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Developmental expression patterns of Arabidopsis *XTH* genes reported by transgenes and Genevestigator

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Abstract

The plant cell wall is the structural basis of cellular form and thus forms a foundation on which morphogenesis builds organs and tissues. Enzymes capable of modifying major wall components are prominent candidates for regulating wall form and function. Xyloglucan endotransglucosylases/hydrolases (XTHs) are predicted to participate in xyloglucan integration and/or restructuring. XTHs are encoded by large gene families in plants; the Arabidopsis genome encodes 33 XTHs. To gain insight into the potential physiological relevance of the distinct members of this family, *GUS* reporter fusion genes were constructed, and plants expressing these transgenes were characterized to reveal spatial and temporal patterns of expression. In addition, Genevestigator sources were mined for comprehensive and comparative *XTH* expression regulation analysis. These data reveal that the Arabidopsis *XTHs* are likely expressed in every developmental stage from seed germination through flowering. All organs show *XTH::GUS* expression and most, if not all, are found to express multiple *XTH::GUS* genes. These data suggest that XTHs may contribute to morphogenesis at every developmental stage and in every plant organ. Different *XTHs* have remarkably diverse and distinct expression patterns indicating that paralogous genes have evolved differential expression regulation perhaps contributing to the maintenance of the large gene family. Extensive overlap in *XTH* expression patterns is evident; thus, XTHs may act combinatorially in determining wall properties of specific tissues or organs. Knowledge of gene-specific expression among family members yields evidence of where and when gene products may function and provides insights to guide rational approaches to investigate function through reverse genetics.

Abbreviations: PCR, polymerase chain reaction; XTH, xyloglucan endotransglucosylase/hydrolase; XET, xyloglucan endotransglucosylase; XEH, xyloglucan endohydrolase; X-gluc, 5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid; GUS, β-glucuronidase

Introduction

The complexity and variety of plant cell, tissue and organ sizes, shapes and mechanical properties requires sophistication of construction and dynamic remodeling of the plant cell wall. The major components of the plant cell wall are known, as are general ideas of the interactions among these components (reviewed in McCann and Roberts, 1991; Carpita and Gibeaut, 1993). In dicotyledenous plants, cellulose microfibrils laid down at the cellular surfaces are noncovalently associated with the hemicellulose xyloglucan released by transport vesicles at the cell surface. Xyloglucan polymers can be found intimately associated with the microfibrils, bound to the surface of the

microfibrils through hydrogen bonding and spanning neighboring microfibrils (Fry, 1989; Hayashi, 1989; McCann *et al*., 1990; Pauly *et al*., 1999). The xyloglucan tethers between the microfibrils are thought to provide integrity and tension resistance to the wall. Pectin polymers, structural proteins and lignin are also wall components that contribute to wall properties.

Despite the wall's putative principal function in providing a reliable structural enclosure for plant cells, the primary wall must be capable of dynamic remodeling to account for cellular growth, division and differentiation (Carpita and McCann, 2000). Indeed, analyses of plant genomes have led to the discovery of large numbers of potential cell wall modifying enzyme encoding genes (Carpita *et al*., 2001; Henrissat *et al*., 2001), some of which may have roles in the biogenesis of wall architecture and remodeling coincident with growth and differentiation.

The xyloglucan endotransglucosylase/hydrolases (XTHs) possess the enzymatic activity of endolytically cleaving xyloglucan polymers and joining the newly generated end to another xyloglucan chain (xyloglucan endotransglucosylase, abbreviated XET, activity) or to water (xyloglucan endohydrolase, XEH, activity) (Rose *et al*., 2002). The abilities of XTHs to modify wall xyloglucan polymers have identified XTHs as potentially important enzymes for wall architecture modifications both during wall synthesis and reinforcement (Fry, 1989; Rose *et al*., 2002). XTHs likely function to integrate nascent xyloglucan and restructure existing wall xyloglucan (Xu *et al*., 1996; Mauch *et al*., 1997; Nishitani, 1997; Fry, 2004). At least some XTHs may function in genesis of secondary walls of vascular tissues (Bourquin *et al*., 2002; Matsui *et al*., 2005).

XTHs are encoded by large gene families, with *Arabidopsis thaliana* (ecotype Col-O) possessing 33 *XTH* genes (Nishitani, 1997; Campbell and Braam, 1999b; Yokoyama and Nishitani, 2001; Rose *et al*., 2002; Yokoyama *et al*., 2004). Sequence similarities among the encoded products and enzymatic analyses of a subset of the proteins to date indicate that the different XTHs have similar, though not identical properties (Campbell and Braam, 1999a; Steele and Fry, 2000; Steele *et al*., 2001). The large number of *XTH* gene family members is consistent with the idea that individual *XTH* genes may have evolved to be expressed with distinct developmental, organ-, tissue- or cell-specific expression or may be upregulated to respond to distinct developmental, hormonal or environmental stimuli. In this way, distinct *XTHs* or combinations of *XTHs* would function with temporal and spatial specificity. Indeed, loss of a single *XTH* can result in significant developmental alterations (Matsui *et al*., 2005). Expression behaviors of small subsets of Arabidopsis *XTH* genes have been reported (e.g., Medford *et al*., 1991; Xu *et al*., 1995, 1996; Akamatsu *et al*., 1999; Hyodo *et al*., 2003; Matsui *et al*., 2005; Nishitani, 2005; Vissenberg *et al*., 2005). In addition, quantitative real-time RT-PCR and microarray analyses have revealed broad expression patterns of Arabidopsis *XTH* gene family members (Yokoyama and Nishitani, 2001; Lee *et al*., 2005; Ma *et al*., 2005). XET activities can be detected *in muro* (Fry, 2004; Vissenberg *et al*., 2005) and, in general, XET activity is detected in organs where *XTH* genes are expressed. However, the extent to which the distinct expression and function assays coincide, especially at a cellular level remains uncertain.

Here, we report the generation of *XTH::betaglucuronidase* (*GUS*) reporter gene transgenics and detection of the *in situ* expression patterns. In general, distinct methods of gene expression detection are consistent with the GUS patterns, indicating that the 5′ sequences present in the reporter gene constructs are likely sufficient to drive appropriate *XTH* expression. Because GUS patterns can report expression pattern distinctions even within organs or domains of tissues, more detailed data on individual *XTH* regulation may be revealed. Overall, we find remarkably divergent *XTH::GUS* transgene expression patterns from the earliest stages of seed germination through flowering. *XTH::GUS* patterns show some overlap which may result in novel combinations of XTH activities in distinct cells or organs. These complex transgene expression patterns implicate *XTH* function in a wide range of developmental processes. In addition, we compile *XTH* expression data available through Genevestigator (http://www.genevestigator.ethz.ch/) and other genome-wide expression sources to provide a summary of the current state of knowledge on the differential expression of the Arabidopsis *XTH* gene family.

Materials and methods

Plant growth conditions

Arabidopsis thaliana (ecotype Col-O) plants for transformation, seed production and analysis of GUS activity in floral organs were grown in soil (Bacto soil, Southwest Fertilizer, Houston, TX) under constant light (~60 μmol m^{-2} s^{-1}) at ~23 °C. Prior to sowing, seeds were stratified at 4 °C for 1–4 days. For generation of sterile plants, seed were surface sterilized with 100% (v/v) Chlorox bleach for 10 min followed by three washes with sterile water and stratified at 4 °C in the dark over night. For GUS analysis of 3-day-old seedlings, sterilized seed were sown in liquid PN growth media (Haughn and Somerville, 1987) in eppendorf tubes (ISC Bioexpress, Kaysville, UT) under constant light (~80 μmol m^{-2} s^{-1}) at ~23 °C. For analysis of 7- and 10-day-old plants, sterilized seed were sown on PN with 1% sucrose (PNS) containing 0.8% agar at ~23 °C under constant light (~40 μmol m^{-2} s^{-1}). To generate etiolated seedlings, seeds were sown on PNS, given a 6-h light (~40 μmol m^{-2} s^{-1}) treatment, then placed in darkness for an additional 6 days at ~23 °C before analysis.

Generation of XTH::GUS *transgene constructs and transgenic lines*

To generate DNA fragments likely to contain transcriptional regulatory sequences of the *XTH* genes, we used Primer3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) to identify potential primer sequences. For all but two genes, an approximately one-kilobase (kb) region with the 3′ end within 70 basepairs (bp) of the translational start site was amplified. For *XTH11*, the 1 kb region included 217 bp of coding sequences and were designed to generate translational fusions. The 5′ oligonucleotide primers were extended to add potential *Pst*I restriction sites and the 3′ oligonucleotide primers were extended to encode *Bam*HI restriction sites; the *XTH9* 5′ and 3′ primers were exceptions in that they were modified to encode *Pst*I sites and *Bgl*II sites, respectively. The oligonucleotides were synthesized by IDT (Coralville, IA). Primer sequences are listed in the Table (Supplementary material). PCR was carried out using the PTC100 Programmable Thermocyler (MJ Research, Incline Village, NV). The *GUS* gene was PCR amplified from the plasmid pGUS358-S (Clontech, Palo Alto, CA) using primers designed with the restriction sites for *Bam*HI and *Sal*I cloning into pCB302 (Xiang *et al*., 1999). The *NOS-T* terminator was also amplified and cloned into pCB302 3′ to the *GUS* gene using *Sal*I and *Sac*I. Amplified *XTH* region DNAs were purified and inserted directionally into the pCB301 vector (Xiang *et al*., 1999) 5′ to the *GUS* gene. The recombinant plasmids were transformed into *E. coli*. Confirmation of cloning was achieved through restriction digests and sequencing of the resulting DNA vectors. Confirmed transgene constructs were purified from *E. coli* and electroporated into *Agrobacterium tumefaciens* strain LBA4404 and GV3101. Confirmation of *Agrobacteria* clones was achieved by re-isolating plasmid DNA from the *Agrobacteria* lines and transforming the plasmid back into *E. coli* for DNA isolation and restriction enzyme analysis. Agrobacteria containing the transgene vectors were used to transform *Arabidopsis thaliana* ecotype Col-O via the floral dip method (Clough and Bent, 1998). Seed resulting from the Agrobacteria-infected plants were sown in soil and seedlings were sprayed repeatedly the herbicide Basta (0.44% v/v, Finale, Montvale, NJ) to select for the resistant plants that had picked up the transgene vector. Between 5 and 15 independently transformed T2 plants per transgene construct were identified for analysis.

GUS histochemical analysis

Plant materials harvested for *GUS* transgene expression were immediately submerged in cyanide-containing buffer (Caissard *et al*., 1994) with 1 mg/ml 5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid (X-gluc) (Gold Biotechnology, St. Louis, MO) and placed under a 600 mm Hg vacuum for 10 min. Incubation was allowed to continue overnight in darkness at 37 °C. Chlorophyll was removed by submerging the tissue in ethanol (70% v/v) (Jefferson *et al*., 1987). Plant tissue was mounted onto glass slides (Fisher, Fair Lawn, NJ) using 80% (v/v) glycerol (Sigma, St. Louis MO). GUS staining was visualized using the MZFIII stereoscope (Leica, Switzerland). In most cases, independent transgenic lines showed similar GUS activity patterns. However, examples of

single lines that showed an expression pattern distinct from other lines were also identified. We reasoned that such lines may have the transgene inserted at a genomic site that aberrantly influenced expression or may have experienced a mutation upon transformation. Thus, *GUS* expression analyses are shown only when at least three independent transgenic lines displayed similar *GUS* expression patterns.

Collection of expression data

Expression data were collected from the Genevestigator (Zimmermann *et al*., 2004, 2005) website (www.genevestigator.ethz.ch). The source code for the Genevestigator data was downloaded and decoded into tab-delimited format for uploading into Microsoft Excel spreadsheet format (Redmon, WA). Data are reported as absolute expression values, and a VBA Macro was developed to color code the data according to a range of absolute expression values rather than by relative expression, as it is reported on the Genvestigator website (www.genevestigator.ethz.ch). In this way, expression magnitude comparisons among the genes can easily be assessed.

Results

Generation of XTH::GUS *transgenic lines*

To assess differential regulation of expression of the Arabidopsis *XTH* family, we took the approach of generating transgenic plants expressing the β-glucuronidase-encoding *GUS* reporter gene under the control of the potential regulatory regions lying upstream of the *XTH* genes. Using gene-specific PCR primers (Table, Supplementary material), we were able to generate DNA products corresponding to an approximately 1 kb region found upstream from the translational start site of 29 of the 33 Arabidopsis *XTH* genes. These PCR fragments were verified by sequencing and ligated upstream of the *GUS* reporter gene in the pCB301 vector (Xiang *et al*., 1999) capable of propagating in *Agrobacterium* and being transformed into plant cells.

Basta-resistant transgenics were obtained for 28 of the 29 vectors transformed. At least three independent lines of each transgene were analyzed for staining patterns. Staining patterns were also compared to expression data obtained from other methods that directly assess *XTH* RNA accumulation, including northerns, microarray analysis and quantitative RT-PCR (Q-RT-PCR) (Medford *et al*., 1991; Xu *et al*., 1995, 1996; Akamatsu *et al*., 1999; Yokoyama and Nishitani, 2001; Hyodo *et al*., 2003; Lee *et al*., 2005; Ma *et al*., 2005; Matsui *et al*., 2005; Nishitani, 2005; Vissenberg *et al*., 2005). *XTH4::GUS*, *XTH6::GUS*, *XTH7::GUS*, *XTH27::GUS* and *XTH31::GUS* transgenics had staining patterns that were not consistent with other expression analyses (data not shown). For example, *XTH6* and *XTH7* transcripts have been detected in many plant organs (Yokoyama and Nishitani, 2001, Ma *et al*., 2005, and as discussed below). However, no GUS staining was found in *XTH7::GUS* transgenics, and *XTH6::GUS* transgenics had detectable GUS activity only in stipules and abscission zones. The limited *XTH6::GUS* staining was lost in later generations. By RNA-based methods of expression analysis, *XTH4* is expressed very strongly and nearly ubiquitously (Akamatsu *et al*., 1999; Yokoyama and Nishitani, 2001, and as discussed below). We found only one *XTH4::GUS* line out of 8 showed very faint GUS activity. Similarly, only one line each of the *XTH27::GUS* and *XTH31::GUS* transgenics stained and activity was limited to anthers and roots, respectively, even though both genes are expected to have more widespread expression based on other RNA-detection methods (Akamatsu *et al*., 1999; Yokoyama and Nishitani, 2001; Ma *et al*., 2005; Matsui *et al*., 2005, and as discussed below). We conclude that either the 5′ upstream regions used in these constructs were insufficient to confer appropriate regulation of *GUS* expression or that transgene silencing affected expression. *XTH2::GUS*, *XTH3::GUS*, *XTH11::GUS* and *XTH20::GUS* transgenics did not exhibit staining under any of the conditions we tested (data not shown); other expression data suggest that these genes are only marginally expressed and thus expression may be too low to generate detectable GUS activity. Further analysis of these lines was not undertaken.

For 16 genes, we obtained multiple independent transgenic lines whose patterns of GUS activity matched aspects of expression expected from direct RNA detection methods. To visualize localized organ and tissue expression of the

distinct *XTH::GUS* transgenes, we stained transgenic plants at early seed germination (approximately 3 days post sowing), light-grown seedling development (approximately 7–10 days post sowing), dark-grown seedling development (approximately 6 days post sowing) and flowering.

XTH::GUS *expression in germinating seeds and young seedlings*

As the seed coat bursts open, expression is detectable in 7 of the *XTH::GUS* transgenics (Figure 1, top two rows). *XTH1::GUS*, *XTH5::GUS*, *XTH29::GUS* and *XTH30::GUS* show expression in both radicles and cotyledons (Figure 1 "*XTH1*a", "*XTH5*a", "*XTH29*a", "*XTH30*a"). *XTH14:GUS* and *XTH15::GUS* expression is more clearly restricted to the radicle and root hairs (Figure 1 "*XTH14*a", *XTH15*a"), whereas *XTH28::GUS* expression is localized primarily to the cotyledons with a minor amount of staining in the hypocotyls (Figure 1 "*XTH28*"). *XTH29::GUS* and *XTH30::GUS* may also have some activity in the seed coat itself (Figure 1 "*XTH29*a", "*XTH30*a").

XTH::GUS *expression patterns in seedlings*

Figure 1 also illustrates expression of *XTH::GUS* transgenes in nascent seedlings fully emerged from the seed coat. *XTH1::GUS* is active in the basal portion of the hypocotyl and in the radicle, with relatively high activity in root hairs (Figure 1 "*XTH1*b"). *XTH5::GUS* and *XTH15::GUS* activity patterns are similar to *XTH1::GUS*, but hypocotyl staining is more uniform up to the apical hypocotyl (Figure 1 "*XTH5*b", "*XTH15*b"). *XTH15::GUS* staining also extends to the vasculature of the cotyledons (Figure 1 "*XTH15*b"). *XTH9::GUS* and *XTH29::GUS* show uniform expression throughout the young seedlings with robust expression in cotyledons, hypocotyls and young roots (Figure 1 "*XTH9*", "*XTH29*b"). *XTH12::GUS*, *XTH14::GUS* and *XTH21:GUS* activities are largely limited to the roots (Figure 1 "*XTH12*", "*XTH14*b"*"XTH21"*). Each root-specific expression pattern, however, is distinct for these three transgenes. Whereas *XTH12::GUS* expression is strongest near the tip (Figure 1 "*XTH12*"), expression of *XTH14::GUS* is nearly absent from the extreme tip, but is high in the differentiation zone, low in the central root portion and somewhat enhanced at the root/shoot junction (Figure 1 "*XTH14*b"). *XTH21::GUS* is relatively uniform throughout the root, except for a lack of expression at the extreme root tip (Figure 1 "*XTH21*"). *XTH15::GUS* also has strong expression in the root (Figure 1 "*XTH15*b"). *XTH24::GUS* activity is limited to the center portion of cotyledons at this stage (Figure 1 "*XTH24*"); *XTH33::GUS* is also in the cotyledons and apical portion of the hypocotyl (Figure 1 "*XTH33*"). *XTH30::GUS* is most strongly expressed in the hypocotyl, however, faint staining is also detected in the cotyledon vasculature (Figure 1 "*XTH30*b").

XTH::GUS *expression in differentiating seedlings and etiolated plants*

Many of the *XTH::GUS* transgenes tend to lose robust expression as seedlings age. *XTH1::GUS*, *XTH30::GUS* and *XTH33::GUS* staining becomes limited to the cotyledon and/or leaf tips (Figure 2 "*XTH1*", "*XTH30*", "*XTH33*"). *XTH9::GUS* has strong relatively constitutive expression before the production of true leaves (Figure 2 "*XTH9*a"), but activity levels fall as the shoot matures (Figure 2 "*XTH9*b"). Similarly, *XTH15::GUS*, *XTH18::GUS*, *XTH24::GUS* and *XTH29::GUS* staining largely disappears from the cotyledons and becomes mostly restricted to the vasculature of the cotyledons and/or rosette leaves (Figure 2 "*XTH15*a", "*XTH18*a", "*XTH24*"). *XTH5::GUS* and *XTH15::GUS* expression is enriched in the hypocotyl at this stage, with strongest staining in the vasculature (Figure 2 "*XTH5*", "*XTH15*a"). *XTH28::GUS* maintains strong GUS activity in the cotyledons, emerging leaves, apical hypocotyl and trichomes (Figure 2 "*XTH28*b"); at later stages *XTH28::GUS* is also expressed strongly in cauline leaves (data not shown). *XTH32::GUS*, which did not show significant activity in seed coat-emerging seedlings, is detected prominently in the shoot apex of older seedlings (Figure 2 "*XTH32*").

XTH9::GUS and *XTH28::GUS* have strong staining in dark-grown seedlings, suggesting that these *XTH* genes are expressed in seedlings regardless of the light conditions (Figure 2 "*XTH9*c", "*XTH28*c"). In contrast, the expression domains of *XTH15::GUS* and *XTH18::GUS*

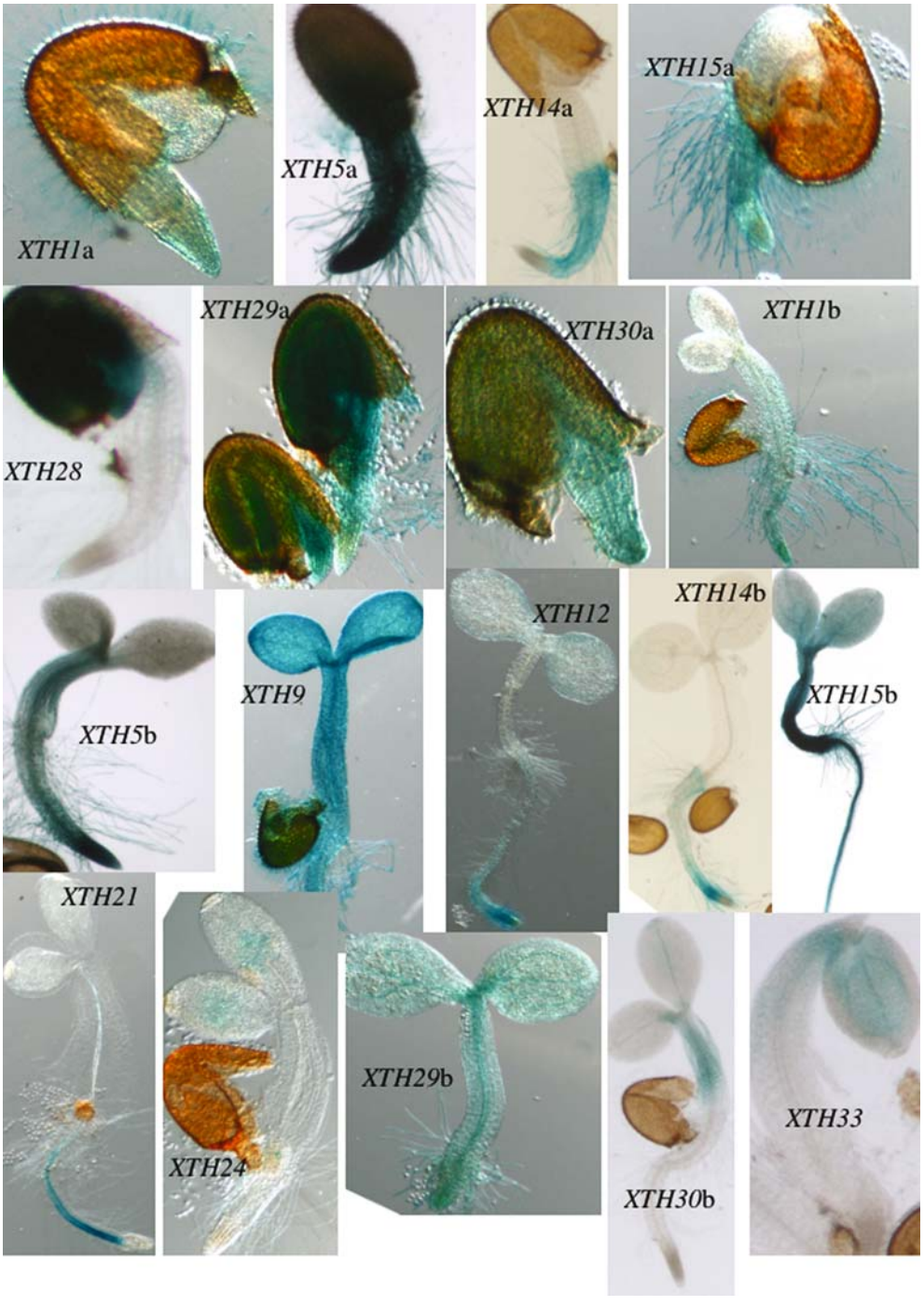


Figure 1. Localization of *XTH::GUS* expression in newly emerging Arabidopsis transgenic seedlings. Labels refer to the *XTH* gene from which the potential regulatory region driving *GUS* was derived. For transgenics for which more than one photo is displayed, lower case letters serve to enable specific referencing in the text.

appear to be extended in seedlings grown in the dark; etiolated *XTH15::GUS* and *XTH18::GUS* have strong GUS staining not only in the etiolated hypocotyl, but also cotyledons (Figure 2 "*XTH15*b", "*XTH18*b"). *XTH15* and *XTH18* are both upregulated in expression by darkness

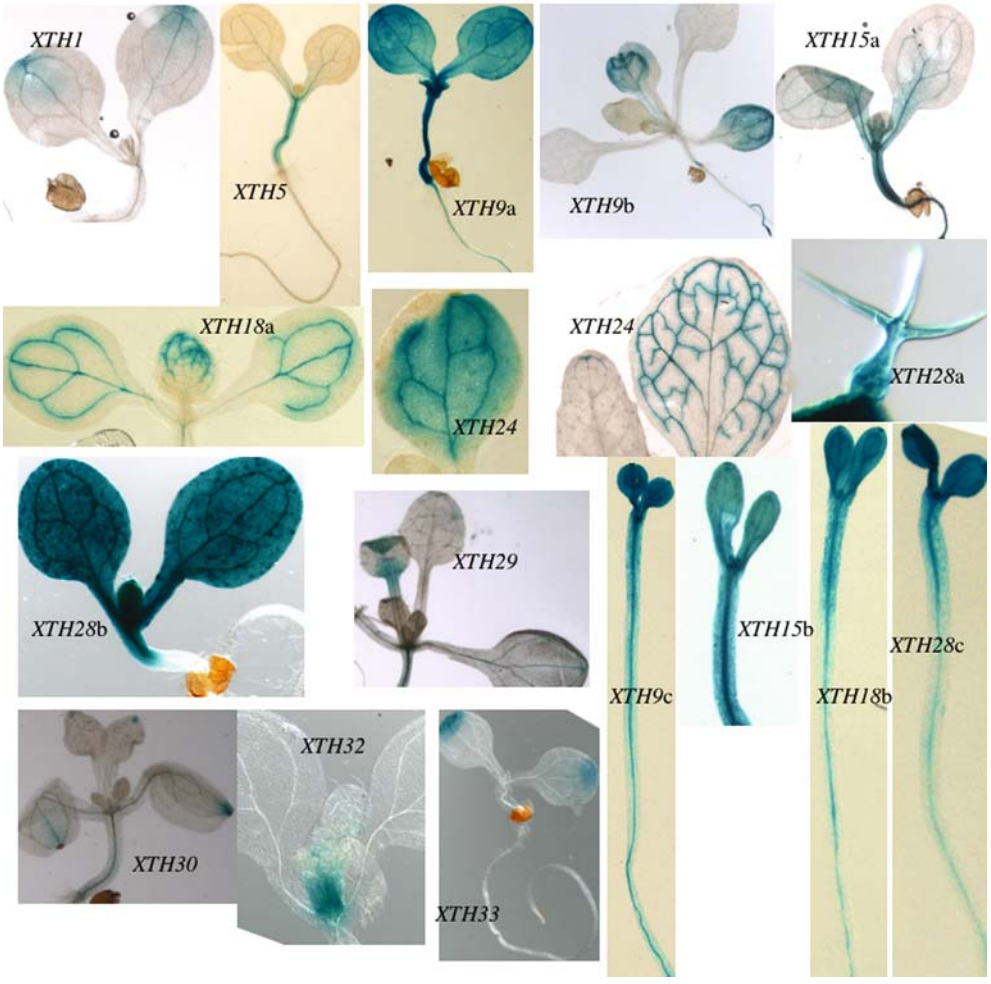


Figure 2. Localization *XTH::GUS* expression in light- and/or dark-grown Arabidopsis seedlings. Photo labeling is described in Figure 1.

(Lee *et al*., 2005). All four of the transgenes expressed in etiolated seedlings show staining throughout the elongated hypocotyl with concentrated staining apparent in the vasculature and additionally strong staining in the cotyledons (Figure 2 "*XTH9*c", *XTH15*b", "*XTH18*b", "*XTH28*c").

Diverse XTH:GUS *expression patterns in mature roots*

Figure 3 illustrates the expression patterns of 11 *XTH::GUS* transgenics that show activity in mature roots. *XTH5::GUS* staining is detected in the extreme root tip and regions of the elongation zone (Figure 3 "*XTH5*"). *XTH9::GUS* expression is excluded from the extreme tip, but has highest expression in the meristematic region and extends into the elongation and differentiation zones (Figure 3 "*XTH9*a"). Root hairs of *XTH9::GUS* plants are also stained (Figure 3 "*XTH9*a"). Many of the *XTH::GUS* genes are expressed in the differentiation zone, as seen in *XTH12::GUS*, *XTH14::GUS*, *XTH15::GUS*, *XTH18::GUS*, *XTH26::GUS*, and *XTH28::GUS* (Figure 3 "*XTH12*", "*XTH14*", "*XTH15*", "*XTH18*a", "*XTH26*", "*XTH28*"). However, distinctions in the staining patterns are apparent; for example, *XTH18::GUS* expression appears largely restricted to inner cell layers (Figure 3 "*XTH18*a, b"). *XTH30::GUS* staining appears to be restricted to both portions of the meristematic and elongation

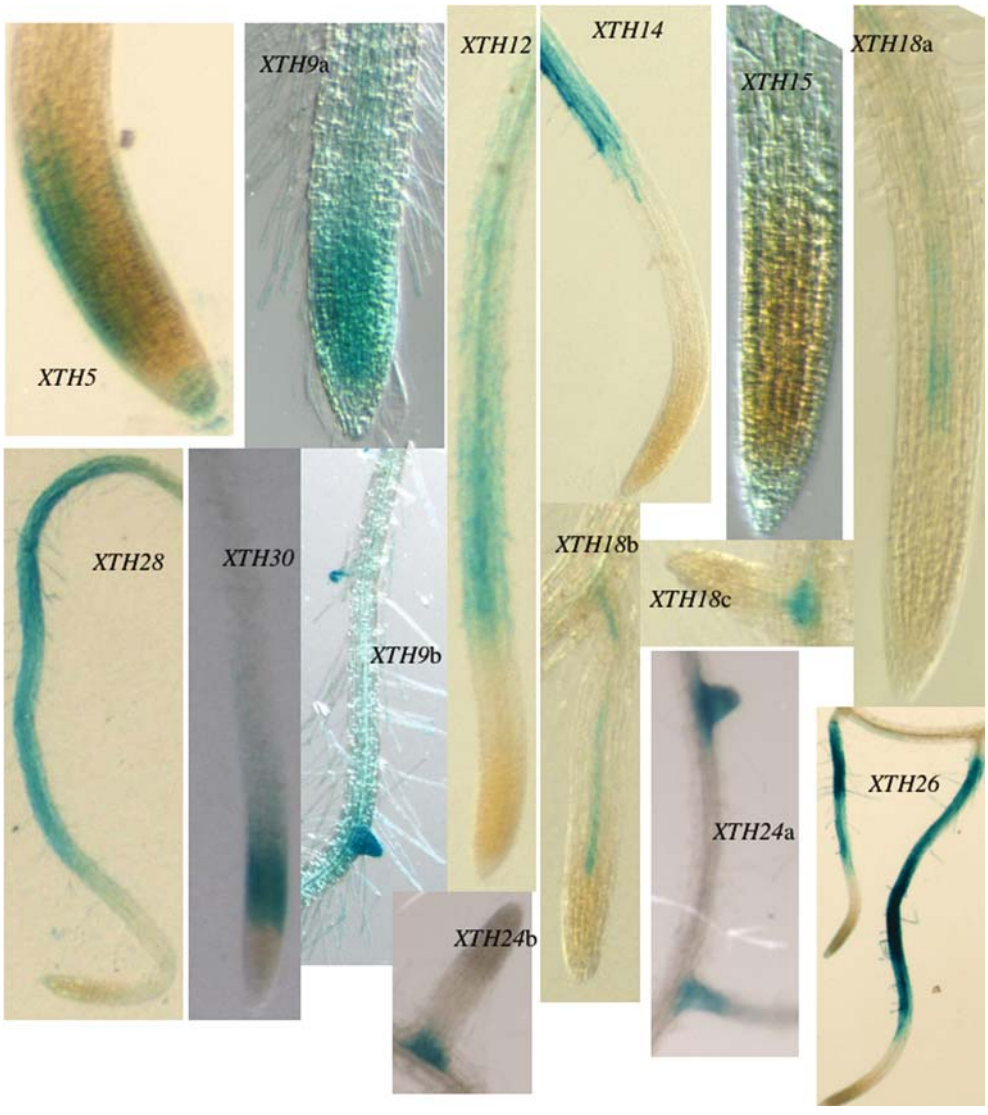


Figure 3. Localization of *XTH::GUS* expression in 7- to 10-day-old Arabidopsis seedling roots. Photo labeling is described in Figure 1.

zones, with a clear absence of expression in the root tip and differentiation zones (Figure 3 "*XTH30*"). *XTH21::GUS* expression continues to also be expressed in mature roots (data not shown) in a pattern similar to that seen in early seedlings (Figure 1 "*XTH21*"). Initiating lateral roots of *XTH9::GUS* transgenics have GUS staining activity (Figure 3 "*XTH9*b"), whereas *XTH24::GUS* activity is seen both at initiated lateral roots and at the base of more extended lateral roots (Figure 3 "*XTH24*a, b"). *XTH18::GUS* staining is seen at the base of young lateral roots (Figure 3 "*XTH18*c") and in the vasculature of more mature laterals (Figure 3 "*XTH18*b"). *XTH26::GUS* is high in lateral roots, but most prominently in lateral roots that have extended differentiation zones (Figure 3 "*XTH26*").

XTH::GUS *expression in floral organs*

Nine *XTH* potential regulatory regions drive *GUS* reporter expression in flower organs (Figure 4). *XTH1::GUS*, *XTH29::GUS*, *XTH30::GUS* and *XTH33::GUS* activity is largely limited to anthers (Figure 4 "*XTH1*a", "*XTH29*b", "*XTH30*a, b",

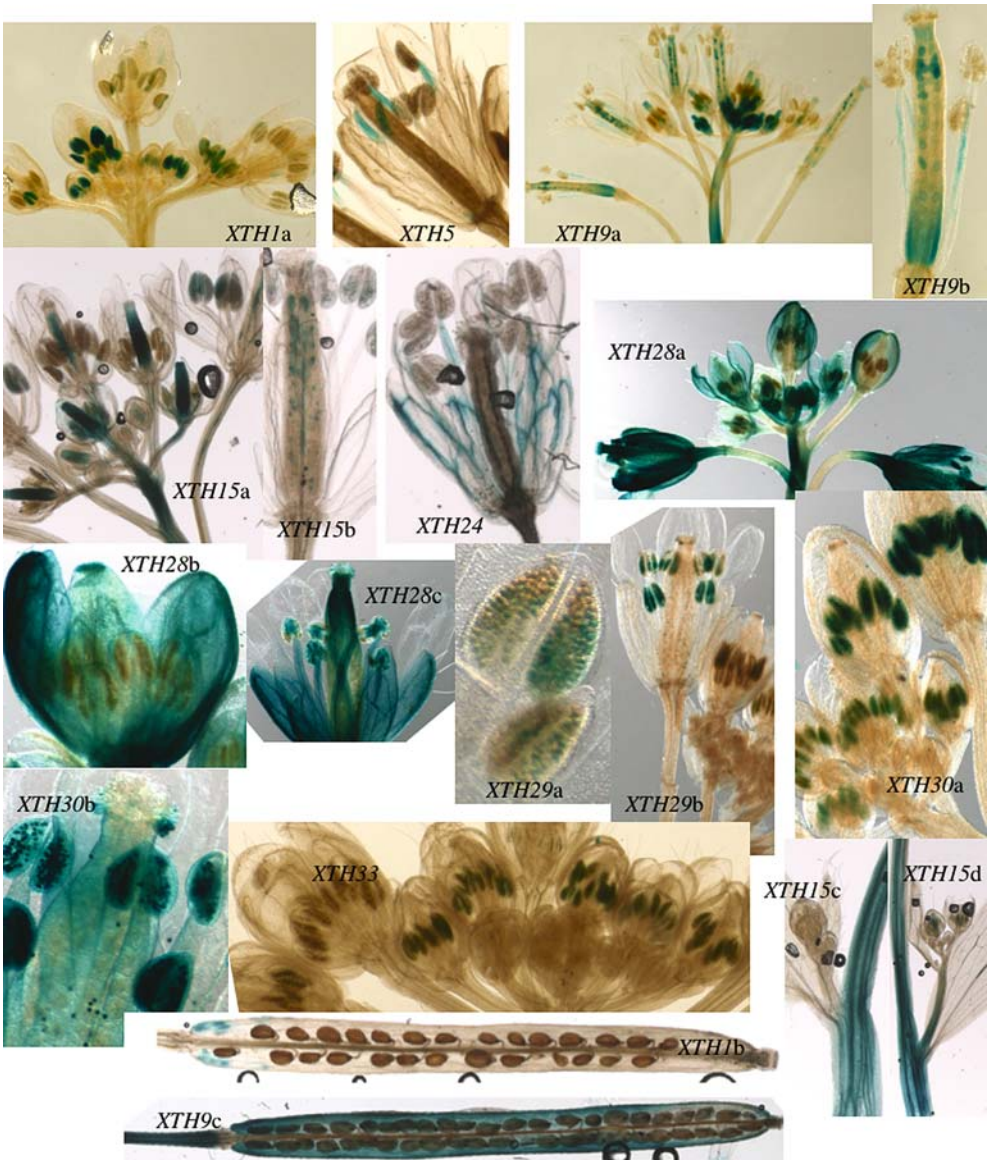


Figure 4. Localization of *XTH::GUS* expression in Arabidopsis floral organs. Photo labeling is described in Figure 1.

"*XTH33*"). Whereas *XTH1::GUS* activity is seen in very young flowers, *XTH33::GUS* staining occurs in intermediate stages of anther development (Figure 4 "*XTH1*a", "*XTH33*"). *XTH30::GUS* expression is also seen in intermediate anther development stages and also mature anthers and pollen (Figure 4 "*XTH30*a, b"). *XTH29::GUS* is expressed in the late stages of pollen development being limited to mature anthers ("*XTH29*a, b"). In young flowers, *XTH28::GUS* is expressed in the outer whorls of sepals and petals (Figure 4 "*XTH28*a, b"); as flowers mature, expression spreads into the stamen filaments and anthers and to the distal portions of the style (Figure 4 "*XTH28*c"). *XTH5::GUS* expression is limited to the distal portions of the stamen filaments (Figure 4 "*XTH5*"). *XTH9::GUS* staining is seen throughout young flower buds (Figure 4 "*XTH9*a") and becomes more restricted in mature flowers where filaments, the base and apex of the style, and developing seed stain for GUS activity (Figure 4 "*XTH9*b"). *XTH15::GUS* expression is most prominent in the styles in both developing and mature flowers

(Figure 4 "*XTH15*a, b"). *XTH24::GUS* is limited to the vasculature of sepals and petals (Figure 4 "*XTH24*"). In maturing siliques, *XTH1::GUS* expression is seen in portions of the silique base (Figure 4 "*XTH1*b"); *XTH9::GUS* staining is seen diffusively in the walls of the silique and within the pedicel (Figure 4 "*XTH9*c").

XTH9::GUS, *XTH15::GUS* and *XTH28::GUS* are also expressed in the inflorescence stem (Figure 4 "*XTH9*a", "*XTH15*c, d", "*XTH28*a"). *XTH15::GUS* expression is detected most strongly in vascular strands (Figure 4 "*XTH15*c") and moves into lateral branches as they extend (Figure 4 "*XTH15*d").

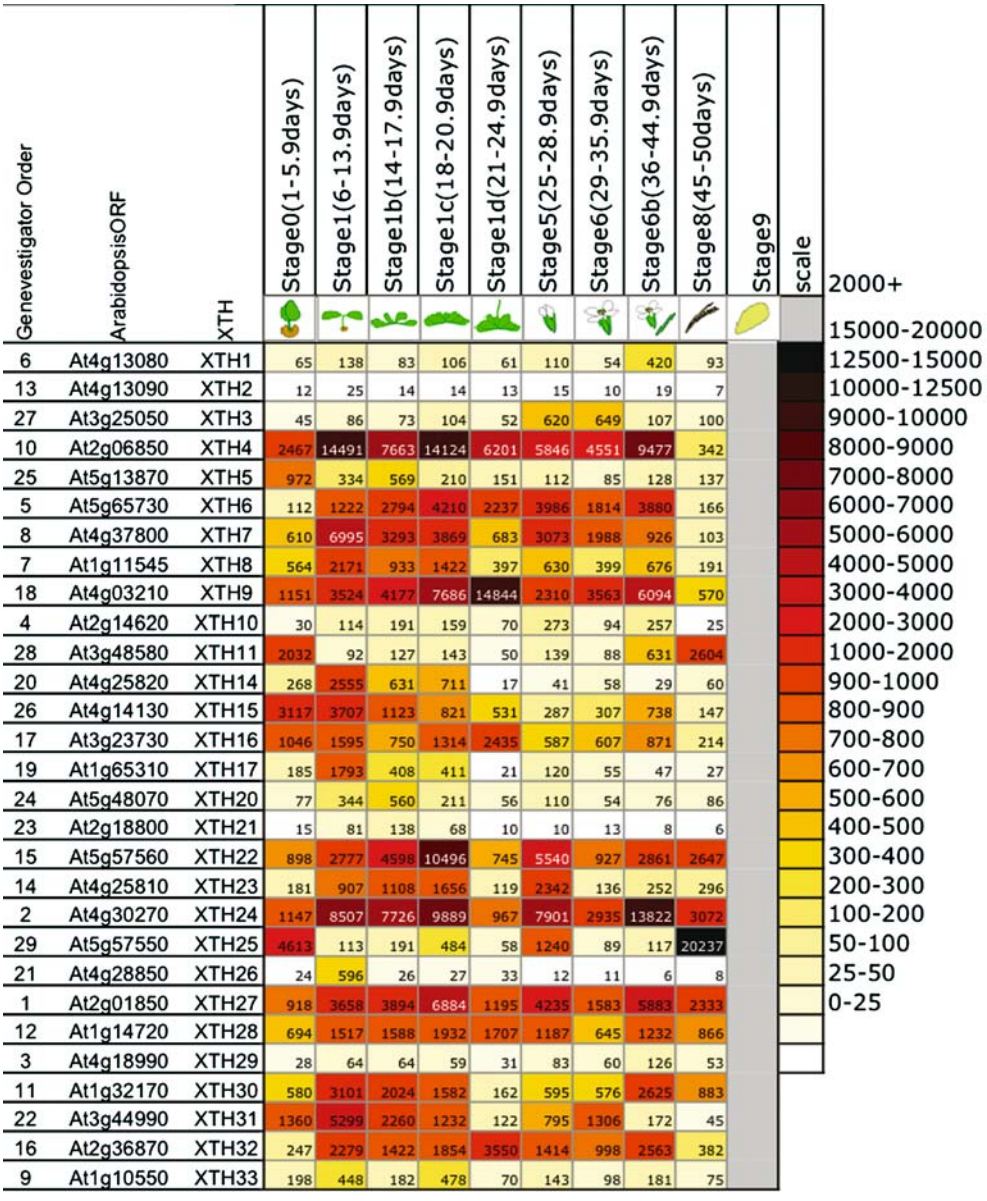
Genevestigator reported XTH *expression*

As we were generating and characterizing the *XTH::GUS* transgenics, additional approaches became available to gain additional insights into expression patterns of Arabidopsis genes. To complement the reporter GUS assays, we extracted Genevestigator (Zimmermann *et al*., 2004, 2005) (www.genevestigator.ethz.ch) data for the Arabidopsis *XTH* gene family. These data are color coded to provide absolute expression levels revealing comparative quantitative assessment of RNA accumulation and, in addition, to indicating broad expression patterns over developmental stages and in distinct organs (Figures 5 and 6). Gene-specific Genevestigator expression data could be obtained for every *XTH* except for the pairs of *XTH12*/*XTH13* and *XTH18*/*XTH19* for which cross reactivity of probes was possible (www.genevestigator.ethz.ch). We have not included expression information for these four genes because of the inability to distinguish gene-specific transcripts derived from these gene pairs.

Figure 5 reports expression of the *XTH* genes over 9 stages of plant development. *XTH2*, *XTH10* and *XTH29* RNAs are found in low abundance over all stages; whereas *XTH27* and *XTH28* have relatively high and constant expression. *XTH1* and *XTH26* transcripts are relatively rare; whereas *XTH1* expression is limited to primarily mature flowering (Figure 5 "Stage 6b"), *XTH26* is largely restricted to young seedlings (Figure 5 "Stage 1"). *XTH1::GUS* patterns are largely consistent with these data, as *XTH1::GUS* has detectable activity only in nascent seedlings and early flower anthers and late-stage siliques (Figure 1 "*XTH1*", Figure 4 "*XTH1*a, b"). *XTH3* expression is also low, with peaks of RNA accumulation during flowering (Figure 5 "Stage 5" and "Stage 6"). *XTH11* and *XTH25* share the characteristic of having maximal expression very early and very late in development; in contrast, *XTH4*, *XTH6* and *XTH32* are expressed at their lowest levels at the earliest and latest developmental stages. *XTH14* and *XTH17* have similar expression behavior over the developmental stages with highest expression in young seedlings (Figure 5 "Stage 1") and much lower levels later in development.

Genevestigator data can be clustered to group genes with related aspects of expression behaviors. Figure 6 summarizes clustered organ-localized expression of the *XTHs*. Clustering genes in this way illustrates examples of specialization among the *XTH*s. For example, expression levels of *XTH1*, *XTH2*, *XTH29* and *XTH30* are very high in pollen and/or stamens relative to the expression of these genes in other organs and/or tissues suggesting a potentially specialized function in pollen development and/or function (Figure 6 "Stamen", " Pollen"). In contrast, the other 25 *XTHs* characterized by Genevestigator have relatively little expression in pollen. *XTH1::GUS*, *XTH29::GUS* and *XTH30::GUS* transgenics anther staining patterns are consistent with the Genevestigator data, however, the GUS activity patterns provide additional information with respect to stage-specific distinctions of expression (Figure 4 "*XTH1*a", *XTH29*a, b" "*XTH30*a, b").

XTH11 and *XTH25* have relatively dedicated expression in seeds (Figure 6 "Seed"). *XTH14*, *XTH17*, *XTH20* and *XTH21* are largely restricted in their expression to seedling radicles and roots (Figure 6 "Radicle", "Roots", "Lateral Roots", "Elongation Zone"). The *XTH14::GUS* and *XTH21::GUS* transgenics stain for activity exclusively in roots and the GUS patterns reveal distinct domains of root-specific expression (Figure 1 "*XTH14*a, b", "*XTH21*" and Figure 3, "*XTH14*"). *XTH12* is reported by the massively parallel signature sequencing technique (MPSS) (Meyers *et al*., 2004) and our *XTH12::GUS* reporter (Figures 1, and 3 "*XTH12*") to be another *XTH* gene with root-specific expression. In sharp contrast to the set of *XTH* genes enriched in root expression, *XTH6* is expressed throughout shoot tissue but its expression is largely absent from roots (Figure 6).



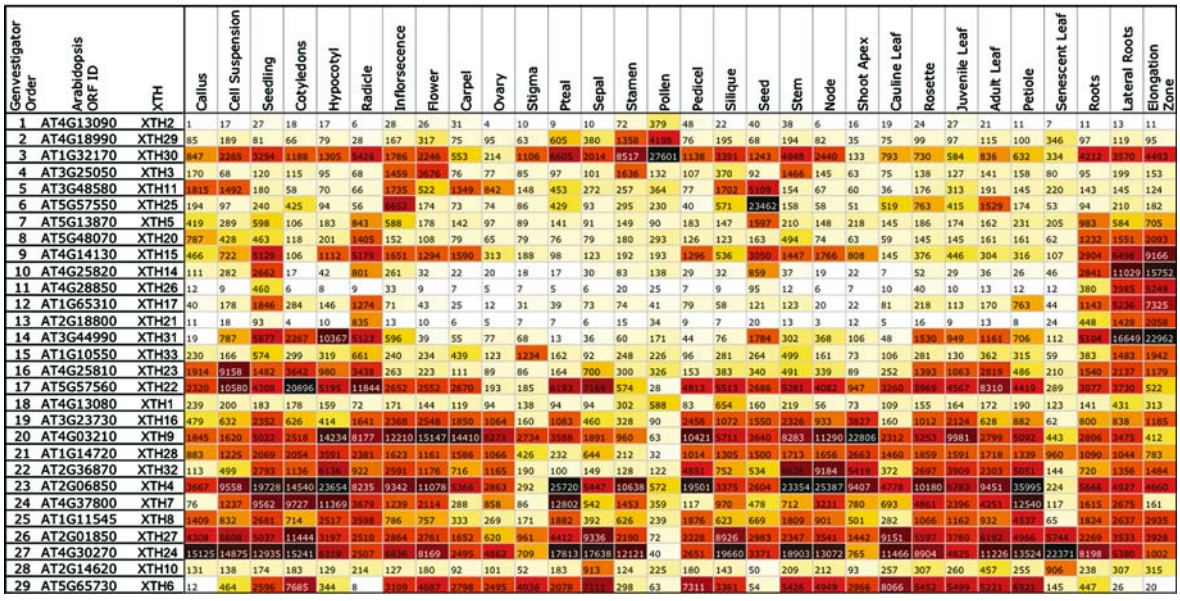
Genevestigator Order	ArabidopsisORF	XTH	Stage0(1-5.9days)	Stage1(6-13.9days)	Stage1b(14-17.9days)	Stage1c(18-20.9days)	Stage1d(21-24.9days)	Stage5(25-28.9days)	Stage6(29-35.9days)	Stage6b(36-44.9days)	Stage8(45-50days)	Stage9
6	At4g13080	XTH1	65	138	83	106	61	110	54	420	93	
13	At4g13090	XTH2	12	25	14	14	13	15	10	19	7	
27	At3g25050	XTH3	45	86	73	104	52	620	649	107	100	
10	At2g06850	XTH4	2467	14491	7663	14124	6201	5846	4551	9477	342	
25	At5g13870	XTH5	972	334	569	210	151	112	85	128	137	
5	At5g65730	XTH6	112	1222	2794	4210	2237	3986	1814	3880	166	
8	At4g37800	XTH7	610	6995	3293	3869	683	3073	1988	926	103	
7	At1g11545	XTH8	564	2171	933	1422	397	630	399	676	191	
18	At4g03210	XTH9	1151	3524	4177	7686	14844	2310	3563	6094	570	
4	At2g14620	XTH10	30	114	191	159	70	273	94	257	25	
28	At3g48580	XTH11	2032	92	127	143	50	139	88	631	2604	
20	At4g25820	XTH14	268	2555	631	711	17	41	58	29	60	
26	At4g14130	XTH15	3117	3707	1123	821	531	287	307	738	147	
17	At3g23730	XTH16	1046	1595	750	1314	2435	587	607	871	214	
19	At1g65310	XTH17	185	1793	408	411	21	120	55	47	27	
24	At5g48070	XTH20	77	344	560	211	56	110	54	76	86	
23	At2g18800	XTH21	15	81	138	68	10	10	13	8	6	
15	At5g57560	XTH22	898	2777	4598	10496	745	5540	927	2861	2647	
14	At4g25810	XTH23	181	907	1108	1656	119	2342	136	252	296	
2	At4g30270	XTH24	1147	8507	7726	9889	967	7901	2935	13822	3072	
29	At5g57550	XTH25	4613	113	191	484	58	1240	89	117	20237	
21	At4g28850	XTH26	24	596	26	27	33	12	11	6	8	
1	At2g01850	XTH27	918	3658	3894	6884	1195	4235	1583	5883	2333	
12	At1g14720	XTH28	694	1517	1588	1932	1707	1187	645	1232	866	
3	At4g18990	XTH29	28	64	64	59	31	83	60	126	53	
11	At1g32170	XTH30	580	3101	2024	1582	162	595	576	2625	883	
22	At3g44990	XTH31	1360	5299	2260	1232	122	795	1306	172	45	
16	At2g36870	XTH32	247	2279	1422	1854	3550	1414	998	2563	382	
9	At1g10550	XTH33	198	448	182	478	70	143	98	181	75	

Figure 5. Genevestigator *Arabidopsis XTH* developmental expression. *XTH* expression table obtained from compiled Arabidopsis microarray experiments as reported by Genevestigator. Data are reported as absolute expression values shaded such that higher expression values are dark red as indicated by the scale. The developmental stages are indicated by the approximate days of growth and a depiction of the growth stage.

Discussion

Methods of determining patterns of XTH *expression and* XTH *activities*

XTHs are encoded by large gene families in plants. The maintenance of such a large number of closely related genes suggests that there may be different functions for the distinct family members. Although subtle enzymatic properties of at least some of the Arabidopsis XTHs have been described (Campbell and Braam, 1999b; Steele and Fry, 2000; Steele *et al*., 2001) and might therefore predict differential physiological functions, these differences probably are not the full basis for continued selection of a large gene family. Direct



Genevestigator Order	Arabidopsis ORF ID	XTH	Callus	Cell Suspension	Seedling	Cotyledons	Hypocotyl	Radicle	Inflorescence	Flower	Carpel	Ovary	Stigma	Pteal	Sepal	Stamen	Pollen	Pedicel	Silique	Seed	Stem	Node	Shoot Apex	Cauline Leaf	Rosette	Juvenile Leaf	Adult Leaf	Petiole	Senescent Leaf	Roots	Lateral Roots	Elongation Zone
1	AT4G13090	XTH2	1	17	27	18	17	6	28	26	31	4	10	9	10	72	379	48	22	40	38	6	16	19	24	27	21	11	7	11	13	11
2	AT4G18990	XTH29	85	189	81	66	79	28	167	317	75	95	63	605	380	1358	4198	76	195	68	194	82	35	75	99	97	115	100	346	97	119	95
3	AT1G32170	XTH30	847	2265	3294	1188	1305	5426	1786	2246	553	214	1106	6605	2014	8517	27601	1138	3391	1243	4848	2440	133	793	730	584	836	632	334	4212	3570	4493
4	AT3G25050	XTH3	170	68	120	115	95	68	1459	3676	76	77	85	97	101	1636	132	107	370	92	1466	145	63	75	138	127	141	158	80	95	199	153
5	AT3G48580	XTH11	1815	1492	180	58	70	66	1735	522	1349	842	148	453	272	257	364	77	1702	5109	154	67	60	36	176	313	191	145	220	143	145	124
6	AT5G57550	XTH25	194	97	240	425	94	56	4452	174	73	74	86	429	93	295	230	40	571	23462	158	58	51	519	763	415	1529	174	53	94	210	182
7	AT5G13870	XTH5	419	289	598	106	183	843	588	178	142	97	89	141	91	149	90	183	147	1597	210	148	218	145	186	174	162	231	205	983	584	705
8	AT5G48070	XTH20	787	428	463	118	201	1405	152	108	79	65	79	76	79	180	293	126	123	163	494	74	63	59	145	145	161	161	62	1232	1551	2093
9	AT4G14130	XTH15	466	722	5128	106	1112	5179	1651	1294	1590	313	188	98	123	192	193	1296	536	3050	1447	1766	808	145	376	446	304	316	107	2904	8498	9166
10	AT4G25820	XTH14	111	282	2662	17	42	801	261	32	22	20	18	17	30	83	138	29	32	859	37	19	22	7	52	29	36	26	46	2841	11029	15752
11	AT4G28850	XTH26	12	9	460	6	8	9	33	9	7	5	7	5	6	20	25	7	9	95	12	6	7	10	40	10	13	12	12	380	3985	5249
12	AT1G65310	XTH17	40	178	1846	284	146	1274	71	43	25	12	31	39	73	74	41	79	58	121	123	20	22	81	218	113	170	763	44	1143	5236	7325
13	AT2G18800	XTH21	11	18	93	4	10	835	13	10	6	5	7	7	6	15	34	9	7	20	13	3	12	5	16	9	13	8	24	448	1428	2058
14	AT3G44990	XTH31	19	787	1877	2267	10367	5123	596	39	55	77	68	13	36	60	171	44	76	1784	302	368	106	48	1530	949	1161	706	112	5104	16649	22962
15	AT1G10550	XTH33	230	166	574	299	319	661	240	234	439	123	1234	162	92	248	226	96	281	264	499	161	73	106	281	130	362	315	59	383	1483	1942
16	AT4G25810	XTH23	1914	9158	1482	3642	980	3438	263	223	111	89	86	164	700	300	326	153	383	340	491	339	89	252	1393	1063	2819	486	210	1540	2137	1179
17	AT5G57560	XTH22	2320	10580	4308	20896	5195	11844	2652	2552	2670	193	185	8193	7169	574	28	4813	5513	2686	6281	4082	947	3260	5969	4567	8310	4419	289	3077	3230	522
18	AT4G13080	XTH1	239	200	183	178	159	72	171	144	119	94	138	94	94	302	588	83	654	160	219	56	73	109	155	164	172	190	123	141	431	313
19	AT3G23730	XTH16	479	632	2352	626	414	1641	2368	2548	1850	1064	160	1083	460	328	90	2458	1072	1550	2326	933	3827	160	1012	2124	628	882	62	800	838	1185
20	AT4G03210	XTH9	1845	1620	5022	2518	14234	8177	12210	15147	14410	6221	2734	3588	1891	960	63	10421	5711	3640	8283	11290	22806	2312	5253	9981	2799	5092	443	2806	3475	412
21	AT1G14720	XTH28	883	1225	2069	2054	3591	2381	1623	1161	1586	1066	426	232	644	212	32	1014	1305	1500	1713	1656	2663	1460	1859	1591	1718	1339	960	1090	1044	783
22	AT2G36870	XTH32	113	499	2793	1136	6136	922	2591	1176	716	1165	190	100	149	128	122	4851	752	534	4626	9184	5419	372	2697	3909	2303	5051	144	720	1356	1484
23	AT2G06850	XTH4	3667	9558	19728	14540	23654	8235	9342	11078	5366	2863	292	25720	3447	10638	572	19501	3375	2604	23354	25387	9407	4276	10180	6783	9451	35995	224	5666	4927	4660
24	AT4G37800	XTH7	76	1237	8562	9727	11369	3679	1239	2114	288	858	86	12802	542	1453	359	117	970	478	712	3231	780	693	4861	2396	4251	12540	117	1615	2675	161
25	AT1G11545	XTH8	1409	832	2681	714	2517	3598	786	757	333	269	171	1882	392	626	239	1876	623	669	1809	901	501	282	1066	1162	932	4537	65	1824	2637	2935
26	AT2G01850	XTH27	4308	5608	5037	11444	3197	2510	2864	2761	1652	620	961	4412	9336	2190	72	2228	8926	2983	2347	3541	1442	8151	5597	3780	6192	4966	5744	2269	3533	3928
27	AT4G30270	XTH24	15125	14875	12935	15241	8319	2507	6836	8160	2495	4862	709	17813	17638	12121	40	2651	19660	3371	18903	13072	765	11466	8904	4825	11226	13524	22371	8198	5380	1002
28	AT2G14620	XTH10	131	138	174	183	129	214	127	180	92	101	52	183	913	124	225	180	143	50	209	212	93	257	307	260	457	255	906	238	307	315
29	AT5G65730	XTH6	12	464	7596	7685	344	8	2109	4687	2798	2495	4026	2078	5111	298	63	7311	3361	54	5426	4949	2966	8066	8452	5499	5221	6921	145	447	26	20

Figure 6. Genevestigator *Arabidopsis XTH* organ-specific expression. *XTH* expression table obtained and reported as described in Figure 5. Organs for which data are included are indicated at the top.

gene-targeted RNA analyses by RNA blots and/or RT-PCR, have found evidence of differential expression of the Arabidopsis *XTH* gene family members (Medford *et al*., 1991; Xu *et al*., 1995, 1996; Akamatsu *et al*., 1999; Hyodo *et al*., 2003; Matsui *et al*., 2005; Nishitani, 2005; Vissenberg *et al*., 2005). A comprehensive RT-PCR approach indicated that the Arabidopsis *XTHs* are differentially expressed among organs, such as root, leaf, stem, flower and siliques (Yokoyama and Nishitani, 2001). These data, along with microarray results (Zimmermann *et al*., 2004; Lee *et al*., 2005; Ma *et al*., 2005), have provided evidence that each of the 33 *XTH* genes is transcribed and therefore this gene family is unlikely to include unexpressed pseudogenes.

Different methods of gene expression detection each have limitations and qualifications. RNA analyses through hybridization are subject to the potential for cross hybridization with transcripts from closely related genes. Oligo-based microarrays are remarkably gene specific and potential inaccuracies can be identified. Sequence-based methodologies are likely to be very specific, but limited either because of the sheer number of sequences required to obtain a global view of expression or because sequence characteristics, such as the availability of a restriction site (Brenner *et al*., 2000), can influence the data. Immunolocalization can be problematic with gene families encoding highly similar proteins; cross reactivity is possible, if not likely. Indeed, antibodies raised against one Arabidopsis XTH (XTH22, also called TCH4) interacted with several other Arabidopsis XTH proteins (Antosiewicz *et al*., 1997). Reporter gene constructs also have limitation in that complete regulatory sequences are required to recapitulate the expression behavior of the native gene. In addition, characteristics of the reporter products, such as stability and diffusibility, can affect results. Therefore, interpretation of reporter gene activity must be cautious; verification of expression behaviors with alternative approaches is important. However, a salient advantage of reporter genes is that the *in situ* expression detection method enables observation of regional expression within organs and other localized expression patterns that are not possible with extracted RNA. In this way, transgene reporter expression analysis, such as that described here, is complementary to other RNA accumulation detection methods.

Reporter transgenes: assaying activity of potential 5′ regulatory sequences

In this report, we find that for 16 *XTH* genes, fusion of approximately 1 kb of sequences found upstream from the translational start site is suffi-

cient to drive expression that matches expression behaviors predicted from direct RNA analyses, such as RT-PCR and/or microarrays. Putative regulatory regions of other Arabidopsis *XTHs* have been localized to comparable 5′ regions (Iliev *et al*., 2002; Vissenberg *et al*., 2005). Whether the putative regulatory sequences used here are sufficient to drive expression fully coincident with the native genes requires further analysis. One approach would be to use these putative regulatory sequences to drive wild-type gene expression in corresponding knock out mutants; if the transgene fully suppresses mutant phenotypes then one could conclude that the putative regulatory region is all that is necessary to drive physiologically relevant expression, at least for those functions that are not redundant with those of other genes.

XTHs *are abundantly and ubiquitously expressed*

Expression from the *XTH* gene family members is widespread with every organ and every developmental stage reporting the presence of *XTH* transcripts (Figures 1–6) (Yokoyama and Nishitani, 2001; Meyers *et al*., 2004; Zimmermann *et al*., 2004; Ma *et al*., 2005). Some organs, such as roots, and developmental stages, such as young seedlings, appear to have the greatest overall *XTH* expression (e.g., Figure 6). Such expression is consistent with numerous reports correlating *XTH* expression and/or encoded activity with cell expansion and tissue growth (Fry *et al*., 1992; Hetherington and Fry, 1993; Pritchard *et al*., 1993; Potter and Fry, 1994; Xu *et al*., 1995; Palmer and Davies, 1996; Antosiewicz *et al*., 1997; Catalá *et al*., 1997; Vissenberg *et al*., 2000, 2001, 2003).

Many organs and developmental stages display overlapping *XTH* expression patterns suggesting a potential for combinational XTH action in the cell walls. Whether these distinct enzyme combinations are required for the specific cellular properties or whether these enzymes may act redundantly remains to be determined.

Specialization of expression

One might predict that paralogous genes that encode closely related proteins may have arisen from relatively recent gene duplications and thus may share regulatory properties as well. Expression of *XTH29::GUS* and *XTH30::GUS* transgenes, derived from two very closely related genes, provide one example of close paralogs sharing similar expression patterns. Both *XTH29::GUS* and *XTH30::GUS* transgenics share expression characteristics from the nascent seedling to flowering stages (Figure 1 "*XTH29*a", "*XTH30*a", Figure 2 "*XTH29*" "*XTH30*", Figure 4, "*XTH29*a, b", "*XTH30*a, b", Figure 6). However *XTH29* is expressed at a much lower magnitude than *XTH30* (Figures 5 and 6). Co-expression of closely related paralogs does not appear to be a prevalent characteristic among the *XTH* gene family members. Expression patterns of closely related *XTH*s are generally distinct, as judged by Genevestigator reported organ-specific expression. Paralogous *XTH* genes that may have evolved from the most recent gene duplication events generally show diversity in expression behavior suggesting acquisition of diverse regulatory sequences. Similarly, Arabidopsis *XTH17*, *XTH18*, *XTH19* and *XTH20* are phylogenetically highly related genes that are all expressed in roots; however, regional specialization of expression has apparently evolved suggesting a specialization in physiological function (Vissenberg *et al*., 2005).

XTH *expression in roots and root hairs*

Many *XTHs* have relatively strong expression in roots. Genevestigator data indicate that 21 of the 29 XTHs analyzed have robust root expression (Figure 6). Of the 11 *XTH::GUS* genes that generate detectable GUS activity in the roots (Figures 1 and 3), most of the patterns can be distinguished. This apparent specialization in expression is consistent with analyses and interpretations of transgene reporters of *XTH17*, *XTH18*, *XTH19* and *XTH20* root-localized expression (Vissenberg *et al*., 2005). Robust XET activity is detected in the elongation zone of roots of a variety of plant species and in the trichoblasts of Arabidopsis during the generation of nascent root hairs (Vissenberg *et al*., 2000, 2001, 2003). Based on the quantitative Genevestigator data (Figure 6), one hypothesis is that XTH14, XTH15 and XTH31 may be the major contributors to XET activity detected in the root elongation zone. The *XTH::GUS* data suggest that *XTH5* and *XTH9* are expressed in the more distal root. The technique determining expression profiles of

isolated root cell types adds corroborating evidence for *XTH5* expression because it is the *XTH* most strongly expressed in the epidermis within 0.30 mm from the root tip (Birnbaum *et al*., 2003) (http://www.arexdb.org/index.jsp). The expression patterns of *XTH18::GUS* and *XTH24::GUS* transgenics suggest that these genes are expressed in nascent lateral roots (Figure 3 "*XTH18*b, c", "*XTH24*a, b"); these data are consistent with previous reports on *XTH18* and *XTH24* expression (Medford *et al*., 1991; Vissenberg *et al*., 2005). In addition, *XTH9::GUS* is expressed throughout emerging lateral roots (Figure 3, "*XTH9*b").

XTH21 and *XTH26* stand out among the root-expressed *XTHs* in that they show virtually no expression in other organs (Figure 6), and this behavior is replicated in the *XTH21::GUS* and *XTH26::GUS* transgenics (Figure 1 "*XTH21*", Figure 3 "*XTH26*"). However, these two root-specialized *XTHs* are distinct in that unlike *XTH21*, as reported by both Genevestigator (Figure 6) and the *XTH21::GUS* analysis (Figure 1 "*XTH21*") to be expressed in both seedling radicle and adult roots, *XTH26* transcripts accumulate (Figure 6) and *XTH26::GUS* expression (Figure 3, "*XTH26*") occurs only in mature roots. These results suggest that XTH21 may have a function common to both radicles and mature roots, whereas XTH26 has a role that may be specific for mature root differentiation.

XET activities are also robust in trichoblasts and emerging root hairs (Vissenberg *et al*., 2001, 2005) and this activity has been proposed to be involved in xyloglucan integration in nonexpanding cells (Vissenberg *et al*., 2005). Based on the *XTH::GUS* transgenic analyses, *XTH5*, *XTH9*, *XTH14*, *XTH15*, *XTH26*, *XTH28*, *XTH29* may contribute to the XET activity observed in Arabidopsis root hairs. Cumulatively, these results suggest that XTHs may function in diverse aspects of root development and/or function.

XTH *expression in vasculature*

XTH::GUS transgenics reveal that for many *XTH*s expression may decrease as seedlings age. The restricted expression seen for *XTH9::GUS*, *XTH15::GUS*, *XTH18::GUS* and *XTH24::GUS* becomes evident in the vasculature of the cotyledons and/or leaves (Figure 2 "*XTH9*a", "*XTH15*a", "*XTH18*a", "*XTH24*a, b"). *XTH::GUS* staining in the vasculature is also apparent in etiolated *XTH9:GUS*, *XTH15::GUS*, *XTH18::GUS* and *XTH28::GUS* hypocotyls (Figure 2 "*XTH9*b", "*XTH15*b", "*XTH18*b", "*XTH28*b") and *XTH5::GUS* photomorphogenic hypocotyls (Figure 2 "*XTH5*"). *XTH24::GUS* vasculature activity is also prominent in the sepals and petals of mature flowers (Figure 4 "*XTH24*"). *XTH15::GUS* is prominently expressed in the vasculature of the inflorescence stems (Figure 4 "*XTH15*c, d"). XTHs may function in vasculature morphogenesis as immunodetection of XTHs, XET activity and derived product has been localized coincident with secondary wall biogenesis in stem xylem and phloem fibers (Antosiewicz *et al*., 1997; Bourquin *et al*., 2002). In addition, Arabidopsis mutants defective in *XTH27* have reduced vein number and altered tracheary element shapes (Matsui *et al*., 2005). Whether the other *XTHs* with strong vasculature tissue expression are also necessary for proper development and/or functioning of this tissue will require additional mutant analyses.

XTH *expression during flower development*

A subset of the *XTH* genes have strong expression in flower organs as indicated by Genevestigator (Figure 6), Q-RT-PCR (Yokoyama and Nishitani, 2001), MPSS (Meyers *et al*., 2004), and microarray experiments (Ma *et al*., 2005). The *XTH::GUS* reported expression shows diverse patterns of flower localized expression. Three *XTH::GUS* genes show expression in anthers, however, the timing of expression is distinct for the three genes (Figure 4). *XTH1::GUS* is expressed in the early stages of flower development, *XTH33::GUS* is expressed exclusively in mid stage flower development anthers and *XTH30::GUS* is expressed in intermediate and late stages and continues to have strong expression in mature pollen (Figure 4, "*XTH1*a", "*XTH33*", "*XTH30*a, b"). In addition *XTH29::GUS* is expressed in pollen (Figure 4, "*XTH29*a, b"). Anther development involves complex wall depositions that may have roles in anther dehiscence and pollen release (Keijzer, 1987; Sanders *et al*., 1999). One possibility is that XTH action is required for such wall modifications

and that subsets of XTHs carry out these processes during distinct developmental stages.

XTH9 has previously been shown to be expressed in inflorescence apices by RNA blotting, RNA *in situ* and immunolocalization (Hyodo *et al*., 2003) and in flowers by quantitative RT-PCR (Yokoyama and Nishitani, 2001). Genevestigator reports that *XTH9* transcript accumulation is highest in the shoot apex and indeed is the *XTH* with the highest expression in the shoot apex (Figure 6). In addition, transcripts from *XTH9* are by far the most abundant *XTH* transcripts during stage1d of development when the inflorescence emergence occurs (Figure 5 ''Stage1d''). The *XTH9::GUS* transgene replicates this expression behavior and reveals details of the localized expression with, for example, highest expression the youngest flowers and loss of generalized expression as the flower matures. *XTH9::GUS* activity remains prominent in the terminal portions of the carpel and throughout the stamen filaments (Figure 4 ''*XTH9*a, b'').

XTH15::GUS and *XTH28::GUS* are also expressed in complex patterns in the developing flower, with prominent expression in the carpel (Figure 4 ''*XTH15*a, b'', ''*XTH28*a, c''); *XTH28::GUS* is also robustly expressed in sepals (Figure 4 ''*XTH28*a, b''). *XTH5::GUS* expression is highly restricted to the distal region of the stamen filament (Figure 4 ''*XTH5*''); one possibility is that *XTH5* is expressed and its gene product may function to modify the filament wall to enable the rapid elongation required for the pollen-rich anthers to reach the stigma.

XTH *expression during leaf senescence and the end of the Arabidopsis life cycle*

Only a small subset of XTHs is expressed at the latest stage of development (Figure 5 ''Stage8''). *XTH25*, for example, may be relatively specialized to be expressed during the very final stages of development as it has the highest magnitude expression of any *XTH* at any developmental stage (Figure 5). Indeed, except for *XTH11* that has significant expression only at the very earliest and latest developmental stages, expression of most other *XTHs* appears to be declining at this final developmental stage (Figure 5). We would predict therefore that XTH25 and perhaps XTH11 play significant roles in the final stage of the Arabidopsis life cycle. The function of XTH25 may be specialized in wall events related to seed development and maturation as *XTH25*'s organ-localized expression is highly limited to seeds (Figure 6 ''Seed''). Silique-specific expression for *XTH25* (previously known as *XTR3*) has also been reported by northern analysis (Hyodo *et al*., 2003) and quantitative RT-PCR (Yokoyama and Nishitani, 2001). Thus, XTH25 may be an XTH specialized in seed-specific XTH function as its expression out surpasses that of any other *XTH*.

Similarly, only a handful of *XTHs* are expressed in senescent leaves, according to the Genevestigator data; these are *XTH10*, *XTH24*, *XTH27* and *XTH28*, with *XTH24* having the overwhelmingly highest expression (Figure 6). Thus, these XTHs may function in the active process of senescence, perhaps in cell wall disassembly and/or degradation.

Expression pattern directed tailoring of reverse genetic studies

Knowledge of genome-wide expression patterns of gene families shed light on potential sites of gene product function. Comprehensive expression analyses are therefore useful for tailoring reverse genetic screens to identify phenotypic consequences of gene-specific mutants. Thus, for example, whereas *XTH5*, *XTH9*, *XTH12*, *XTH14*, *XTH15*, *XTH17*, *XTH18*, *XTH19*, *XTH20*, *XTH21, XTH31* might be predicted to function in distinct domains of primary root morphogenesis or function and *XTH1*, *XTH5*, *XTH9*, *XTH14*, *XTH15*, *XTH26*, *XTH28*, *XTH29* may have specific roles in root hair development, mutants in *XTH25* may be predicted to be defective in seed development and those defective in *XTH10*, *XTH24*, *XTH27* and *XTH28* may fail to undergo productive senescence. *XTH5* knockouts may be defective in filament elongation and *XTH15* mutants may produce defective vascular tissues in the inflorescence. These predictions can now be tested with the reverse genetic approach of probing physiological function of known genes by targeted mutation. For those genes expressed in tissues or organ regions where other *XTHs* are also expressed, mutations in multiple genes may need to be combined to reveal *XTH* function.

Expression analysis of the members of the large Arabidopsis *XTH* family reveals complex and

partially overlapping developmental and organ-specific patterns. These data strongly suggest that XTH action on wall polymers contributes to diverse physiological and morphogenetic processes. Hints of potential function are gleaned from observing sites of expression and can be used as the foundation for a thorough elucidation of XTH function in plant biology.

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