Comparative gene expression analysis in stems of *Dolichos purpureus* and *Arabidopsis thaliana*

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ABSTRACT

The outermost layer epidermis of a plant stem plays an important role in protection and environment-sensing. The mechanisms of sensing and response to the environment through the stem epidermis remain unclear. Here we report enriched expression of genes involved in stress resistance and signal transduction functions in the stem epidermis of both *D. purpureus* and *A. thaliana* by cDNA cloning and QPCR in D. purpureus and by analysis using dataset from a genome-wide comparison with cDNAs differentially expressed between the epidermis and inner parts of top and base stem in *A. thaliana*. Among 188 cDNAs from the stem epidermis of *D. purpureus*, 13% and 17% were related to signal transduction and defense respectively. Most of them were up-regulated more in the stem epidermis than the inner stem, as well as in *A. thaliana*. Also, the distribution of the numbers and specificities of up-regulated genes related to signal transduction and regulatory networks in the epidermis and inner stem revealed the possibility of positional differences in regulation. The results revealed the importance of the epidermis in signal transduction and plant defence.

Keywords: Stem Epidermis; Signal Transduction; Environment; Development

1. INTRODUCTION

The epidermis is a protective layer covering the entire plant, usually comprising one to a few layers of cells: the outmost cuticle layer and the epidermis layer composed of basic cells, trichomes and guard cells. The epidermis begins to differentiate as early as the 8-cell stage, and differentiation of epidermal cell types appears later in embryo development [1-3]. In growing seedlings and mature plants, the epidermal cells of a fully developed plant not only continue to divide and differentiate into specific cell types at specific sites within the epidermis [4], but also secrete cutin and wax to cover the surface, forming a protective layer [5,6]. Also, within the epidermal cells, secondary substances resistant to pathogens and physical stress are produced [7]. The epidermis is also the site at which mechanical and other environmental stimuli are sensed [8-10]. The expression profile of genes in the epidermis changes with developmental stage [11-13] and cell type [7,12-13].

The structure and chemical components of the epidermis layer of the stem are adapted to resist various stresses [7,14]. As the barrier and the site of direct contact between plant and environment, the epidermis is also the most important site for environmental signal perception. How signals are perceived and transduced in the epidermis and conveyed to adjacent tissues has not drawn attention. It remains unknown whether there are receptors sensing stress factors and resistance mechanisms specifically located in the epidermis.

D. purpureus L. which has a purple stem epidermis, grows from spring to early winter. In early winter, the plants become senescent with the cold. Up to that stage, they have experienced pathogens, cold, and various other environmental stresses, so they may have developed some stress responses.

In this study, we sequenced 188 cDNAs from a purple epidermis cDNA library of *D. purpureus* stems collected during early winter and used bioinformatics methods to analyze gene function. In addition,, we grouped, compared and analyzed genes related to signal transduction pathways expressed in the epidermis and the inner stem of both top and base stem using the Arabidopsis stem epidermis gene expression data [15], depicting the spatial and developmental distribution profiles of signal transduction pathway components, thus obtaining indications of a profile of signal perception and transduction in the stem.

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2. MATERIAL AND METHODS

2.1. Stem Epidermis cDNA Library

Total RNA was isolated from the purple epidermis of lentil stem (ca. 1000 μg, collected in November, 2006 in Hangzhou) using the classic guanidine isothiocyanate method [16] followed by treatment with Dnase (1 unit RQ1 Dnase; Promega). Poly(A+) RNA was purified using a biotinylated oligo(dT) primer (Promega), followed by reverse transcription and first strand synthesis. The terminal of the cDNA was blunted and added by an *Eco*R I adapter (5'-EcoR I-GGCACGAGG-3'). After *Eco*R I end phosphorylation and digestion with *Xho* I (5'-Xho I-CTCGAG-3'), all cDNA inserts longer than 500 bp were cloned into a pBluescript® II XR plasmid vector and electroporated into *E. coli* strain DH10B after recollecting the cDNA fragments longer than 500 bp from agarose gel.

2.2. Sequencing and Sequence Annotation

Plasmid DNA was extracted and sequenced using ABI3730XL sequencers. The sequencing primer used was 5'-ATTAACCCTCATCTAAAGGGA-3'. Base-calling was performed with phred. Vector sequences were trimmed using Cross-match. Low-quality bases (quality score <20) were trimmed from both ends of the sequences using Qualtrim and Simpletrim. ESTs longer than 200 bp after both vector and quality trimming were considered "high-quality" ESTs. The repeat sequences in these ESTs were then masked using the RepeatMasker program. The masked sequences were further screened for bacterial chromosomal DNA, RNA, insect viral DNA, rRNA, and mitochondrial DNA using BLASTN [17]. Further screens for possible contaminants were conducted by BLASTN searches of the Non-Redundant Nucleotide Sequences (nt), EST_human, EST_mouse, and EST_others databases. Raw EST data and contigs were compared using BLASTX against the UniProtKB/Swiss-Prot database and BLASTN against EST/N database. The BLAST search was filtered with an e-value set at ≤0.001.

2.3. Quantitative RT PCR

RNA was extracted from triplicate samples of the inner stem and epidermis (each 50 mg, by pealing stem surface skin from stem, the surface skin is the epidemis part, the rest are inner stem) of red stems of *D. purpureus* L. collected in November, 2009 in Wuhan, using TRIZOL reagent (BioTEKE). The mRNAs were reverse transcribed with a reverse transcription kit (BioTEKE). Set I quantitative PCR was conducted by StarGene Co. using a Fluorescent Quantitative Detection System, Model: FQD-48A(A4) (Bioer). The reaction mixture contained cDNA,

specific primers (Supplementary file 1), dNTP, Taq polymerase with buffer (Takara) and SYBRGreen I 1x. The reaction conditions were: 95˚C 3 min, 72˚C 10 s, 95˚C 30 s, Tm (Supplementary file 1) 30 s , 72˚C 30 s, 35 cycles; then 72˚C, 10 min. Set II quantitative PCR was conducted in the CAS Key Laboratory of Genome Science and Information, Beijing Institute of Genomics, using TECHNE TC-512. The reaction mixture contained Primer-F (10 p) and Primer-R (10 p) 0.65 μl each, cDNA 1 μl , SYBR Green/Mix 9 μl, Taq polymerase 0.2 μl, dd H$_2$O to 20 μl. The reaction procedure is described in (Supplementary file 1). Each data is an average of three repeats.

2.4. Analysis of Data from *A. thaliana*

We divided the data from supplementary table 2 in Suh *et al*. [15] into two sets: top and base stem. Genes involved in different signal transduction pathways were selected and compared on the basis of the functions provided. The up-regulation standard was set as the mean ratio of expression level in two tissues >0.5 to cover all possible genes expressed. We defined up-regulation as a signal ratio > 0.5 and down-regulation as a signal ratio < 0.5. Thus, we grouped the data at the whole transcriptome level into four classes: ratio of epidermis to inner stems (epi/inner) > 0.5; <0.5 in both top and base epidermis; and either >0.5 or <0.5 in the top or base epidermis. We also analyzed the genes with up-regulation ratio of epidermis/stem larger than 2.

3. RESULTS AND DISCUSSION

As the defense barrier, the epidermis layer has developed specific surface structures and intracellular chemicals [18, 19,15]. These processes are controlled and directed by internal and environmental signals. The gene expression profile in the stem epidermis reflects the activities of this layer and thus the function of this tissue. In this research, we analyzed the functional pattern of genes expressed in stem epiderm of *D. purpureus* L., further confirmed by QPCR on some representative genes, and compared with the similar genes from *A. thaliana*. Finally, from expression distribution pattern of genes related to signal transduction in the top and base stem, we try to deduct the response pathway of plant to environment.

3.1. Functional Pattern of Genes Expressed in Stem Epidermis of *D. purpureus*

As many as 32% of the 188 sequenced cDNAs in the stem epidermis of *D. purpureus* L. were related to signal transduction and regulation (**Figure 1**), including groups of signal transduction, DNA and RNA processing, regulation which involved in the signal transduction process,
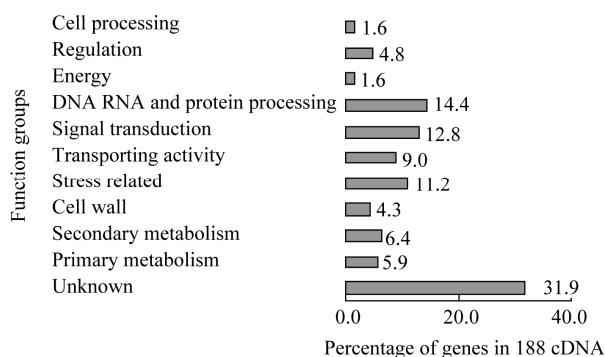
Figure 1. Gene expression profile of 188 cDNAs from purple stem of *D. purpureus* L. Note: some genes are included in different groups, such as cell wall gene CW14s which is water stress related gene, so are included in stress related gene group. Some genes not included in signal transduction group which also play function in final biology effect in pathway of regulation are also considered as in the signal transduction related genes, such as transcription factors (in regulation group), MAPKs (in stress related group), some protein kinase or proteases (in DNA, RNA, protein group).

indicating the importance of signal transduction in this tissue. This result is consistent with that in the stem epidermis of A. thaliana [15].

In the stem epidermis of *D. purpureus*, among known cDNA expressed, the top three classes of expressed cDNAs are stress related, DNA/ RNA/protein processing related and signal transduction related cDNAs. The ratio of them are 17% (6% secondary metabolism plus 11% stress directly related), 14%, and 13% respectively. Transporting related genes are the fourth abundant transcripts in stem epidermis of *D. purpureus* (**Figure 1**). This profile shows that signal transduction and stress resistance are two major functions in stem epidermis. Genes in 13% of the cDNAs expressed in the stem epidermis of *D. purpureus* related to signal transduction were: receptors and membrane proteins, G proteins, MAPK pathway kinases, protein kinases, calcium-signal related, and hormone-signal related (**Table 1**). 17% of the 188 genes expressed in the stem epidermis are directly related to the stress response, among which 6% in secondary pathways and 11% encoding stress resistance proteins (**Figure 1**, **Table 2**).

Expressed genes that function in the regulation of transcription or RNA level constituted 4.8% of the 188 cDNAs of *D. purpureus*. They include *HDZIPIII* family genes, *MYB*103, *WD* family genes, *Knox* gene, *C2H2 zinc finger* family genes, and various genes encoding RNA binding proteins. MYB and WD family proteins are known to form complexes and play key roles in the differentiation of the epidermis [20].

Genes related to various stress resistance include genes encoding the Avr9 elicitor response protein (GR-

463609), which is similar to At1g77810 (87%), LRR Cf2/Cf5 disease resistance protein (GR463639), which is 50% similar to AT5G53890, ERD4 responding to dehydration (GR463575), with 60% similarity to *A. thaliana* ERD4 At1g30360, MLP-like proteins (GR463493, GR-463520, GR463625, GR463645), which are mostly similar to MLP43 in *A. thaliana* (At1g70890), etc. More genes related to stress response such as *AMMECR*1 (*GR-463522 GR463629*), *Steroid sulfotransferase* (*STF*, *GR-463507 GR463615*), *purple acid phosphatase* (*PAP*, *GR-463521 GR463627*), *Chitinase class I* (*GR463543 GR-463595*), *CW*14 (*GR463593*), *Xyloglucan endotransferae* (*GR463567*), *Isoflavone reductase* (*IFR*, *GR463578*), and *Chalcone reductase* (*CHR*, *GR463527*, *GR463635*) are expressed in stem epidermis of *D. purpureus*.

Cf genes are elicited in response to avirulence (Avr) peptides secreted by the pathogen *Cladosporium fulvum*. They encode transmembrane glycoproteins carrying extracytoplasmic Leu-rich repeats (LRRs). Avr9 is one of the Avr peptides; it elicits a set of genes through Cf9 in the hypersensitivity response (HR). These genes are pivotal in the initial defense response characterized by Avr9/Cf9-elicited effects including changes in ion flux, activation of MAPK and a CDPK, and production of active oxygen [21].

ERD4 was induced by various stresses [22,23], and is involved in the brassinosteroid [24], ABA and ethylene [25] hormone regulation pathways.

In humans, AMMECR1 is possibly related to Alport syndrome, mental retardation, midface hypoplasia and eliptocytosis, which involve a contiguous gene deletion syndrome. AMMECR1 is believed to function in RNA base modification [26]. Its sequence is highly similar to ESTs from MOO *Vigna unguiculata*, *Phaseolus vulgaris* and *Glycine max* in plants, which may therefore have a similar catalytic function. GO annotation of AMMECR1 from *A. thaliana* (*AT2G38710*) shows that it responds to salt stress.

Steroid sulfotransferase (STF) catalyzes the sulfonation of steroids. It is reported to be up-regulated in response to cold, NaCl, pathogens, and cell death-triggering reagents [27-30].

The main function of vacular purple acid phosphatase (PAP) is reported to be the release of inorganic phosphates as nutrients from phosphate esters [31,32], cell wall PAP play roles in cell elongation [33,34], some specific PAPs have also been implicated in response to stresses such as drought, heat (At3g17790) [35] and NaCl (GmPAP3) [36,37].

The cell wall is a structural defense layer. Genes for cell wall components were expressed in the epidermis of both *D. purpureus* and *A. thaliana* (**Table 2**). Chitinases are enzymes synthesized by plants that directly target

Table 1. Genes expressed in stem epidermis putatively related to signal transduction in *D. purpureus* and *A. thaliana*.

Possible functions	*D. purpureus*		*A. thaliana*		
	GenBank_Accn	Ratio of epidermis /inner stem	GenBank_Accn	Ratio of epidermis/stem	
				top	base
MAPK4	*GR463588* *GR463529* *R463530*	7.78	*AT4G01370*	0.9	1.0
			AT1G01560	1.9	5.5
Calmodulin	*GR463571*	1.03	*AT3G22930*	0.7	1.1
Calreticulin	*GR463604*	1.16	*AT1G08450*	2.4	1.5
Small Ras-like GTP-binding	*GR463590* *GR463634*	n	*AT5G55190*	0.9	0.9
	GR463526	n	*AT5G20010*	0.5	0.9
avrRpt2-induced gene 1	*GR463469*	−1.20	*AT1G33970*	1.7	1.4
Auxin-repressed DRM1	*GR463562*	n	*AT1G28330*	0.7	1.0
Aux22d	*GR463513*	2.17	*AT5G43700*	1.1	0.7
CKB2	*GR463509* *GR463617*	3.73	*AT4G17640*	1.0	0.9
			AT5G47080	1.2	0.9
Ser/thr phosphatase protein phosphatase-2C	*GR463605*	2.34	*AT1G48040*	1.1	1.3
			AT3G62260	1.0	2.1
			AT3G17250	1.1	0.9
Sae2: sumo-activating enzyme	*GR463597* *GR463545*	1.54	*AT2G21470*	3.1	1.6
RNA-binding protein A KIP1	*GR463550* *GR463608*	n	*AT3G56860*	1.0	1.2
			AT2G41060	1.0	1.4
KNOX STM	*GR463472*	−5.37	*AT1G62360*	0.1	0.4
Homeobox leucine-zipper ATHB-8	*GR463505* *GR463614*	−7.43	*AT4G32880*	0.3	0.2
			AT1G52150	0.2	0.2
14-3-3 GRF2	*GR463601*	n	*AT1G78300*	0.6	0.5
C2H2 Zinc finger	*GR463516*	1.05	*AT5G03150*	0.3	1.3
			AT1G55110	0.6	0.7
GID1-1	*GR463556*	n	*At3g63010**	0.9	1.2
FLA2	*GR463497*	−1.01	*AT4g12730*	1.3	1.4
Receptor-like protein kinase 4	*GR463534*	3.32	*At4g23180*	8.3	5.5

*only present in inner stem. N-not tested.

Table 2. Genes expressed in epidermis of *D. purpureus L.* var. and *A. thaliana* stem putatively related to stress response.

Possible functions	*D. purpureus*		*A. thaliana*		
	GenBank_Accn of	Ratio of epidermis /inner stem	GenBank_Accn	Ratio of epidermis/ top stem	Ratio of epidermis/ base stem
Isoflavone reductase (IFR)	*GR463578*	n	*AT4G39230*	1.1	0.8
Chalcone reductase (CHR)	*GR463527 GR463635*	22.9	*At1g59960*	1.2	1.0
			At4g36350	2.0	10.2
Purple acid phosphatase	*GR463521 GR463627*	1.01	*AT5G34850*	1.5	1.1
Chitinase class I	*GR463543 GR463595*	1.52	*At1g05850*	1.7	1.1
ERD4	*GR463639*	1.06	*At1g30360*	1.3	2.6
CW14	*GR463593 GR463542*	1.75	*At1g10410*	0.7	0.9
Ripening related (MLP43)	*GR463493 GR463520* *GR463645 GR463625*	1.44	*At1g70890*	6.4	5.0
STF	*GR463507 GR463615*	1.44	*At5g07000*	2.4	0.6
Xyloglucan endotransferae	*GR463567*	3.03	*At1g03220*	4.8	7.7
AMMECR1 protein	*GR463522 GR463629*	2.34	*At2g38710*	1.1	0.6
LRR Cf2/Cf5 disease resistance	*GR463639*	1.06	*AT5G53890*	1.7	1.4

n-not tested.

and digest chitin in the cell walls of pathogens and function through both constitutive and inducible pathways as enzyme defenses [38-42]. The structure, function and distribution of chitinases have co-evolved with plant-pathogen interactions, facilitating adaptation to the environment [43]. Sinapyl alcohol dehydrogenase (SAD), catalyzing the reduction of sinapyl aldehyde to sinapyl alcohol, which is a component in lignification [44], together with xyloglucan endotransglycosylase, functions in cell elongation and growth.

It has been reported that UV, environmental stresses and developmental factors stimulate the activation of promoters of chalcone synthase [45,46]. Chalcone synthase is the first step in the flavonoid-specific branch pathway. This enzyme was up-regulated only in the epidermis, indicating that the signals for synthesis of CHS are from the environment, the epidermis being the major site for signal perception and early responses. This is also true for ERD4.

Flavonoids function in various plant development processes such as pigmentation, pollen germination, auxin transport, UV protection, interactions with microbes in the roots of legumes [47-50], and in response to wounding and pathogen invasion [51]. Chalcone reductase (CHR) is a key enzyme in flavonoid and isoflavonone synthesis. Isoflavonone reductase (IFR) is a key enzyme in pathways for the synthesis of phytoalexins and other stress response products. CHR works in harmony with CHS, controlling the direction of the flavonoid pathway [52]. CHR and IFR were expressed in the epidermis of *D. purpureus*. IFR is an NAD(P)H-dependent oxidoreductase, functioning in the synthesis of isoflavonones, which mostly act in plant defense. IFR expression is related to plant resistance to pathogens, UV and other stresses [53-56].

3.2. Comparison Expression Analysis of Genes Related to Signal Transduction and Stress in Stem Epidermis and Inner Stem *D. purpureus* and *A. thaliana*

The expression level of genes related to signal transduction and stress from *D. purpureus* were examined with QPCR method in both stem epidermis and inner stem. Altogether, they are compared with the expression data from *A. thaliana* (**Table 1** and **Table 2**). The results show that most of these genes are up-regulated in stem epidermis.

Receptors and membrane proteins located on stem epidermis cell surfaces are important in responses to environmental signals. Comparison of the differential expression ratio of 13 genes related to signal transduction expressed in stem epidermis to inner stem from *D. purpureus* to the similar genes expressed in stem epidermis

to inner stem from *A. thaliana*, 11 genes have similar differential expression tendency (**Table 1**). With expression ratio in stem epidermis to inner stem larger than 0.5, nine of them are up-regulated in stem epidermis of *D. purpureus*, takes up 69% of the 13 genes. Among 26 genes related to signal transduction expressed in stem of *A. thaliana* listed in **Table 1**, 22 genes in top stem and 23 genes in base stem are up-regulated in stem epidermis at the ratio of 0.5, take up 85% and 88% of 26 genes respectively, only two genes down regulated in stem epidermis of both *D. purpureus* and *A. thaliana*. The two down-regulated genes are *STM* (*GR463472*, *At1g62360*) and a Homeobox gene (*GR463505*, *At4g32880*), which are both transcription factors function in transcription regulation. *STM* is a gene essencial for maintenance of meristem structural organization, which is inside of the epidermis. The function of *At4g32880* is unknown. Most of genes up-regulated in stem epidermis in table 1 are intermediate components such as kinases, phosphatases, protein interacting proteins, RNA-binding proteins, and down-regulated genes are transcription factors. In table 2, All listed genes are up-regulated in stem epidermis than inner stem, most of them are functional enzymes, receptor or other proteins in cytosol. These results indicate that in epidermis responding to environment earlier or more activity than inner stem.

Most of genes related to stress from *D. purpureus* are up-regulated in epidermis than the inner part, and all their orthologs in *A. thaliana* are expressed in both stem epidermis and markedly up-regulated in the epidermis than the inner part (**Table 2**). One Avr9-responsive protein with a glycosyl group-transferring domain encoded by gene *GR463609* was down-regulated in the stem epidermis of *D. purpureus* relative to the inner stem, but its homolog *AT1G77810* was up-regulated in the stem epidermis of *A. thaliana* (**Table 2**). Whether this is a consequence of differences in developmental stage or of some other cause requires further study.

3.3. Expression Analysis of Distribution of Genes Related to Signal Transduction, Stress Response and Development in *A. thaliana*

Up-regulation profile of genes related to signal transduction, stress response, and biotic or abiotic stimulation in stem epidermis and stem from *A. thaliana* were shown in **Figure 2**. It has significant difference between numbers of genes up-regulated in stem epidermis and inner stem. Much more genes related to signal transduction and stress were up-regulated in both top and base stem epidermis than in inner stem.

Distribution of genes related to signal transduction and stress in stem were analyzed by comparing their numbers with expression ratios of signals of epidermis/stem among
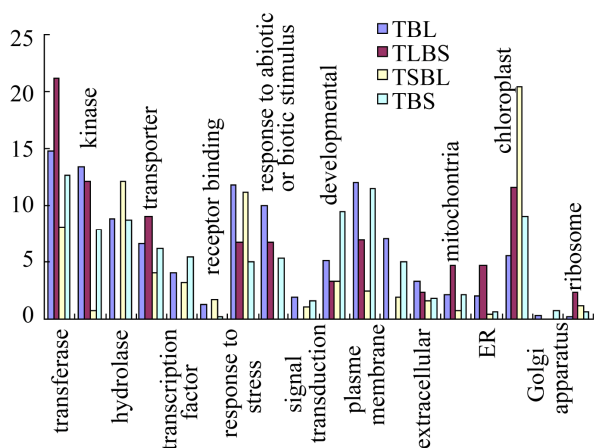

Figure 2. Functional distributions of genes in Arabidopsis stem Note. Y-axial is the percentage of genes up-regulated with ratio of epidermis/stem in each group.

Figure 3. Expression pattern of genes in light signal pathway in Atabidopsis stem.

four groups: 1) in the epidermis to that in inner stems (epidermis/inner) of both top and base stem larger than 0.5 and 2, designated as TBl, 2) in top epidermis larger than 0.5 and 2, in base epidermis less than 0.5 labeled as TlBs,and 3) in top epidermis less than 0.5, in base epidermis larger than 0.5 and 2, labeled as TsBl and 4) in both top and base epidermis less than 0.5; labeled as TBs in base epidermis, (**Tables 3-5**). The expression ratios of signals of epidermis/stem is large than 0.5, indicate the expression preferred in epidermis, otherwise preferred in inner stem. Thus the four groups represent genes of four types of gene expression preferences: 1) genes prefer expressed in both top and base stem epidermis, 2) genes prefer expressed in both top and base inner stem, 3) genes prefer expressed in top epidermis and base stem and 4) genes prefer expressioned in top stem and base epidermis.

Table 3 and Figure 3 listed genes involved in hormone and light signal transduction pathway. The signigicantly more genes prefer expressed in stem epidermis than in inner stem.

In the database, among 13589 genes present in macroarray hybridization, the numbers of up-regulated genes with the ratio of epidermis/inner stem larger than 2 in a, b, c, d four groups of genes are 921, 37, 96 and 363 respectively, takes up 6.78%, 0.27%, 0.71% and 2.67% in 13589 genes respectively. In each group, the function of genes are categoried and compared between four groups as in **Figure 2**.

In **Figure 2**, functional groups were picked up from three different categories by GO annotation using four groups of genes with expression ratio larger than 2 respectively and combined together for comparison and analysis.

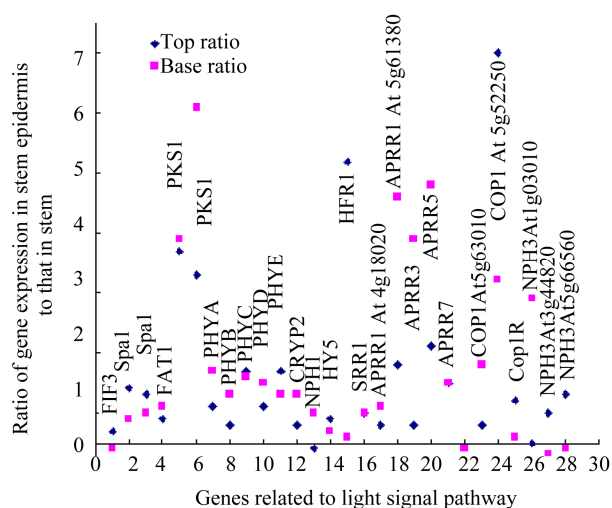Among four groups, TBL group has highest expression ratio of genes in stress responses (11.8%), biotic and abiotic responses (9.9%), plasma membrane (12%), kinases (13.3%), signal transduction (1.9%), extracellular (3.4%), cell wall (7%). The less enrichment of TBL group of genes are in transporters (6.5%), hydrolases (8.8%), transferases (14.8%), receptor binding (1.3%), transcription factors (4.1%), developmental (5.1%). TLBs group has highest expression ratio of genes in transferases (21.2%), transporters (9.1%), mitochontria (4.7%), ER (4.7%), ribosomes (2.3%), receptor binding (1.6%). The less enrichment of TLBs group of genes are in kinases (12.1%), response to abiotic or biotic stimulus (6.7%), extracellular (2.3%), chloroplast (11.6%). TSBL group has highest expression ratio of genes in hydrolases (12.1%) and chloroplast (20.4%). The less enrichment of TsBL group of genes are in response to stress (11.2%). TBs group has highest expression ratio of genes in transcription factors (5.3%), Golgi apparatus (0.8%). The less enrichment of TBs group of genes are in plasma membrane (11.5%), hydrolase (8.7%). The types of genes enriched in epidermis of both top and base stem are more likely function in defense and signal transduction.

Among auxin related genes, the only one auxin binding gene, four auxin effllux carrieries are up-regulated in both top and base stem epidermis, one auxin import transporter PIN3 is specifically expressed in both top and base stem epidermis, and two auxin import transporters PIN7 prefer up-regulated in top stem, base stem and base epidermis respectively (**Table 3**). PIN3 was reported lateral located at epidermis and cortex of apical hooks and mediate the tropism in Arabidopsis [57], but PIN7 seems has stronger signal at epidermis than in cortex at apical hooks of Arabidopsis. It is possible that the distribution of PIN3 and PIN7 are under regulation of complex factors. Among 17 genes which expression up-regulation ratio of epidermis/stem is larger than 2, 6 are *IAA*6, 6 are

Table 3. Distribution of genes expressed in stem related to hormones and light from *A. thaliana* with up-regulation ratio > 0.5 and >2 [15].

Function of genes	TBl		TlBs		TsBl		TBs
	>0.5	>2	>0.5	>2	>0.5	>2	>0.5
Total (13589)	11870	921	571	37	932	96	363
Auxin	105	17	7	2	9	0	11
Auxin-binding	1		0	0	0	0	0
Auxin-regulated protein, IAA	78	17	7	0	7	0	10
Auxin efflux carrier	4	0	0	0	1	0	1
Auxin-independent	12	0	0	0	0	0	0
PIN	PIN3,1	0	0	0	PIN7,1	0	1
Cytokinin	2	2	0	0	0	0	0
ABA	7	0	1	0	0	0	0
Gibberellin	11	2	0	0	0	0	0
Ethylene	55	7	3	0	5	2	0
Ethylene receptor	3	0	0	0	0	0	0
NPH3	3	0	3	0	2	1	1
CIP7	3	0	0	0	0	0	0
CIP8	2	0	0	0	0	0	0

T-Epidermis/stem at top stem B-Epidermis/stem at base stem. L-expression ratio > 0.5, S-expression ratio < 0.5.

Table 4. Distribution of some genes expressed in stem in signal transduction pathway from *A. thaliana* with upregulation ratio > 0.5 and >2 [15].

Function of genes	TBl		TlBs		TsBl		TBs
	> 0.5	>2	> 0.5	>2	>0.5	>2	>0.5
Receptors	169	28	8	0	7	0	5
Membrane receptor	27	4	2	0	1	0	1
LRR	23	17	2	0	1	0	1
Calcium related	77	13	4	0	5	0	2
CDPK	27	8	1	0	0	0	0
Ca transport ATPase	11	3	0	0	0	0	0
MAPK	23	3	1	0	0	0	0

T-Epidermis/stem at top stem B-Epidermis/stem at base stem. L-expression ratio > 0.5, S-expression ratio < 0.5.

Table 5. Distribution of other components in signal transduction and regulation network in stem of A. thaliana with up-regulation ratio > 0.5 and >2 [15].

Function of genes	TBl		TlBs		TsBl		TBs
	> 0.5	>2	>0.5	>2	>0.5	>2	<0.5
Glutamate carboxypeptidase	3	0	0	0	0	0	0
Ser/Thr carboxypeptidase	13	1	3	0	4	0	2
Pro carboxypeptidase	2	0	0	0	0	0	0
Sumo protease	7	0	0	0	0	0	0
Cysteine proteinase	29	1	0	0	2	0	3
MYB type genes	73	18	4	0	7	1	6
14-3-3	9	0	0	0	0	0	0
Homeobox type genes	22	3	4	0	0	0	2
F-box type genes	141	2	1	0	4	0	0
SKIP2	33	1	0	0	0	0	0
SKP1 interacting partner 3	12	0	0	0	0	0	0
SKP1 interacting partner 4	3	0	0	0	0	0	0
SKP1 interacting partner 6	22	0	0	0	0	0	0
Kelch repeat	18	1	1	0	0	0	0
Tubby	8	0	0	0	0	0	0
transport inhibitor response 1	6	0	0	0	0	0	0
WRKY type genes	31	10	1	0	0	0	2

SAURs, 2 *ARG*7, 1 *ARG*, 1 *GH*3, 1 *AIR*12.

Two cytokinin related genes are specifically up-regulated only in epidermis of both top and stem, no other cytokinin related genes up-regulated in other parts. One is *At5g05440* with low similarity to cytokinin-specific binding protein (*Vigna radiata* GI:4190976). The cytokinin-related gene *At3g55950* is specifically up-regulated in the epidermis of both top and base stem, similar to the cytokinin-regulated kinase 1 gene (*Nicotiana tabacum*) *AAG25966*. Cell growth mainly due to expansion can be controlled by a signal (s) initiated from the epidermis, and the epidermis could be a barrier to inner tissue growth; growth activity is coordinated between the shoot epidermis and the inner part [58]. These genes are possibly related to growth regulation.

Three abscisic acid responsive elements-binding factor (ABF) At4g34000, At3g19290, At1g49720, two abscisic acid-activated protein kinases At3g50500, At4g33950, and two abscisic acid-induced proteins At4g24960, At1g74520 are up-regulated in only epidermis of both top and base stem. One RD20 protein encoding gene *At2g33380*, a putative transmembrane channel protein gene, induced by abscisic acid during dehydration, is up-regulated in top stem epidermis and base inner stem. This position specificity indicates the regulation of stem growth by environmental factors.

Twenty gibberellin (GA) related genes are expressed in stem. 11(55%) genes are up-regulated in epidermis of both top and base stem, 2 are up-regulated in only top stem epidermis, not present in base stem epidermis. These genes include gibberellin response modulators *At1g14920* (*GAI/RGA2*), *At1g66350* (*RGAL*), *At2g01570* (*RGA1*), gibberellin signal transduction protein (*SPINDLY*) *At3g11540*, GAST1-related protein *At1g74670*, *At1g22690*, gibberellin 20-oxidase *At3g60290*, *At4g25420*, and GA2-oxidase *At2g34555*. Two genes specifically expressed in top epidermis are gibberellin 2oxidase *At1g30040* and gibberellin response modulator putative member of the VHIID domain transcription factor family *RGAL At5g17490*. GA2-oxidase function in inactivation of Gibberellin.

All three ethylene receptors present in stem are up-regulated in both top and base stem epidermis. In 63 genes related to ethylene response present in stem, 55 genes (87%) are up-regulated in both top and base stem epidermis, only 3 (4.7%) and 5 (7.9%) genes are in groups TLBS and TSBL respectively. No genes related to ethylene response only up-regulated in both top and base inner stem (**Table 3**).

Similarly, in the light signal pathway, the initial steps of light signal transduction in the phytochrome pathway seem mainly to be up-regulated in the stem epidermis. All the five types of phytochromes, the A, B, C, D and E and cryptochrome 2 were absolutely up-regulated in the both top and base epidermis, not in the inner stem (**Figure 3**). Correspondingly, the immediate substrate of PhyA, PKS1, Phytochrome A signal transduction 1 (PAT1), has the same regulation tendency as PhyA; Spa1, APRR1, 3, 5, 7, SRR1, HFR1, COP1, 9, 10, CIP7, 8, COP1R and HY5, the other components in light signal pathway are also up-regulated only in epidermis of both top and base stem. PIF3, which migrates into the nucleus to regulate DNA transcription, is up-regulated in the top stem epidermis and inner parts of base stem (**Figure 3**) .

Blue light receptors CRY1 and 2 interact with COP1, releasing HY5, a transcription factor that directly targets the light-responsive promoters from negative regulation by COP1 [59]. In *A. thaliana* stem, two genes *At5g63010* and *At5g52250* encoded COP1 have opposite expression pattern in top and base stem. *At5g63010* encoded gene has reverse pattern as HY5, possibly function in the same signal pathway (**Figure 3**). The function of far-red light receptors PhyA, PhyC, PhyD and PhyE, are associated with signal transduction by other phytochromes [60,61]. Photoreceptors may function as groups, cooperatively. *PhyA* is known to be up-regulated in the dark and down-regulated under light; *PhyB* shows the reverse pattern. The level of phytochromes is reported to be 23 times lower in the light than in the dark, the A:B:C:D:E ratios being 85:10:2:1.5:1.5 in the dark and 5:40:15:15:25 under light [62]. During *A. thaliana* stem growth, Phy A acts in the initiation stage after light stimulation, and this is followed by PhyB function; they act sequentially and in coordination [63]. In the genome-wise expression data of *A. thaliana* stem, *PhyA*, *B*, *D*, *Cryp*2, *NPH*1 have similar expression distribulation trend between top and base stem, and *PhyC*, *E* have similar expression distribulation trend between top and base stem, the same as *PIF3*, *SPA*1, *PAT*1, *HY*5, *COP*1R, *At5g52250* encoded COP1 (**Figure 3**).

NPHs are factors in phototropin signal pathways that are involved in blue light-induced movement. The distribution of NPH3 in four groups are 3, 1, 3, 2, they are encoded by different genes; for NPH1 is 0, 0, 0, 1. Similarly, the distribution of up-regulated genes reflected the stages or timings of other components in signal transduction pathways. PKS1 (phytochrome kinase substrate 1) is located only in the cytosol, binding directly to Phy A and NPH1 (phototropin1, phot1) [64]. It is up-regulated only in the epidermis of both top and base stem, the position closest to the light source. *NPH*1 is reportedly expressed in both epidermis and inner stem, but the expression level is very weak in the epidermis and strong in inner stem cells [65]. Correspondingly, *NPH*1 is up-regulated in the inner stem at the top stem, the site with active growth and differentiation activities, but at base stem, up-regulated in stem epidermis (**Figure 3**). NPH1 is located on the inner side of the plasma membrane in the

dark, phosphorylated and dissociated from the membrane, then translocated into the cytoplasm under blue light. The NPH1 level in the cells was down-regulated by 24 hours light exposure [65]. NPH3 interacts with NPH1 and is involved in auxin-regulated organogenesis [66-68]. NPH3 encoding genes *At1g03010*, *At1g30440* was up-regulated in epidermis at base stem and inner parts of the top stem, similar to NPH1 (**Figure 3**), in agreement with the optical observations of phototropin1 location in *A. thaliana* [65]. NPH3 encoding *At3g44820* and *At5g-66560* was up-regulated in epidermis of top stem and inner part of base stem, has opposite direction as NPH1, togerher with other NPH3s which constantly preferred at epidermis or inner parts ot both top and base stem, are components of complex regulation network.

The light energy absorbance levels are PhyA< PhyB < Cry2 < NPH1; correspondingly, according to expression ratios of signals of epidermis/stem, the the photoreceptors distribution show the up-regulation tendency as sequences as the top epidermis, top inner, base epidermis, and base inner of the stem (**Figure 3**). Here, two elements could be distinguished: lateral position (or distance from the environment) and developmental stage (top and base). Low energy light receptors are located on the outside of the stem, and higher energy light receptors on the inside. It is possible that light of a higher energy level can penetrate deeper into the plant tissue and that the distribution of up-regulated genes indicates this coordination and adaptation. The distribution of up-regulated receptors may indicate the adaptation of receptors to light penetration.

The distributions of up-regulated light receptors and the components of their signal transduction pathways reveal that the direction of signal transduction in the stem is from the outer layer to the inner part.

Receptors, membrane receptors, calcium related, CDPK, MAPK are important components in many signal transduction pathway, the significantly more numbers of these genes are up-regulated in stem epidermis of both top and base stem (**Table 4**).

The number of total receptors expressed in stem is 243, the number of total calcium related genes expressed in stem is 123, the number of MAPK genes expressed in stem is 27. 28 (11.5%) receptor genes, 17 (6.9%) of LRR type receptors, 13 (11%) of calcium related genes and 3(11%) of MAPK genes are up-regulated in epidermis of both top and base stem at ratio of epidermis/stem larger than 2. Much fewer genes are in TLBs and TSBL types of up-regulation pattern for all these class of genes (**Table 4**). Non-membrane receptors include small molecule receptors such as glutamate, sugar, steroid, S-locus protein, light, retrovirus, protein organelle targeting, probein binding, signal transduction etc., involved in various precess of environmental signal, hormone signal trans-

duction and development process. Somatic embryogenesis receptors CLV1 (At1g60800), CLV2 (At1g-65380), light repressible receptor (At1g48270), and other major receptors all up-regulated in epidermis of both top and base stem.

Table 5 show the maginificent number of genes up-regulated in epidermis of both top and base stem. They are all function in various developmental and defense process. Among total 37 of homeobox genes, 203 of F-box genes, 136 of MYB, 42 of WRKY genes expressed in stem, 22 (59.4%), 141 (69%), 73 (54%), and 31 (74%) were up-regulated in epidermis of both top and base stem, but the genes are up-regulated in epidermis of only top or base stem are much fewer. This suggests that the epidermis layer is not only a very important location for plant sensing environmental stimulation, but also for sensing signals from inner tissues, integreting the effector from various site, directing and initiate morphogenesis of various new organs of the plant. It is a key place for both defense and developmental decision in plant. The much fewer genes up-regulated in epidermis of only top or base stem indicates that the common properties or function of genes required in epidermis function, and those fewer genes up-regulated only in epidermis of top or base stem probably have specific function in top stem or base stem.

The top stem is where the inflorescence rises and flowers and other organs are initiated. Genes that are more up-regulated in the top epidermis of the stem may therefore be related to these initiation and organogenesis activities. Efremova *et al.* [69] showed that class B homeotic genes in *Antirrhinum* and *A. thaliana* control the organ identity by expression initiated from the L1 meristematic layer and, later, to the epidermis and subepidermis of the differentiating organs. Among genes up-regulated in epidermis of both top and base stem with up-regulation ratio of epidermis/stem larger than 2, there are *At1g05230*, *At1g79840* (*GLABRA2*, *GL2*), *At4g21750* (*ATML1*), *At1g01380* (*CPC*), *At2g46410* (*CPC*) [2,15,70, 71], which have been reported specifically located in epidermis and function in meristem organization or initiation of new organs and patterning of epidermis cells. This expression pattern is similar to the distribution pattern of top epidermis, inner top stem, base epidermis, inner base stem noted for some of the genes.

In addition, the sex determination protein tassel seed 2-like genes (*At3g26770*, *At4g03140*) and septum site-determining *MinD* gene (*At5g24020*) are expressed in both top and base stem. This indicates that signaling and early events in flower development may also be located at certain sites in the stem, and this may be related to the regulation of stem growth and organogenesis by a conjunction of light signals and other environmental signals and hormone signals, hinting at positional decisions for

organ formation and flower development on the stem.

　The stem epidermis is a barrier between the plant and its environment. It is important not only in protection and defense, but also as the site for sensing and initiating responses to various signals from environment, including those for development, resistance, and others. The important functions of the epidermis in plant protection and development are far from clear and need more detailed study.

4. CONCLUSIONS

The differential gene expression profiles in epidermis and inner part of stem from both *D. purpureus* L. and *A. thaliana* reaveal that defense and signal transduction is important function in stem epidermis. 13% and 17% of genes from 188 sequenced cDNAs expressed in stem epidermis of *D. purpureus* L. are belong to signal transduction and defense pathways respectively. The results from *D. purpureus* L. were confirmed by QPCR assay. Macroarray data analysis with *A. thaliana* suggest that receptors and components in light and hormone signal pathway are preferred expressed in stem epidermis, reveals that the stem epidermis is not only the barrier between the plant and its environment, but also the sensor for environmental stimuli and signals. In *D. purpureus* L. and *A. thaliana* stems, the gene expression profiles and the distributions of up-regulated genes displayed the predominance of signal transduction processes in stem epidermis function.

5. ACKNOWLEDGEMENTS

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