The bZIP transcription factor PERIANTHIA: a multifunctional hub for meristem control

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As sessile organisms, plants are exposed to extreme variations in environmental conditions over the course of their lives. Since plants grow and initiate new organs continuously, they have to modulate the underlying developmental program accordingly to cope with this challenge. At the heart of this extraordinary developmental plasticity are pluripotent stem cells, which are maintained during the entire life-cycle of the plant and that are embedded within dynamic stem cell niches. While the complex regulatory principles of plant stem cell control under artificial constant growth conditions begin to emerge, virtually nothing is known about how this circuit adapts to variations in the environment. In addition to the local feedback system constituted by the homeodomain transcription factor WUSCHEL (WUS) and the CLAVATA signaling cascade in the center of the shoot apical meristem (SAM), the bZIP transcription factor PERIANTHIA (PAN) not only has a broader expression domain in SAM and flowers, but also carries out more diverse functions in meristem maintenance: *pan* mutants show alterations in environmental response, shoot meristem size, floral organ number, and exhibit severe defects in termination of floral stem cells in an environment dependent fashion. Genetic and genomic analyses indicate that *PAN* interacts with a plethora of developmental pathways including light, plant hormone, and meristem control systems, suggesting that *PAN* is as an important regulatory node in the network of plant stem cell control.

Keywords: *Arabidopsis*, meristem regulation, stem cells, auxin, cytokinin, *PERIANTHIA*, type-A *ARR*, *SHOOTMERISTEMLESS*

INTRODUCTION

In contrast to most animals, plants continue to form new organs throughout their lives. This remarkable capacity is dependent on the continuous presence of undifferentiated and self-renewing stem cells over long periods of time. These stem cells reside at the growing points of a plant, the tips of roots and shoots, and are embedded into specialized structures called meristems (Barton, 2010).

Several genes affecting meristem and stem cell function have been identified by mutant screens in *Arabidopsis thaliana*. Most notably *WUSCHEL* (*WUS*) and *SHOOTMERISTEMLESS* (*STM*) are required for the maintenance of the shoot meristem (Barton and Poethig, 1993; Laux et al., 1996; Long et al., 1996; Mayer et al., 1998). Their inactivation causes premature differentiation and the eventual exhaustion of the stem cell pool, leading to the termination of the shoot meristem. Another group of genes, the *CLAVATA* (*CLV*) genes, have an opposite effect on meristems and if defective, shoot meristems overproliferate and expand inappropriately (Clark et al., 1993, 1995; Kayes and Clark, 1998).

With the exception of *CLV2*, all genes mentioned above are expressed in small domains in the shoot apical meristem (SAM). Elegant genetic studies have shown that *WUS* and *CLV3* are connected by a negative feedback loop to control the size of the stem cell pool. *WUS*, which is expressed in the organizing center, induces the expression of *CLV3* in the overlying true stem cells, which in turn signals back to the organizing center to keep *WUS* expression in check (Brand et al., 2000; Schoof et al., 2000). In addition to these local regulatory interactions, meristem function is affected by global hormone signaling pathways, including auxin and cytokinin circuitries. While *STM* mediates cytokinin biosynthesis (Jasinski et al., 2005; Yanai et al., 2005) to allow cell proliferation in the meristem, its expression is repressed by auxin (Furutani et al., 2004), which in turn allows organ initiation on the flanks of the SAM. In contrast, *WUS* does not interfere with cytokinin biosynthesis, but directly regulates A-type *ARABIDOPSIS RESPONSE REGULATORS* (*ARRs*; Leibfried et al., 2005; Busch et al., 2010) that act in the negative feedback regulation of cytokinin response (To et al., 2004). This feedback system of cytokinin signal transduction is also connected to auxin signaling and *ARR7* and *ARR15* are directly repressed by the AUXIN RESPONSE FACTOR5/MONOPTEROS transcription factor (Zhao et al., 2010). A-type *ARRs* execute important meristematic functions (Leibfried et al., 2005; Buechel et al., 2010; Zhao et al., 2010) by so far undiscovered mechanisms (Leibfried et al., 2005; Zhao et al., 2010).

Cells that leave the shoot meristem during the initial, vegetative phase of the life-cycle give rise to leaves and meristems of axillary shoots. After the transition to the reproductive phase, meristems that newly arise at the flanks of the SAM will develop into flowers instead. This is due to the redundant activity of meristem identity

genes such as *LEAFY* (*LFY*) and *APETALA1* (*AP1*). In contrast to the shoot apex, which is indeterminate, flowers are determinate and stem cell activity ceases after a fixed number of organs have been formed. In plants that lack LFY activity, flowers are converted into partially indeterminate shoot-like structures (Weigel et al., 1992).

One set of genes that is directly controlled by the LFY transcription factor includes homeotic genes that specify the fate of the different floral organs (Parcy et al., 1998; Busch et al., 1999). We have previously shown that *LFY* acts together with *WUS*, which also encodes a transcription factor, to contribute to the transcriptional activation of the homeotic gene *AGAMOUS* (*AG*) in the center of young flowers. AG in turn, not only specifies the fate of the floral reproductive organs, but also terminates stem cell maintenance by negative feedback on *WUS* expression (Lohmann et al., 2001). The bZIP transcription factor PERIANTHIA (PAN) is expressed in the SAM, as well as in developing flowers, where it overlaps with *STM*, *WUS,* the *CLV* transcripts, and *AG*, respectively (Chuang et al., 1999). Loss-of *PAN* function leads to an increase in the number of perianth organs, the sepals and petals, while on a gross morphological level the SAM seems unaffected (Running and Meyerowitz, 1996). In flowers, *PAN* genetically interacts with ABC homeotic genes, however these interactions appear mostly additive (Running and Meyerowitz, 1996). PAN protein expression was shown to be independent of the meristematic regulators *CLV1* and *CLV3* as well as of floral meristem identity genes, such as *LFY* or *AP1*, demonstrating that *PAN* also acts in parallel to these factors (Chuang et al., 1999). It has been shown that PAN interacts with the NPR1-like proteins BLADE ON PETIOLE 1 (BOP1) and BOP2 in yeast and that *bop* mutants share some of *pan* mutant features (Hepworth et al., 2005). However,

their expression domains only overlap marginally, suggesting that PAN primarily acts together with other co-factors. It was shown that *PAN* plays important roles in the activation of *AG* (Das et al., 2009; Maier et al., 2009), which are strikingly modified in various day-length settings. While PAN brings about the termination of floral stem cell fate by the direct transcriptional activation of *AG*, its function in the SAM, where it is also strongly and specifically expressed, remains poorly understood.

RESULTS AND DISCUSSION

Since we had noted before that the floral functions of *PAN* are strongly dependent on the environment (Maier et al., 2009), we carefully analyzed vegetative phenotypes of wild-type Columbia and *pan* mutant plants under various growth conditions and found that day-length had a substantial impact on the penetrance of *pan* related defects. In contrast to the reproductive phase, where *pan* mutants showed the most dramatic aberrations under short-day conditions, *pan* plants at the early vegetative stage were largely undistinguishable from wild-type in short days (SD; **Figures 1A,D**). Conversely, *pan* mutants exhibited pleiotropic phenotypes when exposed to long days (LD), including elongated petioles, curled leaves, and a twisted rosette (**Figures 1B,E**). Under continuous light (CL), Col and *pan* phenotypes were less distinct, but *pan* plants continued to show more extreme leaf-curling and rosette twisting. In addition to the morphological traits, we observed that *pan* mutants flowered slightly early and on average formed 1.5 or 2.5 rosette leaves less than wild-type under LD or CL, respectively (**Figure 3A**; $n = 50$). Furthermore, we realized that *pan* mutants are extremely sensitive to variations in diverse environmental conditions, including water and nutrient availability, as well as biotic and abiotic stress (data not shown). Taken
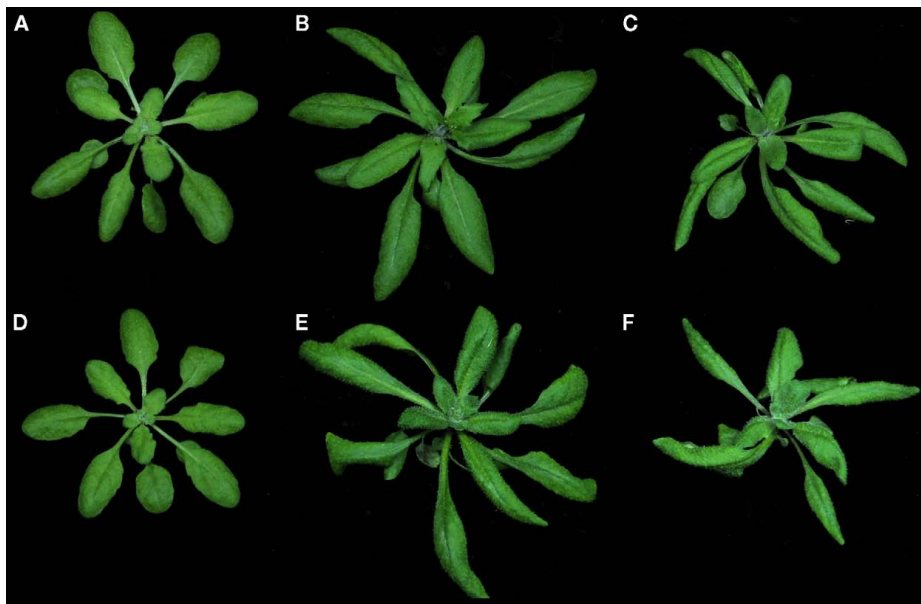

FIGURE 1 | Vegetative phenotypes in response to environmental conditions (A–F). Phenotype of wild-type **(A–C)** and *pan* mutant **(D–F)** plants grown under short-day [SD **(A,D)**], long-day [LD **(B,E)**], and continuous light [CL **(C,F)**] conditions for 21 days. Note leaf-curling, elongated petioles and twisted leaf rosettes under LD and CL conditions.
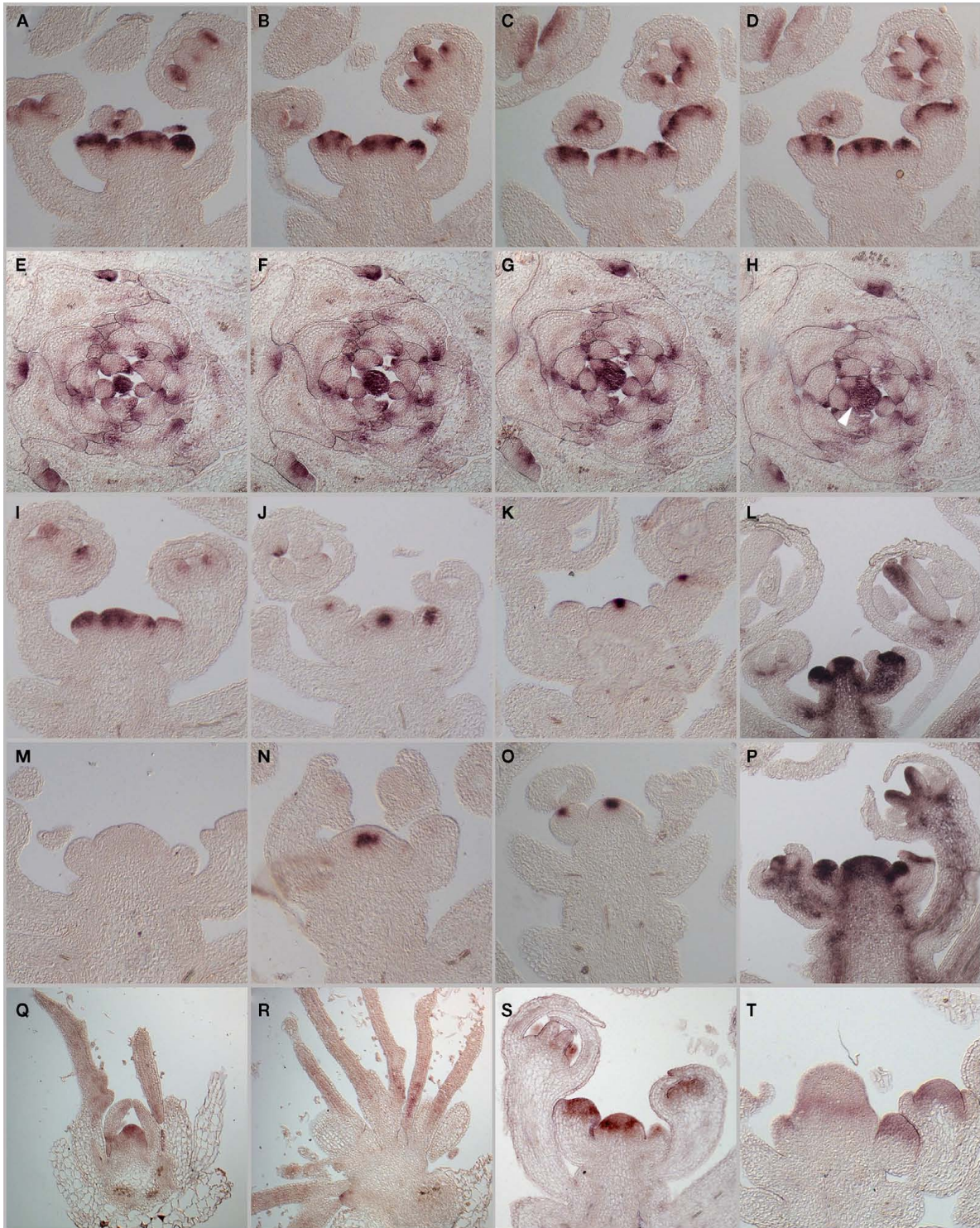
FIGURE 2 | mRNA-expression patterns of *PAN* and SAM regulators *WUS, CLV3, STM*. *In situ* hybridizations were used to analyze *PAN* mRNA-expression patterns. **(A–D)** Serial longitudinal sections of wild-type inflorescence apices after 25 days of growth LD. **(E–L)** Serial cross sections of a vegetative apex grown in 23 days in SD. *PAN* mRNA shows varying expression with a local maximum in a ring domain around the central zone. *PAN* expression is reduced in newly arising organ primordia [P3-P0, see arrowhead in **(H)**]. Expression patterns of *PAN*, *WUS*, *CLV3*, and *STM* in inflorescence apices of wild-type **(I–L)** and *pan* mutant plants **(M–P)**. *PAN* **(I,M)**, *WUS* **(J,N)**, *CLV3* **(K,O)**, and *STM* **(L,P)**. *PAN* mRNA-expression in vegetative tissues of wild-type **(Q)** and *wus* mutants **(R)**. *PAN* expression in wild-type **(S)** and ring-like expression in enlarged floral tissues on *clv3* mutant **(T)**.

FIGURE 3 | Genetic interactions of *PAN* with *CLV3, WUS,* and *STM*.
(A) From left to right the following genotypes are shown: wild-type, *clv3*, *pan clv3*, and *pan*. Top views of inflorescence apices of wild-type **(B)**, *clv3* **(C)**, *pan clv3* **(D)**, and *pan* **(E)** inflorescences. **(F)** Two *wus* mutant plants (left) are shown in comparison to two *pan wus* double mutants (right). Note the inhibition of shoot outgrowth in the double mutant. At later developmental stages a reduced number of shoots grows at a slow rate. **(G)** Two *stm* mutants (left) and two *pan stm* double mutant plants (right). Note the elevated number of shoots and branches, as well as floral buds in the *pan stm* double mutant.

Table 1 | Genes with significantly increased expression in inflorescence apices of *pan* mutants (Rank Products FDR 0.05).

Array element	Fold change	Locus identifier	Annotation
261059_at	2.90	AT1G01250	AP2 domain-containing transcription factor putative
259428_at	2.04	AT1G01560	ATMPK11 (*Arabidopsis thaliana* MAP kinase 11); MAP kinase/kinase
261564_at	1.78	AT1G01720	ATAF1 (Arabidopsis NAC domain-containing protein 2); transcription factor
264606_at	1.90	AT1G04660	Glycine-rich protein
260791_at	1.94	AT1G06250	lipase class 3 family protein
260788_at	1.96	AT1G06260	Cysteine proteinase putative
261077_at	3.17	AT1G07430	Protein phosphatase 2C putative/PP2C putative
261068_at	2.01	AT1G07450	Tropinone reductase putative/tropine dehydrogenase putative
261485_at	1.90	AT1G14360	ATUTR3/UTR3 (UDP-GALACTOSE TRANSPORTER 3); pyrimidine nucleotide sugar transmembrane transporter
261037_at	2.51	AT1G17420	LOX3 (Lipoxygenase 3); iron ion binding/lipoxygenase/metal ion binding/oxidoreductase acting on single donors with incorporation of molecular oxygen incorporation of two atoms of oxygen
260684_at	3.46	AT1G17590	CCAAT-binding transcription factor (CBF-B/NF-YA) family protein
256017_at	1.92	AT1G19180	JAZ1/TIFY10A (JASMONATE-ZIM-DOMAIN PROTEIN 1); protein binding
261221_at	3.37	AT1G19960	Similar to transmembrane receptor [*Arabidopsis thaliana*] (TAIR:AT2G32140.1)
261222_at	2.13	AT1G20120	Family II extracellular lipase putative
261224_at	2.38	AT1G20160	ATSBT5.2; subtilase
264211_at	3.48	AT1G22770	GI (GIGANTEA); binding
265186_at	2.35	AT1G23560	Similar to unknown protein [*Arabidopsis thaliana*] (TAIR:AT1G70480.2); similar to unknown protein [*Arabidopsis thaliana*] (TAIR:AT1G70480.1); similar to unnamed protein product [*Vitis vinifera*] (GB:CAO66084.1); contains InterPro domain Protein of unknown function DUF220 (InterPro:IPR003863)
265002_at	3.00	AT1G24400	LHT2 (LYSINE HISTIDINE TRANSPORTER 2); amino acid transmembrane transporter
261650_at	1.80	AT1G27770	ACA1 (autoinhibited Ca2+ -ATPase 1); calcium-transporting ATPase/calmodulin binding
259607_at	1.96	AT1G27940	PGP13 (P-GLYCOPROTEIN 13); ATPase coupled to transmembrane movement of substances
262736_at	2.12	AT1G28570	GDSL-motif lipase putative
260022_at	3.02	AT1G30020	Similar to unknown protein [*Arabidopsis thaliana*] (TAIR:AT5G46230.1); similar to unnamed protein product [*Vitis vinifera*] (GB:CAO14438.1); contains InterPro domain Protein of unknown function DUF538 (InterPro:IPR007493)
261712_at	2.02	AT1G32780	Alcohol dehydrogenase putative
261191_at	2.11	AT1G32900	Starch synthase putative
256425_at	2.01	AT1G33560	ADR1 (ACTIVATED DISEASE RESISTANCE 1)
261339_at	1.95	AT1G35710	Leucine-rich repeat transmembrane protein kinase putative
245246_at	2.58	AT1G44224	Encodes a ECA1 gametogenesis related family protein
262436_at	1.73	AT1G47610	Transducin family protein/WD-40 repeat family protein
260727_at	2.34	AT1G48100	Glycoside hydrolase family 28 protein/polygalacturonase (pectinase) family protein
256145_at	1.73	AT1G48750	Protease inhibitor/seed storage/lipid transfer protein (LTP) family protein
256181_at	2.04	AT1G51820	Leucine-rich repeat protein kinase putative
262128_at	2.12	AT1G52690	Late embryogenesis abundant protein putative/LEA protein putative
263174_at	1.88	AT1G54040	ESP (EPITHIOSPECIFIER PROTEIN)
263158_at	3.99	AT1G54160	CCAAT-binding transcription factor (CBF-B/NF-YA) family protein
265075_at	2.54	AT1G55450	Embryo-abundant protein-related
264882_at	2.00	AT1G61110	ANAC025 (Arabidopsis NAC domain-containing protein 25); transcription factor
264400_at	1.94	AT1G61800	GPT2 (glucose-6-phosphate/phosphate translocator 2); antiporter/glucose-6-phosphate transmembrane transporter
265122_at	1.93	AT1G62540	Flavin-containing monooxygenase family protein/FMO family protein
265109_s_at	1.78	AT1G63360; AT1G62630	[AT1G63360. disease resistance protein (CC-NBS-LRR class). putative]; [AT1G62630. disease resistance protein (CC-NBS-LRR class). putative]
264636_at	2.57	AT1G65490	Similar to unknown protein [*Arabidopsis thaliana*](TAIR:AT1G65486.1)
259753_at	1.77	AT1G71050	Heavy-metal-associated domain-containing protein/copper chaperone (CCH)-related

(Continued)

Table 1 | Continued

Array element	Fold change	Locus identifier	Annotation
256335_at	2.32	AT1G72110	Similar to unknown protein [*Arabidopsis thaliana*](TAIR:AT2G38995.1); similar to unnamed protein product [*Vitis vinifera*] (GB:CAO48523.1); contains InterPro domain Protein of unknown function UPF0089 (InterPro:IPR004255); contains InterPro domain Protein of unknown function DUF1298 (InterPro:IPR009721)
259852_at	2.20	AT1G72280	AERO1 (ARABIDOPSIS ENDOPLASMIC RETICULUM OXIDOREDUCTINS 1); FAD binding/electron carrier/oxidoreductase acting on sulfur group of donors disulfide as acceptor/protein binding
262378_at	3.32	AT1G72830	HAP2C (Heme activator protein (yeast) homolog 2C); transcription factor
262374_s_at	2.02	AT1G72910; AT1G72930	[AT1G72910. disease resistance protein (TIR-NBS class). putative]; [AT1G72930. TIR (TOLL/INTERLEUKIN-1 RECEPTOR-LIKE); transmembrane receptor]
245734_at	2.26	AT1G73480	Hydrolase alpha/beta fold family protein
260046_at	1.81	AT1G73805	Calmodulin binding
260228_at	3.97	AT1G74540	CYP98A8 (cytochrome P450. family 98. subfamily A. polypeptide 8); oxygen binding
260233_at	2.12	AT1G74550	CYP98A9 (cytochrome P450. family 98. subfamily A. polypeptide 9); oxygen binding
262674_at	4.82	AT1G75910	EXL4 (extracellular lipase 4); acyltransferase/carboxylesterase/lipase
262683_at	2.17	AT1G75920	Family II extracellular lipase 5 (EXL5)
262675_at	5.99	AT1G75930	EXL6 (extracellular lipase 6); acyltransferase/carboxylesterase/lipase
262697_at	2.09	AT1G75940	ATA27 (*Arabidopsis thaliana* anther 27); hydrolase hydrolyzing *O*-glycosyl compounds
261749_at	1.71	AT1G76180	ERD14 (EARLY RESPONSE TO DEHYDRATION 14)
264482_at	2.38	AT1G77210	Sugar transporter putative
259705_at	2.85	AT1G77450	ANAC032 (Arabidopsis NAC domain-containing protein 32); transcription factor
262050_at	4.05	AT1G80130	Binding
267483_at	1.98	AT2G02810	ATUTR1/UTR1 (UDP-GALACTOSE TRANSPORTER 1); UDP-galactose transmembrane transporter/UDP-glucose transmembrane transporter/pyrimidine nucleotide sugar transmembrane transporter
266770_at	1.82	AT2G03090	ATEXPA15 (*ARABIDOPSIS THALIANA* EXPANSIN A15)
263363_at	3.78	AT2G03850	Late embryogenesis abundant domain-containing protein/LEA domain-containing protein
263073_at	1.95	AT2G17500	Auxin efflux carrier family protein
264787_at	2.14	AT2G17840	ERD7 (EARLY-RESPONSIVE TO DEHYDRATION 7)
265983_at	2.58	AT2G18550	ATHB21/HB-2 (homeobox-2); DNA binding/transcription factor
267440_at	2.74	AT2G19070	Transferase family protein
266693_at	2.27	AT2G19800	MIOX2 (MYO-INOSITOL OXYGENASE 2)
265443_at	2.06	AT2G20750	ATEXPB1 (*ARABIDOPSIS THALIANA* EXPANSIN B1)
263739_at	1.74	AT2G21320	Zinc finger (B-box type) family protein
263545_at	2.19	AT2G21560	Similar to unknown protein [*Arabidopsis thaliana*](TAIR:AT4G39190.1); similar to hypothetical protein [*Vitis vinifera*] (GB:CAN77202.1)
265984_at	1.91	AT2G24210	TPS10 (TERPENE SYNTHASE 10); myrcene/(E)-beta-ocimene synthase
265898_at	1.90	AT2G25690	Senescence-associated protein-related
245035_at	1.96	AT2G26400	ARD/ATARD3 (ACIREDUCTONE DIOXYGENASE); acireductone dioxygenase [iron(II)-requiring]/heteroglycan binding/metal ion binding
267595_at	2.30	AT2G32990	ATGH9B8 (*ARABIDOPSIS THALIANA* GLYCOSYL HYDROLASE 9B8); hydrolase hydrolyzing *O*-glycosyl compounds
255795_at	1.74	AT2G33380	RD20 (RESPONSIVE TO DESSICATION 20); calcium ion binding
267429_at	2.81	AT2G34850	MEE25 (maternal effect embryo arrest 25); catalytic
266086_at	1.96	AT2G38060	Transporter-related
257382_at	1.80	AT2G40750	WRKY54 (WRKY DNA-binding protein 54); transcription factor
267083_at	1.73	AT2G41100	TCH3 (TOUCH 3)
266423_at	1.75	AT2G41340	Eukaryotic rpb5 RNA polymerase subunit family protein
266555_at	1.90	AT2G46270	GBF3 (G-BOX BINDING FACTOR 3); transcription factor
266326_at	2.04	AT2G46650	B5 #1 (cytochrome b5 family protein #1); heme binding/transition metal ion binding
266327_at	2.06	AT2G46680	ATHB-7 (*ARABIDOPSIS THALIANA* HOMEOBOX 7); transcription factor
263320_at	1.80	AT2G47180	ATGOLS1 (*ARABIDOPSIS THALIANA* GALACTINOL SYNTHASE 1); transferase transferring hexosyl groups
259352_at	9.07	AT3G05170	Phosphoglycerate/bisphosphoglycerate mutase family protein
258894_at	1.98	AT3G05650	Disease resistance family protein

(Continued)

Table 1 | Continued

Array element	Fold change	Locus identifier	Annotation
258890_at	4.09	AT3G05690	ATHAP2B/HAP2B/UNE8 (HEME ACTIVATOR PROTEIN (YEAST) HOMOLOG 2B); transcription factor
259286_at	3.44	AT3G11480	BSMT1; S-adenosylmethionine-dependent methyltransferase
259937_s_at	1.82	AT3G13080; AT1G71330	[AT3G13080. ATMRP3 (*Arabidopsis thaliana* multidrug resistance-associated protein 3)]; [AT1G71330. ATNAP5 (*Arabidopsis thaliana* non-intrinsic ABC protein 5)]
258370_at	1.93	AT3G14395	Unknown protein
258399_at	1.74	AT3G15540	IAA19 (indoleacetic acid-induced protein 19); transcription factor
257876_at	1.78	AT3G17130	Invertase/pectin methylesterase inhibitor family protein
258158_at	2.80	AT3G17790	ATACP5 (acid phosphatase 5); acid phosphatase/protein serine/threonine phosphatase
257262_at	4.30	AT3G21890	Zinc finger (B-box type) family protein
258321_at	5.37	AT3G22840	ELIP1 (EARLY LIGHT-INDUCABLE PROTEIN); chlorophyll binding
257925_at	2.98	AT3G23170	Similar to unknown protein [*Arabidopsis thaliana*] (TAIR:AT4G14450.1)
257900_at	1.91	AT3G28420	Contains domain PROKAR_LIPOPROTEIN (PS51257)
258003_at	1.81	AT3G29030	ATEXPA5 (*ARABIDOPSIS THALIANA* EXPANSIN A5)
255723_at	1.83	AT3G29575	Similar to TMAC2 (TWO OR MORE ABRES-CONTAINING GENE 2) [*Arabidopsis thaliana*] (TAIR:AT3G02140.1); similar to unnamed protein product [*Vitis vinifera*] (GB:CAO49169.1); contains InterPro domain Protein of unknown function DUF1675 (InterPro:IPR012463)
256940_at	3.17	AT3G30720	Unknown protein
252648_at	1.73	AT3G44630	Disease resistance protein RPP1-WsB-like (TIR-NBS-LRR class) putative
252414_at	2.34	AT3G47420	Glycerol-3-phosphate transporter putative/glycerol-3-phosphate permease putative
252063_at	1.99	AT3G51590	LTP12 (LIPID TRANSFER PROTEIN 12); lipid binding
246302_at	2.39	AT3G51860	CAX3 (cation exchanger 3); cation:cation antiporter
252035_at	1.74	AT3G52160	Beta-ketoacyl-CoA synthase family protein
251928_at	2.78	AT3G53980	Protease inhibitor/seed storage/lipid transfer protein (LTP) family protein
251497_at	1.89	AT3G59060	PIL6 (PHYTOCHROME-INTERACTING FACTOR 5); DNA binding/transcription factor
251400_at	1.93	AT3G60420	Similar to unknown protein [*Arabidopsis thaliana*] (TAIR:AT3G60450.1); similar to unnamed protein product [*Vitis vinifera*] (GB:CAO70569.1); contains InterPro domain Phosphoglycerate mutase (InterPro:IPR013078); contains InterPro domain PRIB5 (InterPro:IPR012398)
251309_at	1.98	AT3G61220	Short-chain dehydrogenase/reductase (SDR) family protein
255575_at	2.00	AT4G01430	Nodulin MtN21 family protein
255302_at	2.34	AT4G04830	Methionine sulfoxide reductase domain-containing protein/SeIR domain-containing protein
254806_at	1.98	AT4G12430; AT4G12432	[AT4G12430. trehalose-6-phosphate phosphatase. putative]; [AT4G12432. CPuORF26 (Conserved peptide upstream open reading frame 26)]
254687_at	1.88	AT4G13770	CYP83A1 (CYTOCHROME P450 83A1); oxygen binding
245329_at	2.27	AT4G14365	Zinc finger (C3HC4-type RING finger) family protein/ankyrin repeat family protein
245306_at	2.15	AT4G14690	ELIP2 (EARLY LIGHT-INDUCIBLE PROTEIN 2); chlorophyll binding
245322_at	2.18	AT4G14815	Protease inhibitor/seed storage/lipid transfer protein (LTP) family protein
245275_at	2.45	AT4G15210	ATBETA-AMY (BETA-AMYLASE); beta-amylase
245465_at	1.86	AT4G16590	ATCSLA01 (Cellulose synthase-like A1); glucosyltransferase/transferase transferring glycosyl groups
245346_at	1.75	AT4G17090	CT-BMY (BETA-AMYLASE 3. BETA-AMYLASE 8); beta-amylase
245389_at	1.87	AT4G17480	Palmitoyl protein thioesterase family protein
254574_at	1.77	AT4G19430	Unknown protein
254321_at	2.62	AT4G22590; AT4G22592	[AT4G22590. trehalose-6-phosphate phosphatase. putative]; [AT4G22592. CPuORF27 (Conserved peptide upstream open reading frame 27)]
254256_at	1.72	AT4G23180	CRK10 (CYSTEINE-RICH RLK10); kinase
254231_at	1.93	AT4G23810	WRKY53 (WRKY DNA-binding protein 53); DNA binding/protein binding/transcription activator/transcription factor
253721_at	2.47	AT4G29250	Transferase family protein
253689_at	1.74	AT4G29770	Similar to unknown protein [*Arabidopsis thaliana*] (TAIR:AT4G29760.1)
253182_at	2.83	AT4G35190	Similar to unknown protein [*Arabidopsis thaliana*] (TAIR:AT2G37210.1); similar to unnamed protein product [*Vitis vinifera*] (GB:CAO47480.1); contains InterPro domain Conserved hypothetical protein CHP00730 (InterPro:IPR005269)

(Continued)

Table 1 | Continued

Array element	Fold change	Locus identifier	Annotation
252870_at	1.97	AT4G39940	AKN2 (APS-KINASE 2); ATP binding/kinase/transferase transferring phosphorus-containing groups
251065_at	1.78	AT5G01870	Lipid transfer protein putative
250688_at	6.33	AT5G06510	CCAAT-binding transcription factor (CBF-B/NF-YA) family protein
250665_at	2.38	AT5G06980	Similar to unknown protein [*Arabidopsis thaliana*] (TAIR:AT3G12320.1)
250637_at	1.76	AT5G07530	GRP17 (Glycine-rich protein 17)
250610_at	3.03	AT5G07550	GRP19 (Glycine-rich protein 19)
250639_at	2.73	AT5G07560	GRP20 (Glycine-rich protein 20); nutrient reservoir
250435_at	2.18	AT5G10380	Zinc finger (C3HC4-type RING finger) family protein
250304_at	2.14	AT5G12110	Elongation factor 1B alpha-subunit 1 (eEF1Balpha1)
246418_at	2.02	AT5G16960	NADP-dependent oxidoreductase putative
250083_at	1.79	AT5G17220	ATGSTF12 (GLUTATHIONE *S*-TRANSFERASE 26); glutathione transferase
246437_at	1.95	AT5G17540	Transferase family protein
249918_at	2.34	AT5G19240	Identical to uncharacterized GPI-anchored protein At5g19240 precursor [*Arabidopsis thaliana*] (GB:Q84VZ5;GB:Q8H7A4); similar to unknown protein [*Arabidopsis thaliana*] (TAIR:AT5G19230.1); similar to unknown [*Populus trichocarpa*] (GB:ABK94712.1)
246071_at	1.77	AT5G20150	SPX (SYG1/Pho81/XPR1) domain-containing protein
246099_at	3.70	AT5G20230	ATBCB (ARABIDOPSIS BLUE-COPPER-BINDING PROTEIN); copper ion binding
249941_at	1.81	AT5G22270	Similar to unknown protein [*Arabidopsis thaliana*] (TAIR:AT5G06270.1); similar to unnamed protein product [*Vitis vinifera*] (GB:CAO15841.1); similar to hypothetical protein [*Vitis vinifera*] (GB:CAN79170.1)
249754_at	2.05	AT5G24530	Oxidoreductase 2OG-Fe(II) oxygenase family protein
246967_at	1.95	AT5G24860	FPF1 (FLOWERING PROMOTING FACTOR 1)
249112_at	2.35	AT5G43780	APS4
248716_at	2.05	AT5G48210	Similar to unknown protein [*Arabidopsis thaliana*] (TAIR:AT3G42565.1)
248638_at	2.85	AT5G49070	Beta-ketoacyl-CoA synthase family protein
248375_at	1.85	AT5G51710	KEA5 (K+ efflux antiporter 5); potassium:hydrogen antiporter
248327_at	2.44	AT5G52750	Heavy-metal-associated domain-containing protein
248160_at	2.74	AT5G54470	Zinc finger (B-box type) family protein
248104_at	1.81	AT5G55250	IAMT1 (IAA CARBOXYLMETHYLTRANSFERASE 1); *S*-adenosylmethionine-dependent methyltransferase
248011_at	2.25	AT5G56300	GAMT2; *S*-adenosylmethionine-dependent methyltransferase/gibberellin carboxyl-*O*-methyltransferase
247718_at	3.69	AT5G59310	LTP4 (LIPID TRANSFER PROTEIN 4); lipid binding
247717_at	3.09	AT5G59320	LTP3 (LIPID TRANSFER PROTEIN 3); lipid binding
247657_at	1.96	AT5G59845	Gibberellin-regulated family protein
247639_s_at	1.92	AT5G60500; AT5G60510	(AT5G60500. undecaprenyl pyrophosphate synthetase family protein/UPP synthetase family protein); (AT5G60510. undecaprenyl pyrophosphate synthetase family protein/UPP synthetase family protein)
247426_at	2.12	AT5G62570	Calmodulin-binding protein
247447_at	1.98	AT5G62730	Proton-dependent oligopeptide transport (POT) family protein
247323_at	4.02	AT5G64170	Dentin sialophosphoprotein-related
247224_at	2.00	AT5G65080	AGL68/MAF5 (MADS AFFECTING FLOWERING 5)
245537_at	3.57	No_match	No_match

together, these phenotypes indicated that *PAN* might act to stabilize the developmental program of the shoot apex and thus buffers the impact of diverse environmental inputs.

Since the activity of the SAM is mainly determined by the *WUS–CLV* feedback system, which acts on the stem cell population, as well as the repression of differentiation throughout the meristem provided by STM, we investigated their regulatory and genetic interaction with *PAN*. Using *in situ* hybridization on serial histological sections, we first analyzed in detail the mRNA-expression patterns of *PAN* in the inflorescence meristem and found that, consistent with a buffering function, *PAN* mRNA is most highly expressed in a ring-shaped domain surrounding the stem cells (**Figures 2A–D**). We detected weaker signals throughout the center of the SAM, suggesting that *PAN* might execute slightly different functions depending on expression levels. Similar to the situation identified for WUS, which was shown to bind to distinct cis-regulatory motifs with different affinity (Busch et al., 2010), these functions could be mediated by distinct sets of PAN downstream targets. However, *in situ* detection of PAN protein on sections of the SAM did not show the ring domain, but rather suggested that PAN is found throughout the meristem (Chuang et al., 1999). Unfortunately, we were unable to resolve whether these

Table 2 | Genes with significantly reduced expression in inflorescence apices of *pan* mutants (Rank Products FDR 0.05).

Array element	Fold change	Locus identifier	Annotation
259445_at	0.54	AT1G02400	ATGA2OX6/DTA1 (GIBBERELLIN 2-OXIDASE 6); gibberellin 2-beta-dioxygenase
261410_at	0.45	AT1G07610	MT1C (metallothionein 1C)
264521_at	0.43	AT1G10020	Similar to unknown protein [*Arabidopsis thaliana*] (TAIR:AT4G29310.1); similar to unnamed protein product [*Vitis vinifera*] (GB:CAO61535.1); contains InterPro domain Protein of unknown function DUF1005 (InterPro:IPR010410)
263236_at	0.53	AT1G10470	ARR4 (RESPONSE REGULATOR 4); transcription regulator/two-component response regulator
256098_at	0.50	AT1G13700	Glucosamine/galactosamine-6-phosphate isomerase family protein
259466_at	0.44	AT1G19050	ARR7 (RESPONSE REGULATOR 7); transcription regulator/two-component response regulator
260662_at	0.49	AT1G19540	Isoflavone reductase. putative
260856_at	0.46	AT1G21910	AP2 domain-containing transcription factor family protein
261926_at	0.57	AT1G22530	PATL2; transporter
264774_at	0.42	AT1G22890	Unknown protein
264901_at	0.51	AT1G23090	AST91 (SULFATE TRANSPORTER 91); sulfate transmembrane transporter
264857_at	0.49	AT1G24170	GATL8/LGT9 (Galacturonosyltransferase-like 8); polygalacturonate 4-alpha-galacturonosyltransferase/transferase. transferring glycosyl groups/transferase. transferring hexosyl groups
255742_at	0.42	AT1G25560	AP2 domain-containing transcription factor. putative
265158_at	0.58	AT1G31040	Zinc ion binding
261193_at	0.51	AT1G32920	Similar to unknown protein [*Arabidopsis thaliana*] (TAIR:AT1G32928.1)
262010_at	0.51	AT1G35612	Transposable element gene
260754_at	0.56	AT1G49000	Similar to unknown protein [*Arabidopsis thaliana*] (TAIR:AT3G18560.1); similar to hypothetical protein [*Vitis vinifera*] (GB:CAN78728.1); similar to unnamed protein product [*Vitis vinifera*] (GB:CAO68009.1)
262399_at	0.34	AT1G49500	Similar to unknown protein [*Arabidopsis thaliana*] (TAIR:AT3G19030.1)
262154_at	0.47	AT1G52700	Phospholipase/carboxylesterase family protein
262226_at	0.57	AT1G53885; AT1G53903	[AT1G53885. senescence-associated protein-related]; [AT1G53903. similar to senescence-associated protein-related [*Arabidopsis thaliana*] (TAIR:AT1G53885.1); similar to Protein of unknown function DUF581 [*Medicago truncatula*] (GB:ABO84791.1); contains InterPro domain Protein of unknown function DUF581 (InterPro:IPR007650)]
263005_at	0.44	AT1G54540	Similar to harpin-induced protein-related/HIN1-related/harpin-responsive protein-related [*Arabidopsis thaliana*] (TAIR:AT1G65690.1); similar to unnamed protein product [*Vitis vinifera*] (GB:CAO62044.1); contains InterPro domain Harpin-induced 1 (InterPro:IPR010847)
256021_at	0.54	AT1G58270	ZW9
260431_at	0.53	AT1G68190	Zinc finger (B-box type) family protein
262232_at	0.53	AT1G68600	Similar to unknown protein [*Arabidopsis thaliana*] (TAIR:AT1G25480.1); similar to unknown protein [*Arabidopsis thaliana*] (TAIR:AT2G17470.1); similar to unnamed protein product [*Vitis vinifera*] (GB:CAO42118.1); contains InterPro domain Protein of unknown function UPF0005 (InterPro:IPR006214)
262278_at	0.03	AT1G68640	PAN (PERIANTHIA); DNA binding/transcription factor
264704_at	0.49	AT1G70090	GATL9/LGT8 (Galacturonosyltransferase-like 9); polygalacturonate 4-alpha-galacturonosyltransferase/transferase. transferring glycosyl groups/transferase. transferring hexosyl groups
259751_at	0.25	AT1G71030	ATMYBL2 (Arabidopsis myb-like 2); DNA binding/transcription factor
260427_at	0.47	AT1G72430	Auxin-responsive protein-related
245777_at	0.50	AT1G73540	ATNUDT21 (*Arabidopsis thaliana* Nudix hydrolase homolog 21); hydrolase
262212_at	0.32	AT1G74890	ARR15 (RESPONSE REGULATOR 15); transcription regulator
261109_at	0.36	AT1G75450	CKX5 (CYTOKININ OXIDASE 5); cytokinin dehydrogenase
259979_at	0.56	AT1G76600	Similar to unknown protein [*Arabidopsis thaliana*] (TAIR:AT1G21010.1); similar to hypothetical protein [*Vitis vinifera*] (GB:CAN67638.1)
264299_s_at	0.41	AT1G78850; AT1G78860	[AT1G78850. curculin-like (mannose-binding) lectin family protein];[AT1G78860. curculin-like (mannose-binding) lectin family protein]
262049_at	0.58	AT1G80180	Similar to unknown protein [*Arabidopsis thaliana*] (TAIR:AT1G15400.2); similar to unknown protein [*Arabidopsis thaliana*] (TAIR:AT1G15400.3); similar to unknown protein [*Arabidopsis thaliana*] (TAIR:AT1G15400.1); similar to hypothetical protein MtrDRAFT_AC148340g12v2 [*Medicago truncatula*] (GB:ABD28396.1)

(Continued)

Table 2 | Continued

Array element	Fold change	Locus identifier	Annotation
260287_at	0.39	AT1G80440	Kelch repeat-containing F-box family protein
263046_at	0.33	AT2G05380	GRP3S (GLYCINE-RICH PROTEIN 3 SHORT ISOFORM)
265511_at	0.16	AT2G05540	Glycine-rich protein
265475_at	0.51	AT2G15620	NIR1 (NITRITE REDUCTASE); ferredoxin-nitrate reductase
265481_at	0.54	AT2G15960	Unknown protein
265821_at	0.54	AT2G17950	WUS (WUSCHEL); DNA binding/transcription factor
267265_at	0.51	AT2G22980	SCPL13; serine carboxypeptidase
245084_at	0.39	AT2G23290	AtMYB70 (myb domain protein 70); DNA binding/transcription factor
266259_at	0.50	AT2G27830	Similar to pentatricopeptide (PPR) repeat-containing protein [*Arabidopsis thaliana*] (TAIR:AT4G22760.1); similar to hypothetical protein [*Catharanthus roseus*] (GB:CAC09928.1)
267497_at	0.40	AT2G30540	Glutaredoxin family protein
267209_at	0.34	AT2G30930	Similar to unknown protein [*Arabidopsis thaliana*] (TAIR:AT1G06540.1)
267461_at	0.23	AT2G33830	Dormancy/auxin associated family protein
267459_at	0.50	AT2G33850	Similar to unknown protein [*Arabidopsis thaliana*] (TAIR:AT1G28400.1); similar to unknown [*Brassica napus*] (GB:AAC06020.1)
267093_at	0.50	AT2G38170	CAX1 (CATION EXCHANGER 1); calcium ion transmembrane transporter/calcium:hydrogen antiporter
267034_at	0.57	AT2G38310	Similar to unknown protein [*Arabidopsis thaliana*] (TAIR:AT5G05440.1); similar to unnamed protein product [*Vitis vinifera*] (GB:CAO48777.1); contains InterPro domain Bet v I allergen; (InterPro:IPR000916); contains InterPro domain *Streptomyces* cyclase/dehydrase (InterPro:IPR005031)
267013_at	0.56	AT2G39180	Protein kinase family protein
267623_at	0.55	AT2G39650	Similar to unknown protein [*Arabidopsis thaliana*] (TAIR:AT4G14620.1); similar to unnamed protein product [*Vitis vinifera*] (GB:CAO69213.1); contains InterPro domain Protein of unknown function DUF506. plant (InterPro:IPR006502)
267357_at	0.57	AT2G40000	Similar to unknown protein [*Arabidopsis thaliana*] (TAIR:AT3G55840.1); similar to unnamed protein product [*Vitis vinifera*] (GB:CAO41329.1); contains InterPro domain Hs1pro-1. C-terminal (InterPro:IPR009743); contains InterPro domain Hs1pro-1. N-terminal (InterPro:IPR009869)
266078_at	0.51	AT2G40670	ARR16 (response regulator 16); transcription regulator/two-component response regulator
257348_at	0.55	AT2G42140	VQ motif-containing protein
265265_at	0.56	AT2G42900	Similar to unnamed protein product [*Vitis vinifera*] (GB:CAO70018.1); contains InterPro domain Plant Basic Secretory Protein (InterPro:IPR007541)
265263_at	0.56	AT2G42940	DNA-binding family protein
266814_at	0.38	AT2G44910	Homeobox-leucine zipper protein 4 (HB-4)/HD-ZIP protein 4
258704_at	0.47	AT3G09780	Protein kinase family protein
256283_at	0.52	AT3G12540	Similar to unknown protein [*Arabidopsis thaliana*] (TAIR:AT2G39690.1); similar to At3g12540-like protein [*Boechera stricta*] (GB:ABB89771.1); contains InterPro domain Protein of unknown function DUF547 (InterPro:IPR006869)
258252_at	0.52	AT3G15720	Glycoside hydrolase family 28 protein/polygalacturonase (pectinase) family protein
257076_at	0.40	AT3G19680	Similar to unknown protein [*Arabidopsis thaliana*] (TAIR:AT1G50040.1); similar to unnamed protein product [*Vitis vinifera*] (GB:CAO61535.1); contains InterPro domain Protein of unknown function DUF1005 (InterPro:IPR010410)
257939_at	0.37	AT3G19930	STP4 (SUGAR TRANSPORTER 4); carbohydrate transmembrane transporter/sugar:hydrogen ion symporter
257985_at	0.57	AT3G20810	Transcription factor jumonji (jmjC) domain-containing protein
257254_at	0.53	AT3G21950	*S*-adenosyl-l-methionine:carboxyl methyltransferase family protein
256766_at	0.45	AT3G22231	PCC1 (PATHOGEN AND CIRCADIAN CONTROLLED 1)
256617_at	0.55	AT3G22240	Unknown protein
258447_at	0.54	AT3G22450	Structural constituent of ribosome
258125_s_at	0.57	AT3G23530; AT3G23510	[AT3G23530. cyclopropane fatty acid synthase. putative/CPA-FA synthase. putative];[AT3G23510. cyclopropane fatty acid synthase. putative/CPA-FA synthase. putative]
252679_at	0.55	AT3G44260	CCR4-NOT transcription complex protein. putative
252374_at	0.55	AT3G48100	ARR5 (ARABIDOPSIS RESPONSE REGULATOR 5); transcription regulator/two-component response regulator

(Continued)

Table 2 | Continued

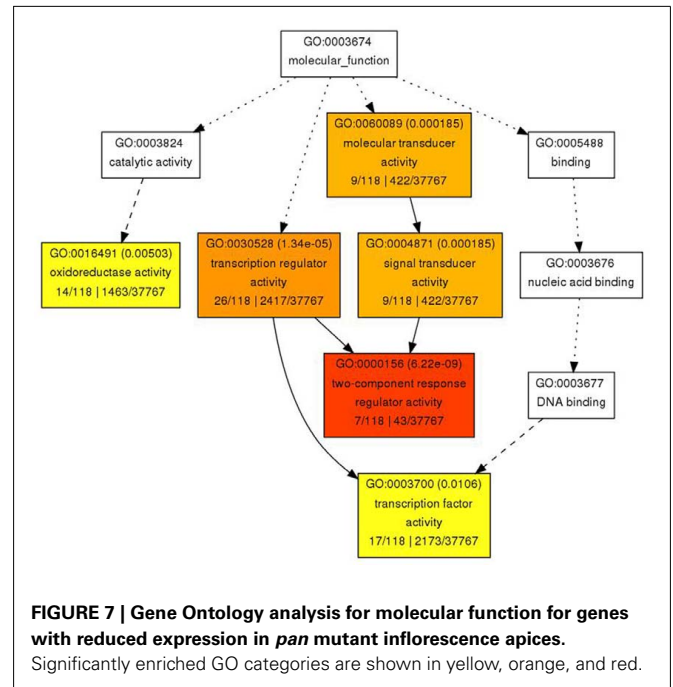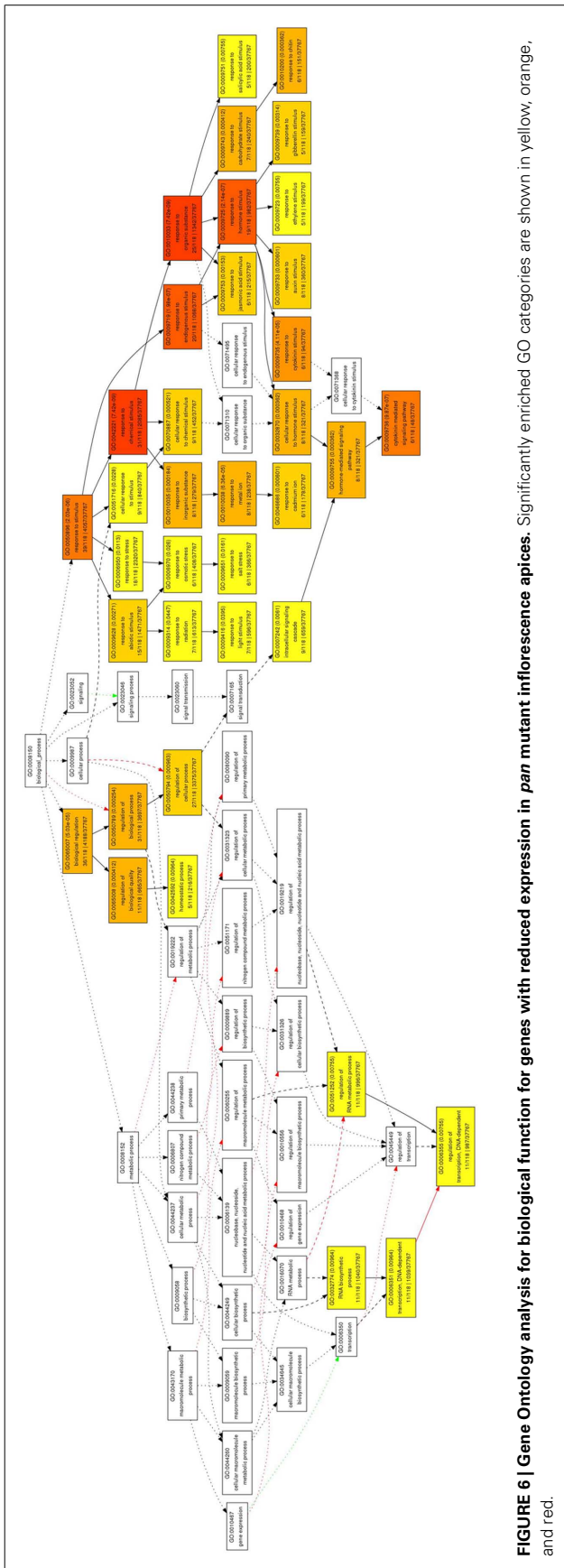| Array element	Fold change	Locus identifier	Annotation
252193_at	0.48	AT3G50060	MYB77; DNA binding/transcription factor
251992_at	0.54	AT3G53350	Myosin heavy chain-related
251791_at	0.25	AT3G55500	ATEXPA16 (*ARABIDOPSIS THALIANA* EXPANSIN A16)
251745_at	0.57	AT3G55980	Zinc finger (CCCH-type) family protein
251723_at	0.53	AT3G56230	Speckle-type POZ protein-related
251704_at	0.56	AT3G56360	Similar to unknown protein [*Arabidopsis thaliana*] (TAIR:AT5G05250.1); similar to unnamed protein product [*Vitis vinifera*] (GB:CAO41488.1)
251575_at	0.55	AT3G58120	bZIP transcription factor family protein
255255_at	0.56	AT4G05070	Unknown protein
255064_at	0.32	AT4G08950	Phosphate-responsive protein. putative (EXO)
254926_at	0.53	AT4G11280	ACS6 (1-AMINOCYCLOPROPANE-1-CARBOXYLIC ACID (ACC) SYNTHASE 6)
254751_at	0.47	AT4G13150	Unknown protein
248692_s_at	0.53	AT4G15070; AT5G48320	[AT4G15070. DC1 domain-containing protein];[AT5G48320. DC1 domain-containing protein]
245441_at	0.55	AT4G16700	PSD1 (PHOSPHATIDYLSERINE DECARBOXYLASE 1); phosphatidylserine decarboxylase
254665_at	0.52	AT4G18340	Glycosyl hydrolase family 17 protein
254098_at	0.55	AT4G25100	FSD1 (FE SUPEROXIDE DISMUTASE 1); iron superoxide dismutase
254057_at	0.53	AT4G25170	Similar to unknown protein [*Arabidopsis thaliana*] (TAIR:AT5G61490.1); similar to unnamed protein product [*Vitis vinifera*] (GB:CAO60860.1); contains InterPro domain uncharacterized conserved protein UCP012943 (InterPro:IPR016606)
253915_at	0.17	AT4G27280	Calcium-binding EF hand family protein
253666_at	0.35	AT4G30270	MERI5B (MERISTEM-5); hydrolase. acting on glycosyl bonds/xyloglucan:xyloglucosyl transferase
253421_at	0.56	AT4G32340	Binding
253439_at	0.50	AT4G32540	YUC (YUCCA); FAD binding/NADP binding/flavin-containing monooxygenase/monooxygenase/oxidoreductase
253317_at	0.53	AT4G33960	Similar to unknown protein [*Arabidopsis thaliana*] (TAIR:AT2G15830.1)
253161_at	0.50	AT4G35770	SEN1 (DARK INDUCIBLE 1)
253125_at	0.54	AT4G36040	DNAJ heat shock N-terminal domain-containing protein (J11)
246200_at	0.44	AT4G37240	Similar to unknown protein [*Arabidopsis thaliana*] (TAIR:AT2G23690.1); similar to unnamed protein product [*Vitis vinifera*] (GB:CAO45438.1); similar to hypothetical protein [*Vitis vinifera*] (GB:CAN61825.1)
246253_at	0.28	AT4G37260	AtMYB73/MYB73 (myb domain protein 73); DNA binding/transcription factor
253061_at	0.47	AT4G37610	BT5 (BTB and TAZ domain protein 5); protein binding/transcription regulator
252997_at	0.48	AT4G38400	ATEXLA2 (*ARABIDOPSIS THALIANA* EXPANSIN-LIKE A2)
251013_at	0.52	AT5G02540	Short-chain dehydrogenase/reductase (SDR) family protein
250777_at	0.42	AT5G05440	Similar to unknown protein [*Arabidopsis thaliana*] (TAIR:AT2G38310.1); similar to unnamed protein product [*Vitis vinifera*] (GB:CAO48777.1); contains InterPro domain Bet v I allergen; (InterPro:IPR000916); contains InterPro domain *Streptomyces* cyclase/dehydrase (InterPro:IPR005031)
250389_at	0.51	AT5G11320	YUC4 (YUCCA4); monooxygenase
250344_at	0.29	AT5G11930	Glutaredoxin family protein
246520_at	0.06	AT5G15790	Zinc finger (C3HC4-type RING finger) family protein
246531_at	0.40	AT5G15800	SEP1 (SEPALLATA1); DNA binding/transcription factor
249996_at	0.36	AT5G18600	Glutaredoxin family protein
246700_at	0.54	AT5G28030	Cysteine synthase. putative/*O*-acetylserine (thiol)-lyase. putative/*O*-acetylserine sulfhydrylase. putative
249645_at	0.39	AT5G36910	THI2.2 (THIONIN 2.2); toxin receptor binding
256356_s_at	0.58	AT5G43620; AT1G66500	[AT5G43620. S-locus protein-related];[AT1G66500. zinc finger (C2H2-type) family protein]
248865_at	0.57	AT5G46790	Similar to unknown protein [*Arabidopsis thaliana*] (TAIR:AT4G17870.1); similar to *Streptomyces* cyclase/dehydrase family protein [*Brassica oleracea*] (GB:ABD65631.1); contains InterPro domain *Streptomyces* cyclase/dehydrase (InterPro:IPR005031)
248163_at	0.49	AT5G54510	DFL1/GH3.6 (DWARF IN LIGHT 1); indole-3-acetic acid amido synthetase
248020_at	0.46	AT5G56490	FAD-binding domain-containing protein
247956_at	0.47	AT5G56970	CKX3 (CYTOKININ OXIDASE 3); cytokinin dehydrogenase

Table 2 | Continued

Array element	Fold change	Locus identifier	Annotation
247925_at	0.13	AT5G57560	TCH4 (TOUCH 4); hydrolase. acting on glycosyl bonds/xyloglucan:xyloglucosyl transferase
247649_at	0.48	AT5G60030	Similar to unknown protein [*Arabidopsis thaliana*] (TAIR:AT1G75335.1); similar to hypothetical protein [*Vitis vinifera*] (GB:CAN66187.1)
247668_at	0.53	AT5G60100	APRR3 (PSEUDO-RESPONSE REGULATOR 3); transcription regulator
247540_at	0.17	AT5G61590	AP2 domain-containing transcription factor family protein
247543_at	0.21	AT5G61600	Ethylene-responsive element-binding family protein
247406_at	0.58	AT5G62920	ARR6 (RESPONSE REGULATOR 6); transcription regulator/two-component response regulator
246987_at	0.21	AT5G67300	ATMYB44/ATMYBR1/MYBR1 (MYB DOMAIN PROTEIN 44); DNA binding/transcription factor
244994_at	0.53	ATCG01010	Chloroplast encoded NADH dehydrogenase unit.

differences were of technical nature, or reflected relevant biology. Hybridizations on cross sections demonstrated that *PAN* mRNA is strongly reduced even in early organ primordia (**Figures 2E–H**). We next investigated how the SAM regulatory system is affected by the loss-of *PAN* function. First, we noticed that the SAM was significantly increased in size (**Figures 2I,M**) and that the *WUS* expression domain is substantially wider compared to the wild-type situation (**Figures 2J,N**). Interestingly, the stem cell domain marked by *CLV3* expression remained largely unaffected despite the expanded stem cell niche (**Figures 2K,O**), suggesting that the regulatory interaction between *WUS* and *CLV3* is partially uncoupled in *pan* mutants. In line with the enlarged meristem, we found expanded *STM* expression in *pan* apices (**Figures 2L,P**) and the absence of *STM* transcripts from emerging organ primordia was less pronounced in *pan* when compared to wild-type. Taken together, these results demonstrate that *PAN* function is required for normal SAM development, which might be mediated by its effects on the expression of the canonical meristem regulators. To address how *PAN* is integrated into the regulatory network of the SAM, we analyzed its expression in *wus* and *clv3* mutants, which represent the extremes in meristem dis-regulation. Since *wus* mutants rarely form inflorescence meristems, we focused our analysis on the seedling stage and found accumulation of *PAN* mRNA mostly in the center of the SAM in wild-type. In addition, we detected weaker signals on the periphery of the meristem and at the adaxial sides of young leaves (**Figure 2Q**). Consistent with the loss-of a fully developed SAM in *wus*, we were unable to detect *PAN* transcripts in central tissue of this mutant, however, strong expression was found in leaf-primordia and young leaves (**Figure 2R**). While Chuang et al. (1999) had reported that *PAN* protein expression is mostly independent of *CLV3*, we observed that *PAN* transcripts accumulated throughout the SAM, with a ring of strong expression toward the base with weaker signals toward the top of the expanded *clv3* meristem (**Figures 2S,T**).

Having shown that PAN is more tightly connected to the regulatory system of the SAM than previously anticipated, we extended our analysis to test the functional interaction of *PAN* with *CLV3*, *WUS*, and *STM* using genetics. Plants that carry mutations in *CLV3* are characterized by an enlarged SAM, an increase in the number of lateral organs developing from the SAM and over-proliferation of floral meristems. When we combined the *clv3–7* loss-of-function allele with *pan*, we observed a substantial
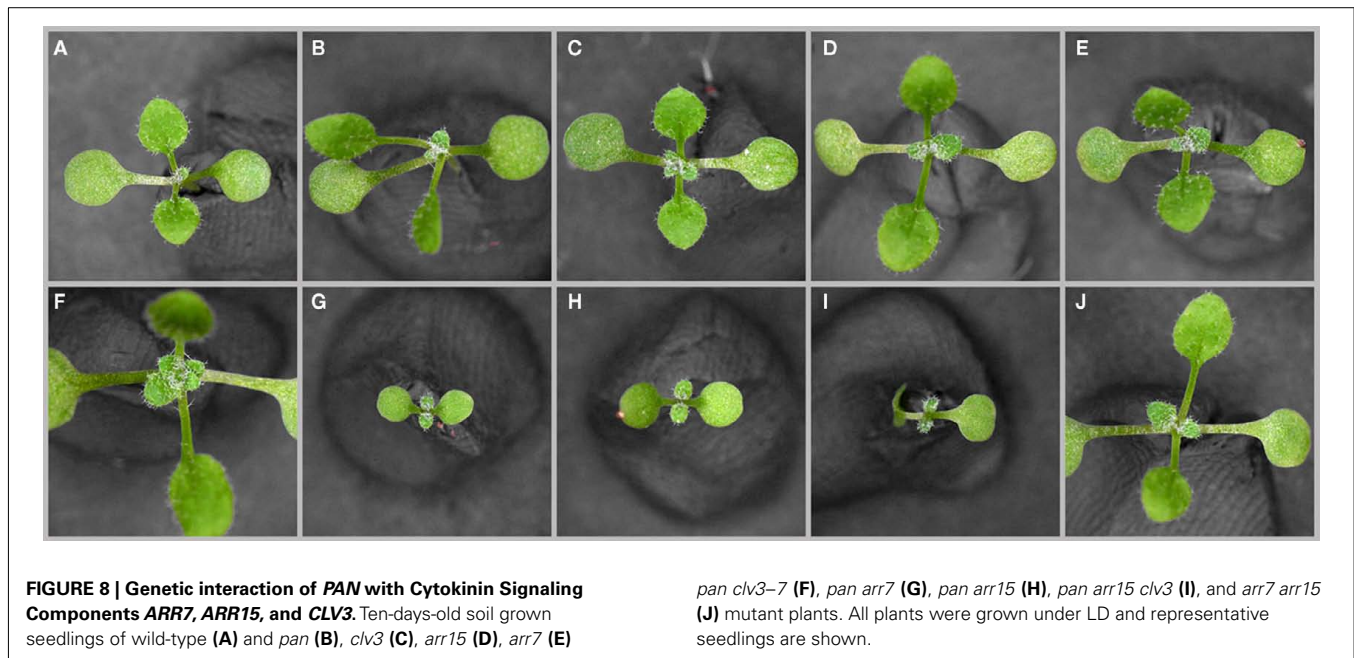
enhancement of the *clv3* phenotype (**Figure 3A**). Compared to *clv3* single mutants, SAMs of *pan clv3* double mutants were even further enlarged (arrowheads in **Figures 3C,D**) and developed even more lateral organs (**Figures 3C,D**). Consistent with an enhancement of meristem phenotypes by the *pan* mutation, we observed a drastic reduction SAM function when we combined *wus* and *pan* (**Figure 3F**). In contrast to *wus* mutants, which develop a bushy stature because of the stop and go phenotype of the meristem (Laux et al., 1996), stem cell activity in *wus pan* double mutants ceased after the formation of leaves and elongated shoots were never formed. Since *CLV3* and *WUS* act in the same pathway and both showed synergistic genetic interactions with *PAN*, we next wondered how *PAN* would interact with *STM*, whose activity is independent of the *WUS–CLV* system. To our surprise we found that the *stm* phenotype was partially suppressed in *pan stm* double mutants, which developed a substantially larger number of lateral organs and shoots compared to *stm* plants (**Figure 3G**). In some cases we even observed flowers with a regular arrangement of floral organs, however these flowers remained sterile. Thus, while in the case of *WUS* and *CLV3 PAN* behaved as a molecular buffer, which is able to stabilize SAM function in the absence of other meristem regulators, this function was not observed when *pan* was combined with *stm*, suggesting that they have antagonistic activities.

To elucidate some of the mechanisms that could underlie these complex meristematic functions of *PAN*, we recorded the molecular phenotype of *pan* single mutants by transcript profiling. Wild-type and *pan* mutants were grown in LD for 25 days before we sampled two independent pools of 50 inflorescence meristems of each genotype by removing developing flowers older than stage 8. After Affymetrix Ath1 profiling we applied GC-RMA to normalize the data and derive expression values (Wu et al., 2004) followed by Rank Products to identify differentially expressed genes at a false discovery rate of 0.05 (Breitling et al., 2004). One hundred sixty transcripts showed increased abundance (**Table 1**), while 120 mRNAs were found to be significantly reduced in inflorescence apices of *pan* mutants compared to wild-type (**Table 2**). To obtain a first insight into the potential function of *PAN* downstream genes we used Gene Ontology (GO) analysis on the level of the annotation of biological function, as well as using molecular function as a readout. Interestingly, we found the "response to stimulus" category as highly enriched among the genes with increased as well as reduced expression. Among the increased

FIGURE 4 | Gene Ontology analysis of biological functions for genes with increased expression in *pan* mutant inflorescence apices. Significantly enriched GO categories are shown in yellow, orange, and red.

FIGURE 5 | Genetic interaction of *PAN* with *GI*. Plants grown for 25 day under LD are shown.

mRNAs we found diverse functional sub-categories indicating that *PAN* plays a role in stress and environmental response (**Figure 4**). A prominent example was *GIGANTEA* (*GI*), whose expression is controlled by the circadian clock and whose activity is necessary for normal clock function and promotion of flowering under LD (Fowler et al., 1999; Park et al., 1999). To test if *GI* plays a relevant role as *PAN* downstream gene, we created *pan gi* double mutants and compared them to the respective parental genotypes. Strikingly, we found that loss-of *PAN* function was able to fully suppress the late flowering phenotype of *gi* mutants in LD (**Figure 5**), demonstrating that *GI* and *PAN* act in the same pathway.

In contrast to the rather diverse GO categories observed in the list of genes with increased expression, the reduced transcripts revealed a much more specific developmental signature. Among them we identified a substantial overrepresentation of genes with annotated functions in hormone signaling, specifically for gibberellin, ethylene, auxin and, most prominently, cytokinin response (**Figure 6**). This developmental signature was also apparent in the GO analysis for molecular functions with "transcription regulator activity" and "two-component response regulator activity" as the most overrepresented annotation terms (**Figure 7**). Two-component response regulators build the backbone of cytokinin signal transduction and response, with B-type *ARRs* acting as cytokinin dependent transcription factors directly upstream of A-type *ARRs* as immediate early cytokinin response genes with roles in negative feedback regulation (Werner and Schmülling, 2009). Strikingly, only the expression of A-type *ARRs* was affected in *pan* mutants and *ARR4*, *ARR5*, *ARR6*, *ARR7*, *ARR15*, and *ARR16*, were among the transcripts with significantly reduced abundance, a result which we independently confirmed using quantitative real-time RT-PCR (data not shown). In addition to cytokinin response genes, we identified two cytokinin oxidases, *CKX3* and *CKX5*, as genes with reduced expression. Since CKX proteins irreversibly degrade cytokinin (Mok and Mok, 2001; Werner et al., 2003) and because A-type ARRs counteract cytokinin signaling (To et al., 2004), a reduction of their expression in *pan* mutants suggests that *PAN* acts to limit cytokinin activity in the SAM. This interpretation is consistent with the finding that SAM size is increased in *pan* mutants reminiscent of plants with increased cytokinin levels (Bartrina et al., 2011). In addition, we had previously identified

FIGURE 6 | Gene Ontology analysis for biological function for genes with reduced expression in _pan_ mutant inflorescence apices. Significantly enriched GO categories are shown in yellow, orange, and red.

FIGURE 7 | Gene Ontology analysis for molecular function for genes with reduced expression in _pan_ mutant inflorescence apices.
Significantly enriched GO categories are shown in yellow, orange, and red.

ARR5, _ARR6_, _ARR7_, and _ARR15_ as direct transcriptional targets of WUS, connecting these cytokinin response genes to the core regulatory system of the SAM. While from the list of genes with reduced expression an antagonistic interaction of _PAN_ and cytokinin could be deduced, it also suggested that _PAN_ acts to stimulate auxin signaling, since it contained _YUCCA1_ and _YUCCA4_, two genes coding for important auxin biosynthesis enzymes (Zhao et al., 2001). Since auxin directly represses transcription of _ARR7_ and _ARR15_ via the Auxin Response Factor MONOPTEROS (Zhao et al., 2010) in the SAM, _PAN_ could act on the expression of A-type _ARRs_ in multiple independent pathways. Strikingly, WUS was identified among the transcriptional regulators with reduced expression, confirming that _PAN_ is intimately connected to the SAM regulatory network.

Having identified cytokinin and auxin signaling as major downstream effector pathways of _PAN_ we next addressed the functional relevance of these regulatory interactions using genetics. We focused our analysis on _ARR7_ and _ARR15_, since both of them were shown to have important meristematic functions (Leibfried et al., 2005; Zhao et al., 2010), and combined these mutants (**Figures 8D,E**) with _pan_ (**Figure 8B**) and _clv3_ (**Figure 8C**) in double and triple mutant combinations. While single A-type _arr_ mutants have no phenotypes or very mild ones (**Figures 8D,E**; To et al., 2004), combination of _arr7_ and _arr15_ with _pan_ lead to severe growth retardation (**Figures 8G,H**). Interestingly, while removing _CLV3_ function in the _pan_ background lead to massive over-proliferation and meristem expansion beyond the regular _clv3_ defect (**Figures 3B–E**), this phenotype was completely suppressed in the _pan clv3 arr7_ combination (**Figures 8F–I**). However, the growth retardation was only transient and _pan arr15_ as well as _pan arr15 clv3_ plants recovered after about 2 weeks and developed plants with pentameric flowers, which closely resembled _pan clv3_ mutants. This capacity to overcome A-type _ARR_ related

FIGURE 8 | Genetic interaction of *PAN* with Cytokinin Signaling Components *ARR7, ARR15,* and *CLV3*. Ten-days-old soil grown seedlings of wild-type **(A)** and *pan* **(B)**, *clv3* **(C)**, *arr15* **(D)**, *arr7* **(E)** *pan clv3–7* **(F)**, *pan arr7* **(G)**, *pan arr15* **(H)**, *pan arr15 clv3* **(I)**, and *arr7 arr15* **(J)** mutant plants. All plants were grown under LD and representative seedlings are shown.

developmental defects was also observed in plants carrying an over-activated form of *ARR7* (Leibfried et al., 2005) and suggest that the cytokinin signaling system has a strong ability to adapt to perturbations. Mutation of multiple A-type *ARRs*, such as in an *arr7 arr15* double mutant did not cause the phenotypes observed in the *pan arr* combinations (**Figure 8J**) underlining the important role of *PAN* in the SAM. Having observed a strong genetic interaction of *PAN* with components of the cytokinin response, we next tested its ability to modify auxin related defects. To this end we analyzed the interaction of *PAN* with *PINFORMED-1* (*PIN1*), the major auxin efflux carrier responsible for generating local auxin maxima at the periphery of the SAM and thus organ initiation during shoot development (Gälweiler et al., 1998; Reinhardt et al., 2000). While *pin1* mutants rarely developed flowers under our growth conditions (**Figures 9A,C**), *pin1 pan* double mutants exhibited a significantly increased number of flowers (**Figures 9B,C**), which were deformed and generally sterile. Again, as in the case of cytokinin signaling, these results demonstrated that *PAN* is able to modulate auxin dependent developmental functions, in line with the hypothesis that *PAN* might act as a multifunctional hub for diverse meristematic functions.

SUMMARY AND OUTLOOK

Taken together, we have shown here by molecular phenotyping and genetics that *PAN* is connected to a plethora of diverse input pathways and may act as an integrator to buffer shoot meristem activity. *PAN* inputs include pathways for environmental sensing, such as day-length and other abiotic factors, as well as hard-wired developmental circuitries, such as the *WUS–CLV* system. Strikingly, the same holds true for the *PAN* output network, which we found to include components of the circadian clock and stress response as examples for modulating environmental interactions. Furthermore, *PAN* downstream genes showed a strong developmental signature, which was most apparently represented by a number

of plant hormone signaling systems. Based on our results we suggest that *PAN* might act as a node between cytokinin and auxin signaling pathways, with cytokinin outputs being repressed and auxin activity being induced by *PAN*. PAN is a member of the D-class of bZIP transcription factors (Jakoby et al., 2002) and thus groups with the TGA regulators, which are involved in mediating pathogen defense (Zander et al., 2010). The sequence similarity of PAN and TGA pathogen response regulators suggests that PAN function might have evolved from an environmental surveillance activity, which was enhanced to include developmental roles to give rise to an integrated buffering system.

MATERIALS AND METHODS
PLANT MATERIAL

Arabidopsis thaliana plants of the Columbia (Col-0) background were grown on soil at 23°C. Analyses were performed after growth under three different light conditions: CL, LD (16 h of light), or SD (8 h light) for 10 days for seedlings and 25 days for vegetative and reproductive tissues if not noted otherwise. The following mutant alleles used: *arr7* (*At1G19050*): WiscDsLox485–488B15; *arr15* (*At1G74890*): WiscDSLox334D02; *clv3* (*At2G27250*): *clv3–7*; pan (*AT1G68640*): Salk N557190; *wus* (*At2G17950*): *wus-4* in Columbia background (*wus-mh*; Leibfried et al., 2005); *stm* (*At1G62360*): GABI-Kat line 100F11; *pin1* (*At1G73590*): GABI-Kat line 051A10; *gi* (*At1G22770*): *gi-201*. Phenotypic characterizations were carried out by growing mutants and controls at least three times independently and analyzing a total of at least 30 individuals for each genotype. Representative plants are shown.

IN SITU HYBRIDIZATIONS

Plant material was fixed and embedded using a Leica ASP300 and hybridized following standard protocols (Weigel and Glazebrook, 2002) adding 10% polyvinylalcohol (PVA) to the staining solution. Digoxigenin-labeled full-length RNA riboprobes were synthesized
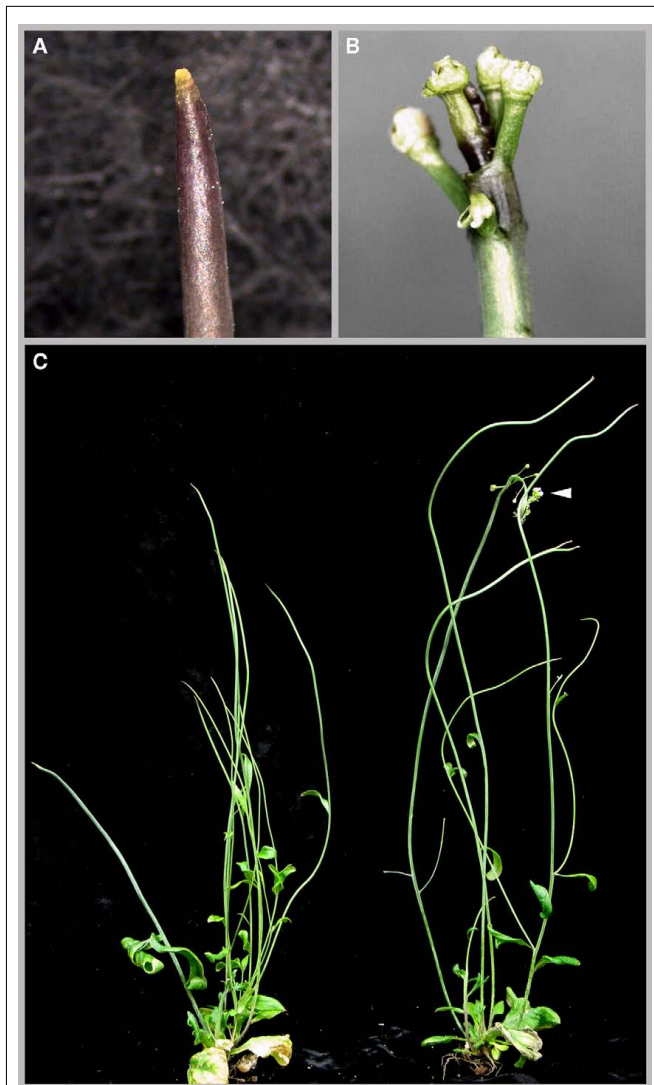
FIGURE 9 | Genetic interaction of *PAN* with *PIN1*. Primary shoot of *pin1* mutant **(A)** and *pan pin1* double mutant **(B)** and whole plant comparison **(C)** of *pin1* (left) and *pan pin1* (right) showing increased development of floral buds on the primary shoot of the *pan pin1* double mutant.

for *CLV3* (*At2G27250*), *PAN* (*AT1G68640*), *STM* (*At1G62360*), and *WUS* (*At2G17950*) as described in Geier et al. (2008) and Maier et al. (2009) according to the manufacturer instruction (Roche).

MICROARRAY EXPERIMENTS

Pools of 50 microscopically dissected inflorescence apices of *pan* mutants and wild-type both carrying the *KB14 AG::GUS* reporter gene (Busch et al., 1999; Lohmann et al., 2001) were grown for 25 days in LD conditions and profiled in duplicate using the Affymetrix ATH1 platform. RNA extraction and microarray analyses were performed as described (Schmid et al., 2005; Buechel et al., 2010). Expression estimates were derived by GC-RMA (Wu et al., 2004) at standard settings implemented in R. We determined significant changes on a per-gene level by applying the Rank products algorithm (Breitling et al., 2004) using 100 permutations and a false discovery rate cut-off of 5%. GO analysis was carried out using AgriGO (Du et al., 2010).

QUANTITATIVE REAL-TIME PCR

Total RNA was extracted from apices of plants grown in an independent experiment using RNeasy Mini columns with on-column DNAse digestion (Qiagen). Reverse transcription was performed with 1 μg of total RNA, using a Reverse Transcription Kit (Fermentas). PCR amplification was carried out in the presence of the double-strand DNA-specific dye SYBR Green (Molecular Probes) using intron spanning primers (Andersen et al., 2008). Amplification was monitored in real-time with the Opticon Continuous Fluorescence Detection System (MJR). *BETA-TUBULIN-2* transcript levels served to normalize mRNA measurements.

REFERENCES

Andersen, S. U., Buechel, S., Zhao, Z., Ljung, K., Novak, O., Busch, W., Schuster, C., and Lohmann, J. U. (2008). Requirement of B2-type cyclin-dependent kinases for meristem integrity in *Arabidopsis thaliana*. *Plant Cell* 20, 88–100.

Barton, M., and Poethig, R. (1993). Formation of the shoot apical meristem in *Arabidopsis thaliana* – an analysis of development in the wild-type and in the shoot meristemless mutant. *Development* 119, 823–831.

Barton, M. K. (2010). Twenty years on: the inner workings of the shoot apical meristem, a developmental dynamo. *Dev. Biol.* 341, 95–113.

Bartrina, I., Otto, E., Strnad, M., Werner, T., and Schmülling, T. (2011). Cytokinin regulates the activity of reproductive meristems, flower organ size, ovule formation, and thus seed yield in *Arabidopsis thaliana*. *Plant Cell* 23, 69–80.

Brand, U., Fletcher, J. C., Hobe, M., Meyerowitz, E. M., and Simon, R. (2000). Dependence of stem cell fate in Arabidopsis on a feedback loop regulated by CLV3 activity. *Science* 289, 617–619.

Breitling, R., Armengaud, P., Amtmann, A., and Herzyk, P. (2004). Rank products: a simple, yet powerful, new method to detect differentially regulated genes in replicated microarray experiments. *FEBS Lett.* 573, 83–92.

Buechel, S., Leibfried, A., To, J. P. C., Zhao, Z., Andersen, S. U., Kieber, J. J., and Lohmann, J. U. (2010). Role of A-type Arabidopsis response regulators in meristem maintenance and regeneration. *Eur. J. Cell Biol.* 89, 279–284.

Busch, M. A., Bomblies, K., and Weigel, D. (1999). Activation of a floral homeotic gene in Arabidopsis. *Science* 285, 585–587.

Busch, W., Miotk, A., Ariel, F. D., Zhao, Z., Forner, J., Daum, G., Suzaki, T., Schuster, C., Schultheiss, S. J., Leibfried, A., Haubeiss, S., Ha, N., Chan, R. L., and Lohmann, J. U. (2010). Transcriptional control of a plant stem cell niche. *Dev. Cell* 18, 849–861.

Chuang, C. F., Running, M. P., Williams, R. W., and Meyerowitz, E. M. (1999). The PERIANTHIA gene encodes a bZIP protein involved in the determination of floral organ number in *Arabidopsis thaliana*. *Genes Dev.* 13, 334–344.

Clark, S. E., Running, M. P., and Meyerowitz, E. M. (1993). CLAVATA1, a regulator of meristem and flower development in Arabidopsis. *Development* 119, 397–418.

Clark, S. E., Running, M. P., and Meyerowitz, E. M. (1995).

CLAVATA3 is a specific regulator of shoot and floral meristem development affecting the same processes as *CLAVATA1*. *Development* 121, 2057–2067.

Das, P., Ito, T., Wellmer, F., Vernoux, T., Dedieu, A., Traas, J., and Meyerowitz, E. M. (2009). Floral stem cell termination involves the direct regulation of AGAMOUS by PERIANTHIA. *Development* 136, 1605–1611.

Du, Z., Zhou, X., Ling, Y., Zhang, Z., and Su, Z. (2010). agriGO: a GO analysis toolkit for the agricultural community. *Nucleic Acids Res.* 38, W64–W70.

Fowler, S., Lee, K., Onouchi, H., Samach, A., Richardson, K., Morris, B., Coupland, G., and Putterill, J. (1999). GIGANTEA: a circadian clock-controlled gene that regulates photoperiodic flowering in Arabidopsis and encodes a protein with several possible membrane-spanning domains. *EMBO J.* 18, 4679–4688.

Furutani, M., Vernoux, T., Traas, J., Kato, T., Tasaka, M., and Aida, M. (2004). PIN-FORMED1 and PINOID regulate boundary formation and cotyledon development in Arabidopsis embryogenesis. *Development* 131, 5021–5030.

Gälweiler, L., Guan, C., Müller, A., Wisman, E., Mendgen, K., Yephremov, A., and Palme, K. (1998). Regulation of polar auxin transport by AtPIN1 in Arabidopsis vascular tissue. *Science* 282, 2226–2230.

Geier, F., Lohmann, J. U., Gerstung, M., Maier, A. T., Timmer, J., and Fleck, C. (2008). A quantitative and dynamic model for plant stem cell regulation. *PLoS ONE* 3, e3553. doi:10.1371/journal.pone.0003553

Hepworth, S. R., Zhang, Y., McKim, S., Li, X., and Haughn, G. W. (2005). BLADE-ON-PETIOLE-dependent signaling controls leaf and floral patterning in Arabidopsis. *Plant Cell* 17, 1434–1448.

Jakoby, M., Weisshaar, B., Dröge-Laser, W., Vicente-Carbajosa, J., Tiedemann, J., Kroj, T., Parcy, F., and bZIP Research Group. (2002). bZIP transcription factors in Arabidopsis. *Trends Plant Sci.* 7, 106–111.

Jasinski, S., Piazza, P., Craft, J., Hay, A., Woolley, L., Rieu, I., Phillips, A., Hedden, P., and Tsiantis, M.

(2005). KNOX action in Arabidopsis is mediated by coordinate regulation of cytokinin and gibberellin activities. *Curr. Biol.* 15, 1560–1565.

Kayes, J. M., and Clark, S. E. (1998). CLAVATA2, a regulator of meristem and organ development in Arabidopsis. *Development* 125, 3843–3851.

Laux, T., Mayer, K. F., Berger, J., and Jürgens, G. (1996). The WUSCHEL gene is required for shoot and floral meristem integrity in Arabidopsis. *Development* 122, 87–96.

Leibfried, A., To, J. P. C., Busch, W., Stehling, S., Kehle, A., Demar, M., Kieber, J. J., and Lohmann, J. U. (2005). WUSCHEL controls meristem function by direct regulation of cytokinin-inducible response regulators. *Nature* 438, 1172–1175.

Lohmann, J. U., Hong, R. L., Hobe, M., Busch, M. A., Parcy, F., Simon, R., and Weigel, D. (2001). A molecular link between stem cell regulation and floral patterning in Arabidopsis. *Cell* 105, 793–803.

Long, J. A., Moan, E. I., Medford, J. I., and Barton, M. K. (1996). A member of the KNOTTED class of homeodomain proteins encoded by the STM gene of Arabidopsis. *Nature* 379, 66–69.

Maier, A. T., Stehling-Sun, S., Wollmann, H., Demar, M., Hong, R. L., Haubeiss, S., Weigel, D., and Lohmann, J. U. (2009). Dual roles of the bZIP transcription factor PERIANTHIA in the control of floral architecture and homeotic gene expression. *Development* 136, 1613–1620.

Mayer, K. F., Schoof, H., Haecker, A., Lenhard, M., Jürgens, G., and Laux, T. (1998). Role of WUSCHEL in regulating stem cell fate in the Arabidopsis shoot meristem. *Cell* 95, 805–815.

Mok, D. W., and Mok, M. C. (2001). Cytokinin metabolism and action. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 52, 89–118.

Parcy, F., Nilsson, O., Busch, M. A., Lee, I., and Weigel, D. (1998). A genetic framework for floral patterning. *Nature* 395, 561–566.

Park, D. H., Somers, D. E., Kim, Y.-S., Choy, Y. H., Lim, H. K., Soh, M. S., Kim, H. J., Kay, S. A., and Nam,

H. G. (1999). Control of circadian rhythms and photoperiodic flowering by the Arabidopsis GIGANTEA gene. *Science* 285, 1579–1582.

Reinhardt, D., Mandel, T., and Kuhlemeier, C. (2000). Auxin regulates the initiation and radial position of plant lateral organs. *Plant Cell* 12, 507–518.

Running, M. P., and Meyerowitz, E. M. (1996). Mutations in the PERIANTHIA gene of Arabidopsis specifically alter floral organ number and initiation pattern. *Development* 122, 1261–1269.

Schmid, M., Davison, T. S., Henz, S. R., Pape, U. J., Demar, M., Vingron, M., Schölkopf, B., Weigel, D., and Lohmann, J. U. (2005). A gene expression map of *Arabidopsis thaliana* development. *Nat. Genet.* 37, 501–506.

Schoof, H., Lenhard, M., Haecker, A., Mayer, K. F., Jürgens, G., and Laux, T. (2000). The stem cell population of Arabidopsis shoot meristems in maintained by a regulatory loop between the CLAVATA and WUSCHEL genes. *Cell* 100, 635–644.

To, J. P. C., Haberer, G., Ferreira, F. J., Deruère, J., Mason, M. G., Schaller, G. E., Alonso, J. M., Ecker, J. R., and Kieber, J. J. (2004). Type-A Arabidopsis response regulators are partially redundant negative regulators of cytokinin signaling. *Plant Cell* 16, 658–671.

Weigel, D., Alvarez, J., Smyth, D. R., Yanofsky, M. F., and Meyerowitz, E. M. (1992). LEAFY controls floral meristem identity in Arabidopsis. *Cell* 69, 843–859.

Weigel, D., and Glazebrook, J. (2002). *Arabidopsis: A Laboratory Manual*, 1st Edn. Cold Spring Harbor, NY: CSHL Press.

Werner, T., Motyka, V., Laucou, V., Smets, R., Van Onckelen, H., and Schmülling, T. (2003). Cytokinin-deficient transgenic Arabidopsis plants show multiple developmental alterations indicating opposite functions of cytokinins in the regulation of shoot and root meristem activity. *Plant Cell* 15, 2532–2550.

Werner, T., and Schmülling, T. (2009). Cytokinin action in plant development. *Curr. Opin. Plant Biol.* 12, 527–538.

Wu, Z., Irizarry, R., Gentleman, R., Martinez-Murillo, F., and Spencer, F. (2004). A model-based background adjustment for oligonucleotide expression arrays. *J. Am. Stat. Assoc.* 99, 909–917.

Yanai, O., Shani, E., Dolezal, K., Tarkowski, P., Sablowski, R., Sandberg, G., Samach, A., and Ori, N. (2005). Arabidopsis KNOXI proteins activate cytokinin biosynthesis. *Curr. Biol.* 15, 1566–1571.

Zander, M., La Camera, S., Lamotte, O., Métraux, J.-P., and Gatz, C. (2010). *Arabidopsis thaliana* class-II TGA transcription factors are essential activators of jasmonic acid/ethylene-induced defense responses. *Plant J.* 61, 200–210.

Zhao, Y., Christensen, S. K., Fankhauser, C., Cashman, J. R., Cohen, J. D., Weigel, D., and Chory, J. (2001). A role for flavin monooxygenase-like enzymes in auxin biosynthesis. *Science* 291, 306–309.

Zhao, Z., Andersen, S. U., Ljung, K., Dolezal, K., Miotk, A., Schultheiss, S. J., and Lohmann, J. U. (2010). Hormonal control of the shoot stem-cell niche. *Nature* 465, 1089–1092.