, 2001) but positive input is also provided by PAN (Das *et al.*, 2009; Maier *et al.*, 2009). Similar to *pan-2 lfy* mutants, unfused carpels

occur in *bop1 bop2 lfy-1* flowers (Das *et al.*, 2009; Figure 3h) indicating that BOP1/2 may likewise fine-tune *AG* expression in developing flowers, but this remains to be tested. Unlike flowering-time regulators, BOP1/2 are broadly expressed in initiating lateral organs and their loss-of-function affects leaf, fruit, and floral architecture (e.g. Ha *et al.*, 2003; Hepworth *et al.*, 2005; McKim *et al.*, 2008). Plants over-expressing BOP1 or 2 are reduced in stature with clustered or downward-pointing siliques, similar to mutation of *BP* or *PENNYWISE* (*PNY)* (Norberg *et al.*, 2005; Ha *et al.*, 2007). Interestingly, double mutants of *pny* and the related gene *poundfoolish* (*pnf*) cannot complete floral evocation: flowering signals direct the up-regulation of floral integrators *FT, SOC1,* and *FUL* but *LFY* and *AP1* are not activated nor is an inflorescence generated (Smith *et al.*, 2004; Kanrar *et al.*, 2008) indicating that PNY/PNF co-ordinate shoot architecture and flowering. Our work indicates that BOP1/2 are likely also involved in this co-ordination and illustrates how modulation of determinacy contributes to shoot architectural diversity.

EXPERIMENTAL PROCEDURES

Plant material and growth conditions

Plants were grown on agar plates or in soil at 21°C in long-day (16 h) or continuous (24 h) light. Wild-type was the Columbia-0 (Col-0) ecotype of *Arabidopsis thaliana.* Mutant alleles were obtained from the Arabidopsis Biological Resource Center unless otherwise stated. *bop1-3 bop2-1* and *pan-1* mutants were previously described (Hepworth *et al.*, 2005). The *ap1-1* allele was introgressed into Col-0. The *agl24–3* allele (SALK_095007) was provided by Richard Amasino and *ft-1* (introgressed into Col-0), *fd-2,* and *fd-2 lfy-12* were gifts from Hao Yu and Philip Wigge. Mitsuhiro Aida provided the *bop1 bop2 puchi* mutant and Detlef Weigel provided *spl9* and *spl9 pSPL9:GFP-SPL9* lines. All mutant combinations were constructed by crossing and confirmed by genotyping. For genotyping *lfy-2,* a 168-bp product was amplified by PCR; *Bam*HI cleaves only the wild-type product. For genotyping *ft-1,* a 183-bp product was amplified by PCR; *Sac*I cleaves only the wild-type product. Floral stages were determined according to Smyth *et al.* (1990). Primers are listed in Table S1.

Scanning electron microscopy (SEM)

Samples were prepared for SEM as described (Hepworth *et al.*, 2005). Images were acquired on Hitachi VP-6400 (http://www.hitachi-hitec.com) or Tescan VegaII XMU VPSEM (http://www.tescan-usa.com) microscopes.

BOP2::GUS reporter lines, GUS staining and *in situ* hybridization

pBOP2::GUS containing the *BOP2* 5′-UTR (nt −4015 to +16) fused in-frame with the GUS gene *uidA* was created using the strategy described (Hepworth *et al.*, 2002). Wild-type plants were transformed by floral dipping (Clough and Bent, 1998). Tissues were stained for GUS activity, fixed, embedded, and sectioned as described in Sieburth and Meyerowitz (1997). Sections were adhered to glass slides and dewaxed with tert-butanol prior to imaging. *In situ* hybridization and probe synthesis was performed essentially as described (Hepworth *et al.*, 2005). The *AG* probe was based on Drews *et al.* (1991).

RT-PCR and quantitative RT-PCR (qPCR)

Total RNA was isolated from inflorescence apices using Trizol© reagent (Invitrogen, http://www.invitrogen.com). Total cDNA was synthesized from 1 μg of RNA template using Superscript III reverse transcriptase (Invitrogen) followed by RT-PCR using *Taq* polymerase (Invitrogen). *GAPC* served as a control transcript (Hepworth *et al.*, 2005). Band intensities were quantified with Alphalmager software (Cell Biosciences, http://www.cellbiosciences.com). qPCR was performed in triplicate with SYBR® Green (Sigma, http://www.sigmaaldrich.com) and IQ Supermix (BioRad, http://www.bio-rad.com) using a Rotor-Gene 6000 (Qiagen, http://www.qiagen.com) thermocycler. Conditions were optimized for each primer pair and data quality was verified by melting curve analysis. Relative transcript levels were calculated from threshold cycle values and standard curves. Values were normalized to *GAPC* and then to the wild-type control. Experiments were repeated twice with independently-isolated RNA to ensure reproducibility.

ChIP assay

1 g of 25-day-old *bop1 bop2* BOP1:BOP1–GFP apices were used for ChIP as described in Saleh *et al.* (2008) omitting the nuclear isolation step. Anti-GFP antibodies were used for immunoprecipitation (ab290; Abcam, http://www.abcam.com). Fold-enrichment of DNA sequences was determined by qPCR in triplicate with *ACTIN2/7* as a control according to Liu *et al.* (2008). Primers are listed in Table S2. Assays were repeated twice to ensure reproducibility.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article:

Figure S1. Scanning electron micrographs of WT and mutant inflorescence apices.

Figure S2. Expression of *LFY* in WT and mutant inflorescence apices.

Figure S3. Expression of *SOC1* in WT and mutant inflorescence apices.

Figure S4. Expression of *FUL* in WT and mutant inflorescence apices.

Figure S5. Floral branching in *bop1 bop2 lfy-2* triple mutants is suppressed by loss-of-function *agl24.*

Figure S6. Analysis of BOP interactions with PAN and direct regulators of *AP1* and *AGL24* expression.

Data S1. Materials and methods.

Table S1. List of primers.

Table S2. List of primers for ChIP qPCR.

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