Functional analysis of nine cotton genes related to leaf senescence in *Gossypium hirsutum* L

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Abstract Leaf senescence is defined as a deterioration process that continues to the final developmental stage of leaf. This process is usually regulated by both external and internal factors. There are about 5356 senescence associated genes belonging to 44 plant species. A great number of these genes were identified in Arabidopsis. Leaf senescence can be regulated by many transcription factors. In this study, nine gene families were selected according to their expression levels during leaf senescence from our laboratory database. Phylogenetic tree was constructed by MEGA6. Cultivated cotton CCRI-10 seeds were sown in the experimental field of Institute of Cotton Research of CAAS for profiling and leaf development stages analysis. For abiotic (drought and salt) stress and phytohormone (ABA, SA, ET and JA) treatments, CCRI-10 seeds were sown in potting soil at 25 °C in a chamber room. Total RNA was isolated from various samples and the cDNA prepared for qRT-PCR. The comparative CT method was applied to calculate the relative expression levels of genes. For phylogenetic tree, nine cotton genes were divided into two groups, most of homologous genes in previous studies showed roles in phytohormones and abiotic stress. Expression profiling of the nine genes showed different patterns of tissue specific expression. In leaf development stages, majority of cotton genes showed high expression in early and complete senescence stage. Furthermore, most of

cotton genes have positive or negative response to phytohormones and abiotic stress. Based on the results of this study, we found four cotton genes CotAD_07559, CotAD_37422, CotAD_21204 and CotAD_54353 as candidate genes for leaves senescence and abiotic stress.

Keywords Gene expression · Profiling · Leaf development · QRT-PCR · Phylogenetic tree

Introduction

Upland cotton (*Gossypium* spp.) is one of global principal natural fiber crops and is a major source of protein meal and seed oil (Han et al. 2004; Mei et al. 2004). Cotton production is constrained by many biotic and abiotic factors. Abiotic stress negatively influences plant development and production, resulting in huge economic losses. During evolution, plants have evolved to have diverse mechanisms to adapt to environmental stresses at both physiological and biochemical levels (Munns and Tester 2008). However, in cotton, leaf senescence has become most important factor decreasing cotton yield and quality (Dong et al. 2006). Premature leaf senescence is a programmed cell deterioration process that constitutes the final developmental stage of leaf (Lim et al. 2003). The changes most closely associated with senescence are decline in total protein and RNA levels and chloroplast break down. Chlorophyll loss and concomitant leaf yellowing are most visible symptoms of leaf senescence (Weaver et al. 1998). At the final stage, leaf senescence ultimately causes leaf death, a process that is regulated by many external and internal factors. Internal factors include age, phytohormones level and developmental processes. Although leaf senescence is age-dependent, there are a variety of phytohormones which influence

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the initiation and progression of senescence (Hensel et al. 1993; Lim et al. 2003); phytohormones such as ethylene, jasmonates (JAs), abscisic acid (ABA) and salicylic acids (SA) are known to enhance the process of leaf senescence. For instance, Jasmonates could induce the expression levels of a number of senescence associated genes (SAGs) (Schenk et al. 2000). The external factors generally include biological and environmental stresses such as pathogen attack, drought, salinity stress, extreme temperature, wounding, oxidative stress and nutrient limitation (Guo and Gan 2012; Lim et al. 2007). Like other developmental processes, leaf senescence can also be regulated by numerous transcription factors (TF) (Fan et al. 2015). Detection of senescence associated genes (SAGs) is considered fundamental to elucidate the molecular mechanism of leaf senescence. There are 5356 SAGs that belong to 44 plant species. The greater number (over 3745) of these genes were found in *Arabidopsis* (Lin et al. 2015) and the majority of them were known to encode hydrolases, lipases, proteases, nucleases, stress responsive proteins, enzymes involving transcriptional regulation and nutrient recycling (W 2006). Senescence Associated Genes are classified into up- and down-regulated SAGs; Developmental SAGs, with predicted up-regulation roles contain TFs genes (148), signal transduction pathways components (185), transporters and cellular transport components (102), metabolic enzymes (257), enzymes involved in degradation of macromolecules (98), defense related proteins (93), lipids (21), nucleic acids (6), cell wall degradation enzymes (24) and others (Guo and Gan 2012). In the contrary, the function of down-regulated SAGs significantly differs from these up-regulated SAGs. Their predicted functions are in the functional category of energy and cell division as well as growth. The category includes predominantly components of photosynthesis (Lim et al. 2003). However, several studies were focused on the function of transcription factors such as NAC and WRKY families (Dou et al. 2014; Ondati et al. 2016; Shah et al. 2014). Therefore, we decided to examine the potential role of other new genes related to leaf senescence in cotton. These genes and their predicted function were listed in supplementary Table 1. The selection criterion was based on the expression levels during leaf senescence as earlier indicated by our lab database (*Accession number SRR656612*). Our analysis established that these genes belong to different families. For example, CotAD_07559 belonged to Branched chain aminotransferase2 (BCAT-2) that localized in chloroplasts, and contributes in branched-chain amino acids (BCAA) synthesis that is derived from metabolites for instance fatty acids, sugars and glucosinolates. These metabolites have roles in plant growth, development and defense (Diebold et al. 2002). CotAD_13315 gene with sterile alpha motif (SAM)

domain, interact with proteins, DNA, RNA and lipids, and is involved in cellular functions such as signal transduction and transcription regulation (The et al. 2016). CotAD_21204 belong to Ribosomal protein L2 family (RPL2). Ribosomes are significant to protein biosynthesis process. Ribosomes are composed of small (S) and Large (L) units. According to localization, there are three types of ribosomal proteins (RPs) chloroplastic, cytosolic and mitochondrial. RPs are essential for growth, cell division, metabolism and as regulatory in developmental processes (Marty and Meyer 1992). CotAD_75839 is associated with Mildew resistance locus o (MLO8). Seven transmembrane family protein localized in plasma membrane, modulates defense to the powdery mildew fungus, and its expression was up-regulated by biotic and abiotic stress (Chen et al. 2006b). CotAD_37422 is associated with Growth regulating factors (GRF2) which are plant transcription factors that were originally known for their functions in developmental processes in various tissues such as leaf, stem, root, flower and seed. Apparently, they play key role in organizing the growth processes under unfavorable environmental conditions (Omidbakhshfard et al. 2015). CotAD_56419 (GhSPL13) classified to Squamosa promoter binding protein-like (SPL) which are plants transcription factors that contain SQUAMOSA promoter binding protein (SBP) domain (X. Zhang et al. 2014). SPL genes have several roles in tissue developmental processes and plant hormone signaling (Gene et al. 2016). CotAD_54353 is associated with Chloroplastic drought induced stress protein of 32KD(CDSP32) which is a member of thioredoxin family which play many roles during plant life, such as plant development, metabolisms, hormone synthesis, biotic and abiotic stress responses (Nuruzzaman et al. 2012). CotAD_09457 is associated with laccase-4(LAC4), this group has various physiological functions in plants according to different subcellular localization. Also LACs have a role in detoxification; that is, ability of degrading toxins and a variety of environmental pollutants therefore plants growth and development continue with environmental contaminants and stress (Cai et al. 2006). CotAD_30878; unknown protein hits to 121 proteins in 42 species.

In this study qRT-PCR analyses, showed that majority of these cotton genes have spatial and temporal expression patterns in specific tissues, indicating a high possibility of involvement in various aspects of physiological and developmental processes. Additionally these genes showed high level of expression during different stages of leaf senescence, a result that was attributed to their role in leaf senescence. Furthermore, these genes were responsive to phytohormones and abiotic stress as indicated by qRT-PCR analysis. Our work provides a subset of possible candidate

new families' gene for further studies for stresses and leaf senescence in upland cotton.

Materials and methods

Phylogenetic analysis

The 17 Arabidopsis, Rice, Maize, Glycine max and Sorghum protein amino acid sequences were obtained from the NCBI website (<http://www.ncbi.nlm.nih.gov/>). A phylogenetic tree was constructed using the complete amino acid sequences of the nine cotton proteins and 17 proteins of other species. The nine protein amino acid sequences were aligned by Clustalx1.81 program and a phylogenetic tree was constructed using MEGA6 software with the neighbor joining (NJ) method and internal branch support was estimated with 1000 bootstrap replicates.

Plant material and growth conditions

In this study, CCRI-10 seeds were sown in the experimental field of Institute of Cotton Research of CAAS, Anyang, Henan, P. R. China in April 2015. For profiling expressions, cotyledons, leaves, stems, roots and flowers were collected during different development stages, and then all collected samples were quickly put in liquid nitrogen and stored at -80°C for RNA extraction. To investigate the effect of natural leaf senescence, leaves were harvested from cotton seedlings at five developmental stages; a non-senescent leaf stage (NS), initial senescent stage (IS), early senescent stage (ES), late senescent stage (LS) and a completely senescent stage (CS) (about 90% yellowing of the leaf surface) (Ondati et al. 2016). At each senescence stage, the RNA was isolated and labeled. The cDNAs were prepared for qRT-PCR analysis.

Abiotic stress and phytohormone treatments

To investigate the expression level of these genes under various abiotic stresses and phytohormone treatments, CCRI-10 seeds were germinated in potting soil at 25°C in a growth chamber with a 16 h light/8 h dark cycle. Seedlings at the cotyledon stage were subjected to different treatments. Seedling leaves were sprayed with $200\text{ }\mu\text{M}$ ABA, 2 mM SA, $100\text{ }\mu\text{M}$ MeJA and ethylene (0.5 mMethephon). Ethephon, which emits ethylene when dissolved in water, was used as a substitute for ethylene (Jin and Liu 2008). Drought and salinity treatments were applied by submerging plant roots in aqueous solutions of 15% PEG 6000, 200 mM NaCl respectively. Wounding was inflicted by injuring leaves from the apex. Seedlings treated with sterile water were used as control. After

treatments of 0, 6, 12, 24, 36 and 48 h, leaves were collected and quickly frozen in liquid nitrogen and stored at -80°C for RNA extraction.

Extraction total RNA, and cDNA synthesis

Total RNA was isolated from various samples with RNA prep pure Plant Kit (TIANGEN, China). The RNA concentration and the purity were determined by NANO-Drop2000 spectrophotometer and gel electrophoresis. The cDNA for gene cloning and qRT-PCR was synthesized by a Prime Script TM 1st Strand cDNA Synthesis Kit (Takara, Japan) according to the manufacturer's instructions.

Cloning of nine DNA and cDNA sequences

Candidate gene sequences were selected from our lab gene database related to leaf senescence. According to the obtained unigenes, specific primers for these genes were designed using oligo6 software (Supplemental Table 2). The generated cDNAs were used as templates to amplify these gene sequences from CCRI-10. PCR was performed using Prime STAR Max cDNA Polymerase (Takara, Japan) in PCR reaction to amplify full length of these genes and the thermal cycling parameters were as follows: 98°C , 2 min; 30 cycles of 98°C , 10 s, 57°C , 15 s, and 68°C , 1 min; followed by a extension step of 72°C , 10 min. The PCR products were detected on a 1.5% agarose gel and then purified with a Hipure Gel mini Kit (Magen, China). The purified PCR products were cloned into the pMD18-T vector (TaKaRa, Japan) and transformed into *Escherichia coli* competent cells and sequenced by GeneWiz (<https://www.genewiz.com>).

Quantitative RT-PCR assays

For qRT-PCR, oligo nucleotide primers were designed according to the untranslated regions of all genes with OLIGO6 (version 6.71) as described in supplementary Table 3. All primers were tested with melting peaks and dissociation curves to confirm that there was only one product for each pair of primers. GhActin1 was used as a housekeeping gene to standardize the amount of template cDNA in each amplification reaction (Meng et al. 2011). The qRT-PCR was performed using SYBR Premix EX Taq Kit (TaKaRa, Japan). For each $20\text{ }\mu\text{l}$ reaction, $2\text{ }\mu\text{l}$ of sample cDNA was mixed with $10\text{ }\mu\text{l}$ of a SYBR Premix Ex Taq, $0.8\text{ }\mu\text{l}$ ($10\text{ }\mu\text{M}$) of forward primer, $0.8\text{ }\mu\text{l}$ ($10\text{ }\mu\text{M}$) of reverse primer, $0.4\text{ }\mu\text{l}$ ROX reference dye II and $6\text{ }\mu\text{l}$ of sterile water. ABI 7500 Sequence detection system (Applied Biosystems) machine was used. The following thermal cycle conditions were used: 95°C for 2 min, followed by 40 cycles of 95°C for 5 s, products collected at 60°C

for 34 s. All reactions were performed in triplicate, and melting curve analysis performed. The comparative CT method was used to calculate the relative expression levels (Livak and Schmittgen 2001).

Results

Phylogenetic analysis of cotton genes

In order to establish the functional relationship between these nine cotton genes and other plant species, we conducted NCBI search. A phylogenetic tree was constructed with similar Arabidopsis, Rice, Maize, Glycine max and cotton proteins sequences using the neighbor joining (NJ) method in MEGA6. The results showed 17 genes had high identity (80–98%) with cotton genes. This allowed us to divide our genes into two sub groups (Fig. 1). Sub- Group 1 contained five cotton genes. Among these five, four of them; CotAD_37422, CotAD_56419 (GhSPL13), CotAD_21204 and CotAD_07559 grouped into four different families (GRF2, SPL13, RPL2 and BCAT respectively) and were homologous to the four Arabidopsis AT2G06200, AT5G50670.2.1, AT4G36130.1 and AT1G10070.1 genes respectively. Sub- Group 2 contained

four cotton genes CotAD_54353, CotAD_75839, CotAD_09457 and CotAD_13315 representing four families (CDSP32, MLO8, LAC4 and SAM) were homologous to Arabidopsis AT1G76080.1, AT2G17480.1, AT2G38080.1 and AT1G70180 genes respectively.

Profiling gene expression

Gene expression is associated with specific physiological and developmental functions; therefore we examined the expression patterns of these genes in various tissues, including cotyledon, young leaf, true leaf, stem, root, flower parts and fiber by using qRT-PCR. Obviously, the expression of these genes showed different expression patterns at each tissue (Fig. 2) implying different functions for these genes may be due to genes belonging to different families. Gene CotAD_21204 showed higher expression levels in the root and stem compared to other tissues. Three cotton genes, CotAD_13315, CotAD_37422 and CotAD_75839 showed highest expression in the stem, whereas three genes (CotAD_07559, CotAD_30878 and GhSPL13) were highly induced at floral parts. Two genes CotAD_54353 and CotAD_09457 were highly expressed in true and young leaf respectively.

Leaf development stages

To study the role of these genes in senescence, their expression was studied in senescing leaf at different stages of natural leaf senescence (from non senescent to completely senescent leaf) (Fig. 3). CotAD_21204 gene showed highest expression level at late senescent leaf stage. Four genes (CotAD_13315, CotAD_37422, CotAD_75839 and CotAD_54353) showed highest expression at early senescing leaf (ES) and decreased at late stages. Two genes CotAD_07559 and CotAD_09457 expression level was increased with increasing leaf development stages until they reached highest expression level at completely senescent leaf (CS). Two genes GhSPL13 and CotAD_30878 expression levels was higher at two (Initial and complete senescence) stages.

Expression of the cotton genes under phytohormones treatment conditions

To study the relationship between these genes and phytohormones, cotton seedlings were treated with ABA, SA, MeJA and ETH. qRT-PCR analysis results indicated that ABA induced four genes (CotAD_21204, GhSPL13, CotAD_07559 and CotAD_75839) which were down-regulated. CotAD_75839 was gradually expressed with peak expression at 12 h. Three genes (CotAD_13315, CotAD_37422 and CotAD_09457) were gradually

Fig. 1 Phylogenetic relationships of cotton and Arabidopsis, rice, maize and glycine max proteins. The complete amino acid sequences of nine cotton genes and 17 proteins were aligned by Clustalx1.81, and the neighbor-joining tree was constructed using MEGA6 with 1000 bootstrap replicates

Fig. 2 Profiling tissue specific gene expression in cotton. The mRNA expression patterns in cotyledon, young leaf, true leaf, stem, root, fiber, anther, ovule, petal, pistal and sepal tissues of cotton were examined by qPCR assay. CT method is used to determine the relative expression

expressed acquiring peak at 6 h post abscisic acid treatment. Gene CotAD_54353 showed no response to ABA treatment. CotAD_30878 expression was down-regulated at early stages and up-regulated at 48 h post (ABA) abscisic acid treatment (Fig. 4). For SA treatment, three genes (CotAD_21204, CotAD_37422 and GhSPL13) expression were down-regulated while all the other six genes (CotAD_13315, CotAD_09457, CotAD_07559, CotAD_75839, CotAD_54353 and CotAD_30878) were up-regulated, reaching their maximum at 36 h post salicylic acids treatment. However, gene CotAD_13315 expression level peaked at 12 h (Fig. 4). For MeJA treatment, generally, all genes expression was up-regulated except genes CotAD_07559 and CotAD_30878 (Fig. 5). All genes were up-regulated with ethylene (ETH) treatment, relative to control (Fig. 5).

Expression of cotton genes under abiotic stress

To analyze the regulation of these genes under abiotic stress (Salt and drought), cotton seedlings were treated with NaCl and PEG6000 respectively. To examine the expression patterns of these genes, qRT-PCR analysis was performed. The results indicated that majority of these genes were induced by salt and drought stress (Fig. 6). For Salt (NaCl) treatment; six genes (CotAD_21204,

CotAD_13315, CotAD_37422, CotAD_75839, CotAD_30878 and CotAD_07559) were highly expressed at late stages (36 and 48 h) post NaCl treatment, while the expression of the other three genes (GhSPL13, CotAD_54353 and CotAD_09457) was high at 6 and 24 h (Fig. 5). For drought (PEG6000), four genes CotAD_13315, CotAD_75839, CotAD_07559 and CotAD_30878 showed no obvious change (Fig. 6) while four genes CotAD_21204, CotAD_37422, GhSPL13 and CotAD_09457 were up-regulated whereas Gene CotAD_54353 was down-regulated.

Expression of cotton genes under wounding (W)

The expression level of majority genes was up-regulated by wounding (Fig. 7). Four genes (CotAD_09457, CotAD_30878, CotAD_07559 and CotAD_13315) expression level peaked at 12 h post wounding treatment, while three genes CotAD_21204, CotAD_75839, CotAD_54353 were up-regulated at 48 h. Two genes (CotAD_37422 and GhSPL13) were down-regulated.

Fig. 3 Expression of cotton genes during different stages of leaf senescence. Samples were taken at five stages (non senescent NS, initial senescent IS, early senescent ES, late senescent LS and completely senescent CS) of leaf in cotton

Discussion

In this study, we selected nine genes from our lab database using their expression levels in cotton. The putative functions of these genes were identified by carrying out phylogenetic tree and expression profiling analysis.

Phylogenetic analysis of cotton genes

Phylogenetic tree was constructed to investigate the putative functions of cotton genes which were divided into two sub groups; group1 (red color) and group 2 (blue color)

which provided more information about the functions of these gene families. Result established that CotAD_37422 aligned with gene AT4G37740.1, that was previously reported to have been induced by abiotic stress (Omidbakhshfard et al. 2015). Similarly, our results showed that CotAD_37422 was highly expressed in abiotic stress implying that this gene might be candidate gene for abiotic stress. GhSPL13 is homologous to AT5G50670.2.1, which play key role in stress (Wang et al. 2009). CotAD_21204 homologous to AT4G36130.1 was highly expressed at wounding, but showed low expression in drought (Wang et al. 2013). CotAD_07559 homologous to AT1G10070.1,

Fig. 4 Absciscic acid (ABA) and salicylic acid (SA) treatment, expression was monitored by qRT-PCR in cotton

Fig. 5 MeJa and ethylene (1 mMethphon) treatments, expression was monitored by qRT-PCR in cotton

which showed in previous study response to abiotic stress (Peng et al. 2015). CotAD_54353 homologous to AT1G76080.1, both related to CDSP32 family which have function in stress responses (Chen et al. 2006a; Choi et al. 2004). CotAD_75839 homologous to AT2G17480.1,

which showed high expression under salt and wounding stress (Chen et al. 2006a). CotAD_09457 was highly expressed in drought and salt, suggesting that this gene may be involved in both drought and salt stress. Its homologous is AT2G38080.1, which was responsive to

Fig. 6 Effect of drought PEG6000 (15%) and salt NaCl (200 mM) on cotton genes expression in cotton

Fig. 7 Effect of wounding on cotton genes expression in cotton

abiotic stress (Cai et al. 2006). CotAD_13315 homologous to AT1G70180, a gene that was highly expressed in shoot and plant development (<http://www.arabidopsis.org>). These

results might provide significant support to confirm that these cotton genes may play key roles in the response to abiotic stress.

The tissue-specific

The tissue-specific analysis for genes predicted possible biological functions of the genes during plant growth and development (Shah et al. 2014). Four genes CotAD_07559, GhSPL13, CotAD_09457 and CotAD_30878 were highly expressed in the flower, implying that they may have a role in late growth development. Earlier studies reported similar genes; AT1G10070 and ATSPL13 were highly expressed in flower and have roles in many fundamental developmental processes (Chen et al. 2010; Peng et al. 2015). Furthermore, At2g38080 was highly expressed in flower, root, stem and leaf (Cai et al. 2006). CotAD_54353 was highly expressed in true leaf. Four genes CotAD_75839, CotAD_37422, CotAD_21204 and CotAD_13315 showed relationship with different families (MLO8, GRF2, RPL2 and SAM respectively), the expression of these four genes was high in the stem, which is in agreement with previous studies which revealed that At2g17480 (MLO8) was highly expressed in stem (Chen et al. 2006a; Choi et al. 2004). Two genes, At4g37740 (GRF2) and RPL2 were involved in growth process (Choi et al. 2004; Zheng et al. 2016). The above results confirm that these cotton genes functions in various specific tissues may be for growth and development, stress regulation or defense mechanisms.

Leaf development stages

In experiment of leaf senescence, expression levels of nine cotton genes were significantly enhanced during one or more of leaf senescence stages indicating their involvement in leaf senescence. Two genes, CotAD_37422, CotAD_21204 were highly expressed at early and late senescence and were up-regulated in stem, explaining their possible role in growth. CotAD_37422 is homologous to AT2G06200, which was highly expressed at various development stages in Arabidopsis (Choi et al. 2004). Four genes, CotAD_07559, GhSPL13, CotAD_09457 and CotAD_30878 were up-regulated at complete senescence stage and highly expressed in flower, indicating their role in late growth development. Gene CotAD_54353 was up-regulated at early senescence stage while highly expressed in true leaf. This may be attributed to the function of this family (CDSP32) in chloroplast protection against oxidative damage (Pérez-Ruiz et al. 2006). In general, four genes, CotAD_07559, CotAD_37422, CotAD_21204 and CotAD_54353 showed highest expression levels in leaves senescence indicating that they may be involved in regulation of cell maturation and death.

Expression of the cotton genes under different phytohormones treatment conditions

To further confirm the role of these nine genes in leaf senescence, seedlings were subjected to ethylene, ABA, JA and SA treatments. These phytohormones were identified as plant growth regulators which have been known as key factors for leaf senescence (Zhang and Zhou 2013). Results showed that, most gene expressions were induced with at least one or more hormones. Genes CotAD_54353 and GhSPL13 were highly expressed by both JA and ET, while CotAD_21204 was highly induced by ET. Noteworthy, jasmonic acid and ethylene were known as most important phytohormones or growth regulators (Cramer et al. 2011) and promoted leaf senescence by different mechanisms (Hung and Kao 1996).

Expression of cotton genes under abiotic stress

Molecular responses to drought and salt stress have been evaluated by monitoring genes with enhanced expressions in cotton under environmental stresses (Nakashima et al. 2009). Majority of cotton gene expressions were induced by abiotic stress. For instance, gene CotAD_37422 expression level was up-regulated with both drought and salt stress, gene CotAD_07559 was up-regulated with salt stress whereas gene CotAD_21204 was induced by salt stress and down-regulated with drought stress. Induction of these genes by stress explain their participation in drought and osmotic regulation in plants. CotAD_54353 was highly expressed in salt treatment but showed low expression in drought, this result was not consistent with homologous AT1G76080.1, which is induced by drought whereas did not respond to salinity (Wong et al. 2006). The phytohormones (ABA, SA JA and ET) signal transduction of abiotic stress responses is complex and crosstalk with each other (Zhang et al. 2012). ABA is an important hormone. It plays an important role in regulating the expression of many genes controlling dehydration (Saad et al. 2013). Abiotic stress responses are mainly regulated by abscisic acid, while biotic stress responses; defense against different biotic attacks, is specified by antagonism between the salicylic acid and jasmonic acid/ethylene signaling pathways (Wong et al. 2006). Our result found that, gene CotAD_37422 was highly induced by ABA and abiotic treatment which suggest that this gene may be involved in interaction of various abiotic stresses. Most of cotton gene expressions were highly induced by SA at 48 h post treatment concluding that these genes may play important roles in plant defense response.

Expression of cotton genes under wounding (W)

Wounding is physical damage that occurs to plants as a result of abiotic (wind, rain and hail) and biotic factors (insect feeding) (Cheong et al. 2002). Results showed that, majority of cotton genes were induced by wounding; three genes, CotAD_09457, CotAD_07559 and CotAD_21204 were highly up-regulated by wounding. Gene CotAD_21204 was homologous to AT4G36130.1 which in previous study was highly expressed at wounding (Wang et al. 2013). There is interaction between wounding and abiotic stress responses. Other several plant hormones that are important in wounding include jasmonic acid (JA), salicylic acid (SA) and ethylene (Diaz et al. 2002). Three genes, CotAD_09457, CotAD_07559 and CotAD_21204 were highly expressed by wounding, SA and ET as compared to control, this result might provide some evidence about an interaction between plant hormones and wounding stress. Our results showed that the majority of cotton genes were significantly induced by salt, drought and phytohormones treatments, implying that, there is extensive overlap in the responses to leaf senescence and stress (Guo and Gan 2012; Lim et al. 2003). Based on the results of this study, we found four cotton genes, CotAD_07559, CotAD_37422, CotAD_21204 and CotAD_54353 as candidate genes for leaves senescence and can be added to SAGs genes to design future study.

Conclusions

In this study, nine cotton genes were related to different genes families (GRF2, SPL13, RPL2, BCAT, CDSP32, MLO8, LAC4 and SAM). According to phylogenetic tree, most of homologous genes have functions at least one or more in phytohormones regulation and abiotic stress responses, this supports our findings that these new cotton genes could have important role in leaf senescence and stress responses in upland cotton. Additionally, their expressions attest to the fact that they play specific roles at specific tissues and during leaf senescence. Finally, four genes apparently indicate their involvement in leaf senescence; they include CotAD_07559, CotAD_37422, CotAD_21204 and CotAD_54353. These genes may be considered for further studies to establish detailed functions in senescence and stress responses in cotton. The keys to study new families could be related to leaf senescence and abiotic stress. This study spot light on new genes families, so more studies will be needed to understand their functions well.

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References

- Cai X, Davis EJ, Ballif J, Liang M, Bushman E, Haroldsen V, Wu Y (2006) Mutant identification and characterization of the laccase gene family in Arabidopsis. *J Exp Bot* 57(11):2563–2569. <https://doi.org/10.1093/jxb/erl022>
- Chen Y, Takita J, Hiwatari M, Igarashi T, Hanada R, Kikuchi A, Hayashi Y (2006a) Mutations of the PTPN11 and RAS genes in rhabdomyosarcoma and pediatric hematological malignancies. *Genes Chromosom Cancer* 45(6):583–591. <https://doi.org/10.1007/s11103-005-5082-x>
- Chen Z, Hartmann HA, Wu M, Friedman EJ, Chen J, Pulley M, Jones AM (2006b) Expression analysis of the AtMLO gene family encoding plant-specific seven-transmembrane domain proteins. <https://doi.org/10.1007/s11103-005-5082-x>
- Chen X, Zhang Z, Liu D, Zhang K, Li A, Mao L (2010) SQUAMOSA promoter-binding protein-like transcription factors: star players for plant growth and development. *J Integr Plant Biol* 52(11):946–951. <https://doi.org/10.1111/j.1744-7909.2010.00987.x>
- Cheong YH, Chang H-S, Gupta R, Wang X, Zhu T, Luan S (2002) Transcriptional profiling reveals novel interactions between wounding, pathogen, abiotic stress, and hormonal responses in Arabidopsis. *Plant Physiol* 129(2):661–677. <https://doi.org/10.1104/pp.002857.1>
- Choi D, Jeong HK, Kende H (2004) Whole genome analysis of the OsGRF gene family encoding plant-specific putative transcription activators in rice (*Oryza sativa* L.). *Plant Cell Physiol* 45(7):897–904. <https://doi.org/10.1093/pcp/pch098>
- Cramer GR, Urano K, Delrot S, Pezzotti M, Shinozaki K (2011) Effects of abiotic stress on plants: a systems biology perspective. *BMC Plant Biol* 11(1):163. <https://doi.org/10.1186/1471-2229-11-163>
- Diaz J, ten Have A, van Kan JAL (2002) The role of ethylene and wound signalling in resistance of tomato to *Botrytis cinerea*. *Plant Physiol* 129:1341–1351. <https://doi.org/10.1104/pp.001453.1>
- Diebold R, Schuster J, Da K, Binder S (2002) The branched-chain amino acid transaminase gene family in Arabidopsis encodes plastid and mitochondrial. *Proteins*. <https://doi.org/10.1104/pp.001602.540>
- Dong H, Li W, Tang W, Li Z, Zhang D, Niu Y (2006) Yield, quality and leaf senescence of cotton grown at varying planting dates and plant densities in the Yellow River Valley of China. *Field Crops Res* 98(2–3):106–115. <https://doi.org/10.1016/j.fcr.2005.12.008>
- Dou L, Zhang X, Pang C, Song M, Wei H, Fan S, Yu S (2014) Genome-wide analysis of the WRKY gene family in cotton. *Mol Genet Genomics*. <https://doi.org/10.1007/s00438-014-0872-y>
- Fan K, Bibi N, Gan S, Li F, Yuan S, Ni M, Wang X (2015) A novel NAP member GhNAP is involved in leaf senescence in *Gossypium hirsutum*. *J Exp Bot* 66(15):4669–4682. <https://doi.org/10.1093/jxb/erv240>
- Gene P, Mao H, Yu L, Li Z, Yan Y, Han R, Ma M (2016) Genome-wide analysis of the SPL family transcription factors and their responses to abiotic stresses in maize. *Plant Gene* 6:1–12. <https://doi.org/10.1016/j.plgene.2016.03.003>
- Guo Y, Gan SS (2012) Convergence and divergence in gene expression profiles induced by leaf senescence and 27 senescence-promoting hormonal, pathological and environmental

- stress treatments. *Plant Cell Environ* 35(3):644–655. <https://doi.org/10.1111/j.1365-3040.2011.02442.x>
- Han Z-G, Guo W-Z, Song X-L, Zhang T-Z (2004) Genetic mapping of EST-derived microsatellites from the diploid *Gossypium arboreum* in allotetraploid cotton. *Mol Genet Genom* 272(3):308–327
- Hensel LL, Grbić V, Baumgarten D, Bleecker AB (1993) Developmental and age-related processes that influence the longevity and senescence of photosynthetic tissues in *Arabidopsis*. *Plant Cell* 5(May):553–564. <https://doi.org/10.1105/tpc.5.5.553>
- Hung KT, Kao CH (1996) Promotive effect of jasmonates on the senescence of detached maize leaves. *Plant Growth Regul* 19(1):77–83. <https://doi.org/10.1007/BF00024405>
- Jin LG, Liu JY (2008) Molecular cloning, expression profile and promoter analysis of a novel ethylene responsive transcription factor gene GhERF4 from cotton (*Gossypium hirsutum*). *Plant Physiol Biochem* 46(1):46–53. <https://doi.org/10.1016/j.plaphy.2007.10.004>
- Komili S, Farny NG, Roth FP, Silver PA (2008) Functional specificity among ribosomal proteins regulates gene expression. *Cell* 131(3):557–571
- Lim PO, Woo HR, Nam HG (2003) Molecular genetics of leaf senescence in *Arabidopsis*. *Trends Plant Sci*. [https://doi.org/10.1016/S1360-1385\(03\)00103-1](https://doi.org/10.1016/S1360-1385(03)00103-1)
- Lim PO, Kim HJ, Nam HG (2007) Leaf senescence. *Annu Rev Plant Biol* 58:115–136. <https://doi.org/10.1146/annurev.arplant.57.032905.105316>
- Lin M, Pang C, Fan S, Song M, Wei H, Yu S (2015) Global analysis of the *Gossypium hirsutum* L. Transcriptome during leaf senescence by RNA-Seq. *BMC Plant Biol* 15:43. <https://doi.org/10.1186/s12870-015-0433-5>
- Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and. *Methods* 25:402–408. <https://doi.org/10.1006/meth.2001.1262>
- Marty I, Meyer Y (1992) CDNA nucleotide sequence and expression of a tobacco cytoplasmic ribosomal protein L2 gene. *Nucleic Acids Res* 20(7):1517–1522. <https://doi.org/10.1093/nar/20.7.1517>
- Mei M, Syed NH, Gao W, Thaxton PM, Smith CW, Stelly DM, Chen ZJ (2004) Genetic mapping and QTL analysis of fiber-related traits in cotton (*Gossypium*). *TAG Theoretical Appl Genet* 108(2):280–291
- Meng Y, Liu F, Pang C, Fan S, Song M, Wang D, Yu S (2011) Label-free quantitative proteomics analysis of cotton leaf response to nitric oxide. *J Proteome Res* 10(12):5416–5432. <https://doi.org/10.1021/pr200671d>
- Munns R, Tester M (2008) Mechanisms of salinity tolerance. *Annu Rev Plant Biol* 59:651–681. <https://doi.org/10.1146/annurev.arplant.59.032607.092911>
- Nakashima K, Ito Y, Yamaguchi-Shinozaki K (2009) Transcriptional regulatory networks in response to abiotic stresses in *Arabidopsis* and grasses. *Plant Physiol* 149(January):88–95. <https://doi.org/10.1104/pp.108.129791>
- Nuruzzaman M, Sharoni AM, Satoh K, Al-Shammari T, Shimizu T, Sasaya T, Kikuchi S (2012) The thioredoxin gene family in rice: genome-wide identification and expression profiling under different biotic and abiotic treatments. *Biochem Biophys Res Commun* 423(2):417–423. <https://doi.org/10.1016/j.bbrc.2012.05.142>
- Omidbakhshfard MA, Proost S, Fujikura U, Mueller-Roeber B (2015) Growth-regulating factors (GRFs): a small transcription factor family with important functions in plant biology. *Mol Plant* 8(7):998–1010. <https://doi.org/10.1016/j.molp.2015.01.013>
- Ondati E, Lingling D, Yaning G, Chaoyou P, Hengling W, Meizhen S, Shuxun Y (2016) GhNAC18, a novel cotton (*Gossypium hirsutum* L.) NAC gene, is involved in leaf senescence and diverse stress responses. *Afr J Biotech* 15(24):1233–1245. <https://doi.org/10.5897/AJB2016.15224>
- Peng C, Uygun S, Shiu S-H, Last RL (2015) The impact of the branched-chain ketoacid dehydrogenase complex on amino acid homeostasis in *Arabidopsis*. *Plant Physiol* 169(3):1807–1820. <https://doi.org/10.1104/pp.15.00461>
- Pérez-Ruiz JM, Spínola MC, Kirchsteiger K, Moreno J, Sahrawy M, Pe JM, Cejudo FJ (2006) Rice NTRC is a high-efficiency redox system for chloroplast protection against oxidative damage. *Society* 18:2356–2368. <https://doi.org/10.1105/tpc.106.041541>
- Saad ASI, Li X, Li HP, Huang T, Gao CS, Guo MW, Liao YC (2013) A rice stress-responsive NAC gene enhances tolerance of transgenic wheat to drought and salt stresses. *Plant Sci* 203–204:33–40. <https://doi.org/10.1016/j.plantsci.2012.12.016>
- Schenk PM, Kazan K, Wilson I, Anderson JP, Richmond T, Somerville SC, Manners JM (2000) Coordinated plant defense responses in *Arabidopsis* revealed by microarray analysis. *Proc Natl Acad Sci USA* 97(21):11655–11660. <https://doi.org/10.1073/pnas.97.21.11655>
- Shah ST, Pang C, Hussain A, Fan S, Song M, Zamir R, Yu S (2014) Molecular cloning and functional analysis of NAC family genes associated with leaf senescence and stresses in *Gossypium hirsutum* L. *Plant Cell Tissue Organ Cult* 117(2):167–186. <https://doi.org/10.1007/s11240-014-0430-7>
- The E, Gre L, Sayou C, Nanao MH, Jamin M, Pose D, Tichtinsky G (2016) A SAM oligomerization domain shapes the genomic binding landscape of the LEAFY transcription factor. *Nat Commun*. <https://doi.org/10.1038/pj.2016.37>
- W SR (2006) A novel nuclear-localized CCCH-type zinc finger protein, OsDOS, is involved in delaying leaf. *Plant Physiol* 141:1376–1388. <https://doi.org/10.1104/pp.106.082941.1376>
- Wang Y, Hu Z, Yang Y, Chen X, Chen G (2009) Function annotation of an SBP-box gene in *Arabidopsis* based on analysis of co-expression networks and promoters. *Int J Mol Sci* 10(1):116–132. <https://doi.org/10.3390/ijms10010116>
- Wang J, Lan P, Gao H, Zheng L, Li W, Schmidt W (2013) Expression changes of ribosomal proteins in phosphate- and iron-deficient *Arabidopsis* roots predict stress-specific alterations in ribosome composition. *BMC Genomics* 14(1):783
- Weaver LM, Gan S, Quirino B, Amasino RM (1998) A comparison of the expression patterns of several senescence-associated genes in response to stress and hormone treatment. *Plant Mol Biol* 37(3):455–469. <https://doi.org/10.1023/A:1005934428906>
- Wong CE, Li Y, Labbe A, Guevara D, Nuin P, Whitty B, Moffatt BA (2006) Transcriptional profiling implicates novel interactions between abiotic stress and hormonal responses in *Thellungiella*, a close relative of *Arabidopsis*. *Plant Physiol* 140(April):1437–1450. <https://doi.org/10.1104/pp.105.070508>
- Zhang H, Zhou C (2013) Signal transduction in leaf senescence. *Plant Mol Biol* 82(6):539–545. <https://doi.org/10.1007/s11103-012-9980-4>
- Zhang Z, Liu X, Wang X, Zhou M, Zhou X, Ye X, Wei X (2012) An R2R3 MYB transcription factor in wheat, TaPIMP1, mediates host resistance to *Bipolaris sorokiniana* and drought stresses through regulation of defense- and stress-related genes. *New Phytol* 196(4):1155–1170. <https://doi.org/10.1111/j.1469-8137.2012.04353.x>
- Zhang X, Dou L, Pang C, Song M, Wei H, Fan S, Yu S (2014) Genomic organization, differential expression, and functional analysis of the SPL gene family in *Gossypium hirsutum*. *Mol Genet Genomics* 290(1):115–126. <https://doi.org/10.1007/s00438-014-0901-x>
- Zheng M, Wang Y, Liu X, Sun J, Wang Y, Xu Y, Wan J (2016) The RICE MINUTE-LIKE1 (RML1) gene, encoding a ribosomal large subunit protein L3B, regulates leaf morphology and plant architecture in rice. *J Exp Bot* 67(11):3457–3469. <https://doi.org/10.1093/jxb/erw167>