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 FACTORS/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 (API). 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 API, 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 indistinguishable 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.
Maier et al. PERIANTHIA: a multifunctional hub for meristem control

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.
| 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-rich protein 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 (lipoxygcnase 3); iron ion binding/lipoxygcnase/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/TFIY10A (JASMONATE-ZIM-DOMAIN PROTEIN 1); protein binding |
| 261221_at     | 3.37        | AT1G19960       | Similar to transmembrane receptor [Arabidopsis thaliana] (TAIR:AT2G31240.1) |
| 261222_at     | 2.13        | AT1G20120       | Family II extracellular lipase putative |
| 261224_at     | 2.38        | AT1G20160       | ATSBT5.2; subtilase |
| 264211_at     | 3.48        | AT1G22770       | pG (pIGANTEA); 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       | LH2 (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        | AT1G42224       | Encodes a ECA1 gametogenesis related family protein |
| 262436_at     | 1.73        | AT1G47610       | Transducin family protein/VWD-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 |
| 265181_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 (EPITHIOPESPECIFIER PROTEIN) |
| 263158_at     | 3.99        | AT1G54160       | CCAAT-binding transcription factor (CBF-B/NF-YA) family protein |
| 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; [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)
| 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:CAO488523.1); contains InterPro domain Protein of unknown function UPF0089 (InterPro:IPR004258); 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 |
| 262060_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) |
| 266984_at     | 1.91        | AT2G24210       | TPS10 (TERPENE SYNTHASE 10); myrcene/(E)-beta-ocimene synthase |
| 266898_at     | 1.90        | AT2G25690       | Senescence-associated protein-related |
| 245035_at     | 1.96        | AT2G26400       | ARD/ATARD3 (ACIREDUCTONE DIOXYGENASE); acireductone dioxygenase [iron(III)-requiring]/heteroglycan binding/metal ion binding |
| 267595_at     | 2.30        | AT2G29990       | ATGH888 (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 |
| 266066_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 ribosome 5S 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 HOMEBOX 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 |
| Array element | Fold change | Locus identifier | Annotation |
|---------------|-------------|-----------------|------------|
| 258890_at     | 4.09        | AT3G05690       | ATHAP2B/HAP2B/JNE8 (HEME ACTIVATOR PROTEIN (YEAST) HOMOLOG 2B); transcription factor |
| 259296_at     | 3.44        | AT3G11480       | BSM1; S-adenosylmethionine-dependent methyltransferase |
| 259937_s_at   | 1.82        | AT3G13080;      | [AT3G13080. ATMRP3 (Arabidopsis thaliana multidrug resistance-associated protein 3)]; [AT1G71330. ATNAP5 (Arabidopsis thaliana non-intrinsic ABC protein 5)] |
| 258370_at     | 1.93        | AT3G14395       | Zinc finger (B-box type) family protein |
| 258399_at     | 1.74        | AT3G15540       | IAA19 (indoleacetic acid-induced protein 19); transcription factor |
| 257876_at     | 1.78        | AT3G17130       | Invertase/pectin methylsterase inhibitor family protein |
| 258158_at     | 2.80        | AT3G17790       | ATACPS (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-INDUCIBLE 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:AT3G02140.1); similar to unnamed protein product [Vitis vinifera] (GB:CAO49169.1); contains InterPro domain Protein of unknown function DUF1675 (InterPro:IPR012463) |
| 251309_at     | 1.98        | AT3G61220       | Short-chain dehydrogenase/reductase (SDR) family protein |
| 255675_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;      | [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 |
| 246322_at     | 2.18        | AT4G14815       | Protease inhibitor/seed storage/lipid transfer protein (LTP) family protein |
| 245275_at     | 2.45        | AT4G15210       | ATBETA-AMY (BETA-AMYLASE); beta-amylace |
| 245465_at     | 1.86        | AT4G16590       | ATCSLA01 (Cellulose synthase-like A1); glucosyltransferase/transferring glycosyl groups |
| 245346_at     | 1.75        | AT4G17090       | CTBMY (BETA-AMYLASE 3. BETA-AMYLASE 8); beta-amylace |
| 245389_at     | 1.87        | AT4G17480       | Palmityl protein thioesterase family protein |
| 254574_at     | 1.77        | AT4G19430       | Unknown protein |
| 254321_at     | 2.62        | AT4G22590;      | [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       | WRYKYS (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:AT4G37210.1); similar to unnamed protein product [Vitis vinifera] (GB:CAO47480.1); contains InterPro domain Conserved hypothetical protein CHP00730 (InterPro:IPR005269) |
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 |
| 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        | AT5G07660       | 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 (eEF1alpha) |
| 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       | Transferrase family protein |
| 246071_at     | 1.77        | AT5G20150       | SPX (SYG1/Pho81/XPR1) domain-containing protein |
| 246009_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 |
| 245537_at     | 2.12        | AT5G26570       | Calmodulin-binding protein |

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 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 DUF1006 (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       | PITL2; 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       | GALTL8/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: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 | Similar to unknown protein (*Arabidopsis thaliana*) (TAIR:AT1G53903.1); similar to senescence-associated protein-related (*Arabidopsis thaliana*) (TAIR:AT1G53903.1); similar to Protein of unknown function DUF581 (*Medicago truncatula*) (GB:ABO84791.1); contains InterPro domain Protein of unknown function DUF581 (InterPro:IPR007650) |
| 263006_at     | 0.44        | AT1G54540       | Similar to harpin-induced protein-related/MIN1-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       | GALTL9/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        | AT1G73840       | ATNUDT21 (*Arabidopsis thaliana* Nudix hydrolase homolog 21); hydrolase |
| 262212_at     | 0.32        | AT1G74890       | ARR15 (RESPONSE REGULATOR 15); transcription regulator |
| 261109_at     | 0.36        | AT1G75450       | CKX6 (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:CAN78638.1) |
| 264299_s_at   | 0.41        | AT1G78850; AT1G78860 | [AT1G78860. curculin-like (mannose-binding) lectin family protein]; [AT1G78860. curculin-like (mannose-binding) lectin family protein] |
| 260249_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 MrDRAFT_AC148340g12v2 (*Medicago truncatula*) (GB:ABD28396.1) |

(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        | AT2G00540       | Glycine-rich protein |
| 265475_at     | 0.51        | AT2G15620       | NITR1 (NITRITE REDUCTASE); ferredoxin-nitrate reductase |
| 268481_at     | 0.54        | AT2G15860       | Unknown protein |
| 265821_at     | 0.54        | AT2G22980       | SCPL13; serine carboxypeptidase |
| 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:ACO06020.1) |
| 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 1 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        | AT2G424140      | 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:IPR010401) |
| 257939_at     | 0.37        | AT3G19930       | STP4 (SUGAR TRANSPORTER 4); carbohydrate transmembrane transporter/sugar:hydrogen ion symporter |
| 257965_at     | 0.57        | AT3G20810       | Transcription factor jumonji (jmj)C 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_a_t    | 0.57        | AT3G23530;       | Similar to pentatricopeptide (PPR) repeat-containing protein [Arabidopsis thaliana] (TAIR:AT4G22760.1); similar to hypothetical protein [Catharanthus roseus] (GB:CAC09928.1) |
| 25279_at      | 0.55        | AT3G44260       | CCR4-NOT transcription complex protein. putative |
| 252374_a_t    | 0.55        | AT3G48100       | ARRS (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) |
| 251723_at     | 0.53        | AT3G56230       | Speckle-type POZ protein-related |
| 251704_at     | 0.56        | AT3G56850       | 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 |
| 255064_at     | 0.32        | AT4G05070       | Unknown protein |
| 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 | DC1 domain-containing protein;[AT5G48320. DC1 domain-containing protein] |
| 245441_at     | 0.55        | AT4G16700       | PSD1 (PHOSPHATIDYLSTERINE DECARBOXYLASE 1); phosphatidylinositol deoxyribosyltransferase |
| 254665_at     | 0.52        | AT4G18340       | Glycosyl hydrolase family 17 protein |
| 254098_at     | 0.55        | AT4G25100       | ACS6 (1-AMINOCYCLOPROPANE-1-CARBOXYLIC ACID (ACC) SYNTHASE 6) |
| 254751_at     | 0.47        | AT4G13150       | Unknown protein |
| 253915_at     | 0.17        | AT4G22780       | Calcium-binding EF hand family protein |
| 253666_at     | 0.35        | AT4G30270       | MER15B (MERISTEM-5); hydrolase. acting on glycosyl bonds/xyloglucan:xyloglucosyl transferase |
| 254241_at     | 0.56        | AT4G32340       | Binding |
| 253439_at     | 0.50        | AT4G32540       | YUC (YUCCA); FAD binding/NADP binding/ADP binding/flavin-containing monoxygenase/monoxygenase/oxidoreductase |
| 253337_at     | 0.53        | AT4G33960       | Similar to unknown protein [Arabidopsis thaliana] (TAIR:AT2G15830.1) |
| 253161_at     | 0.50        | AT4G35770       | SEN1 (DARK INDUCIBLE 1) |
| 251013_at     | 0.54        | AT4G36040       | DNA 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       | B7 (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 uncharacterized conserved protein UCP01293 (InterPro:IPR016606) |
| 250389_at     | 0.51        | AT5G11320       | YUC4 (YUCCA4); monoxygenase |
| 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 |
| 249966_at     | 0.36        | AT5G18600       | Glutaredoxin family protein |
| 246700_at     | 0.54        | AT5G28030       | Cysteine synthase, putative/O-acetylserine (thiol)-lyase, putative/O-acetylserine sulphydrylase, putative |
| 249645_at     | 0.39        | AT5G36910       | THI2.2 (THIONIN 2.2); toxin receptor binding |
| 256356_s_at   | 0.58        | AT5G43620:AT1G66500 | 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 cyclosporin gene family protein [Brassica oleracea] (GB:ABD65563.1); contains InterPro domain Streptomyces cyclosporin gene family;[AT5G46790. cyclase/dehydrogenase family protein] |
| 248163_at     | 0.49        | AT5G5410        | DFL1/GH3.6 (DWARF IN LIGHT 1); indole-3-acetic acid amido synthetase |
| 248020_at     | 0.46        | AT5G56490       | FAD-binding domain-containing protein |
| 247966_at     | 0.47        | AT5G56970       | CKX3 (CYTOKININ OXIDASE 3); cytokinin dehydrogenase |
We next investigated how the SAM regulatory system is affected (CL V3) with \( CL V3 \) extended our analysis to test the functional interaction of \( CL V3 \) and young leaves (\( PAN \)) reported that protein expression is mostly independent of \( WUS \). First, we noticed that the SAM was drastically reduced \( SAM \) function when we combined \( WUS \) and \( PAN \). 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 \( wus \) and \( PAN \) double mutants ceased after the formation of leaves and elongated shoots were never formed. Since CL V3 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–CL V3 system. To our surprise we found that the stem cell domain 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
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...
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
developmental defects was also observed in plants carrying an over-activated form of \textit{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 \textit{ARRs}, such as in an \textit{arr7 arr15} double mutant did not cause the phenotypes observed in the \textit{pan arr} combinations (Figure 8J) underlining the important role of \textit{PAN} in the SAM. Having observed a strong genetic interaction of \textit{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 \textit{PAN} with \textit{PINFORMED-1} (\textit{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 (Galweiler et al., 1998; Reinhardt et al., 2000). While \textit{pin1} mutants rarely developed flowers under our growth conditions (Figures 9A,C), \textit{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 \textit{PAN} is able to modulate auxin dependent developmental functions, in line with the hypothesis that \textit{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 \textit{PAN} is connected to a plethora of diverse input pathways and may act as an integrator to buffer shoot meristem activity. \textit{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 \textit{WUS–CLV} system. Strikingly, the same holds true for the \textit{PAN} output network, which we found to include components of the circadian clock and stress response as examples for modulating environmental interactions. Furthermore, \textit{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 \textit{PAN} might act as a node between cytokinin and auxin signaling pathways, with cytokinin outputs being repressed and auxin activity being induced by \textit{PAN}. \textit{PAN} is a member of the D-class of \textit{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 \textit{PAN} and TGA pathogen response regulators suggests that \textit{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**

\textit{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: \textit{arr7} \textit{(At1G19050)}; WiscDsLox485–488B15; \textit{arr15} \textit{(At1G74890)}; WiscDsLox334D02; \textit{clv3} \textit{(At2G27250)}; \textit{clv3–7}; \textit{pan} \textit{(AT1G62360)}; \textit{Salk N557190}; \textit{wus} \textit{(At2G17950)}; \textit{wux-4} in Columbia background \textit{(wux-mh)}; Leibfried et al., 2005); \textit{stm} \textit{(At1G62360)}: GABI-Kat line 100F11; \textit{pin1} \textit{(At1G73590)}: GABI-Kat line 051A10; \textit{gi} \textit{(At1G22770)}; \textit{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.
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 AC::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 (Ferments). 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.

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