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Transgenic *Arabidopsis* plants over-expressing cotton *DELLA* or *DELLA* domain deletion: insights into *DELLA* gene function

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Abstract

Gibberellic acid (GA) promotes cotton fiber cell elongation under *in vitro* culture conditions and GA signal transduction is mediated by DELLA proteins. Our laboratory has cloned several *DELLA* genes from cotton and generated transgenic lines in *Arabidopsis* over-expressing the *DELLA* gene (*GhRGL*) and the DELLA-domain deletion mutant gene (*Ghrgl*). Comparative transcriptome profiling was performed on *Arabidopsis* transgenic lines over-expressing both *GhRGL* and *Ghrgl*. *KEGG* pathway analysis indicated that the differential genes participated in many important pathways, such as response to gibberellic acid, fatty acid metabolism, *etc.*, and the expression patterns of 93 genes were validated by RT PCR in all transgenic lines and the wild-type plants. Gene ontology annotation identified that these genes were involved in sucrose synthesis, actin nucleation, root hair development, and various hormone signal responses.

Additional key words: fiber development, gibberellic acid, *Gossypium hirsutum*, metabolic pathways, RT PCR.

Introduction

The gibberellic acid (GA) signaling pathway is repressed by DELLA proteins which have highly conserved N-terminal DELLA and VHYNP domains (Pysh *et al.* 1999, Willige *et al.* 2007). In *Arabidopsis*, deletion mutants of the N-terminal DELLA and VHYNP structural domains are extreme dwarfs and insensitive to exogenous GA. These two structural domains are essential for reception of GA signal (Sun 2004).

There are five highly homologous DELLA protein repressors in *Arabidopsis thaliana* genome including GA INSENSITIVE (GAI) REPRESSOR OF *ga1-3* (RGA), RGL1 (RGA-like 1), RGL2, and RGL3 (Peng *et al.* 1997, Silverstone *et al.* 1998, Richards *et al.* 2001, Willige *et al.* 2007). GAI and RGA have overlapping functions in inhibiting cell elongation in *Arabidopsis* (Willige *et al.* 2007). RGL1 and RGL2 act in combination to control petal and stamen development in response to GA (Dill and Sun 2001, King *et al.* 2001, Lee *et al.* 2002, Cheng *et al.* 2004, Tyler *et al.* 2004). DELLA proteins in *Arabidopsis* are degraded by the ubiquitin proteolytic system which then allows the GA response (Silverstone *et al.* 2001, Fu *et al.* 2002, McGinnis 2003, Sasaki *et al.* 2003, Willige *et al.* 2007).

Several DELLA proteins have been identified in other plant species, such as height1 (Rht1) from wheat, dwarf8 (d8) from maize (Peng *et al.* 1999), Slender1 (Sln1) from barley (Chandler *et al.* 2002), Slr1 from rice (Ikeda *et al.* 2001), Vvgai1 from grape (Boss and Thomas 2001), and dwf2 from *Brassica rapa* (Muangprom and Osborn 2004). All these DELLA proteins are negative regulators of GA action. The homologous *DELLA* genes from *Arabidopsis* and other species play a common role on repressing GA signaling. This has led to the suggestion that the components of the GA signal transduction signal pathway are highly conserved (King *et al.* 2001, Willige *et al.* 2007).

Cotton is the most widely used fiber plant in the world (Wahid *et al.* 2003, Shi *et al.* 2006). Cotton fibers

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Abbreviations: GA - gibberellic acid; *GhRGL* - DELLA gene from *Gossypium hirsutum*; *Ghrgl* - DELLA-domain deletion mutant gene from *Gossypium hirsutum*.

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are extremely elongated single trichomes that are derived from the ovule epidermis (Shi *et al.* 2006). During fiber development, the ovule epidermal cells go through several independent but overlapping stages including fiber initiation, fiber expansion, secondary cell wall formation, and fiber cell maturation (Basra *et al.* 1984, Tiwari *et al.* 1995, Wilkins *et al.* 1999, Kim *et al.* 2001, Yang *et al.* 2006). However, the precise mechanism of fiber formation is still not fully understood. Experiments using *in vitro* culture of cotton ovule have demonstrated that plant hormones such as GA and IAA play key roles in fiber development (Beasley 1974).

Previous studies have identified seven *DELLA* genes in the cotton genome (Yang *et al.* 2006). Our laboratory has cloned a cotton *DELLA* gene (*GhRGL*) which was found to have higher expression during fiber initiation and expansion (Yang *et al.* 2006, Aleman *et al.* 2008, Liao *et al.* 2009). These findings suggest that *DELLA* genes could play a regulatory role in fiber development. When the *GhRGL* and its *DELLA*-domain deletion mutant gene (*Ghrgl*) were over-expressed in *Arabidopsis*, *Ghrgl* was extremely dwarfed. This suggests that both the *Arabidopsis* and cotton DELLA affect plant elongation growth (Aleman *et al.* 2008, Liao *et al.* 2009).

In order to determine the function of *GhRGL* and *Ghrgl* and their impact on downstream metabolic pathways, both of these were over-expressed in *Arabidopsis*. Gene expression profiles in the two types of transgenic plants were compared. Bioinformatic analyses of KEGG metabolic pathway and biological functions aimed to elucidate the association of *DELLA* genes with cotton fiber growth and development.

Materials and methods

Arabidopsis thaliana L. plants over-expressing *GhRGL* and *Ghrgl* used in this experiment were created previously (Liao *et al.* 2009). Four events for each constructs were used in this experiment and five T3 homozygous lines from each event were sampled for RNA isolation. *Arabidopsis* wild-type (WT) plants were used as controls. The plants were grown in *Vermiculite* potting mix in a greenhouse set at constant temperature of 22 °C, relative humidity of 75 %, a 16-h photoperiod, and irradiance of 100 μmol m^{-2} s^{-1}. Leaves were sampled from 30-d-old plants (20 for each genotype including WT control). Samples were ground in liquid N_2 and total RNA was extracted using an RNA plant extraction kit (*Tiangen*, *Biotech Co*., Beijing, China). These samples were considered as one set and a total of three replicates were taken for the whole experiment.

The *Arabidopsis* genome *Oligo Set* v. 3.0 array (*Operon*, *CapitalBio,* Beijing, China) comprising 29 110 of 70-mer *Oligo* DNA probes was used. Each chip represents 28 964 transcripts and 26 173 protein encoding genes. Details about the oligo array can be found at http://www. Operon.com.

For semi-quantitative reverse trascriptase-polymerase chain reaction, total RNA (5 μg) was converted into cDNA by reverse transcription using *T7-Oligo* (dT) primer (*Promega*, Madison, USA). The cRNA was prepared by *in vivo* transcription from double stranded cDNA using the *T7 RiboMAX* express large scale RNA production system (*Promega*). Labeled cDNA molecules were generated by reverse transcription of cRNA (2 μg) by *CbcScript II* enzyme (*Promega*) with random primer and by subsequent *Klenow* fragment polymerase labeling. The transgenic plant samples were labeled with Cy5 and the wild type with Cy3. The labeled cDNA was dissolved in 0.035 cm^3 of hybridization buffer consisting of 3× saline-sodium citrate (SSC), 0.12 % (m/v) sodium dodecyl sulphate (SDS), 5× *Denhart* and 25 % (v/v) formamide. Microarray hybridization was conducted overnight at 42 °C. Upon completion of hybridization, the slides were washed in a buffer (0.12 % SDS, 2× SSC solutions) at 42 °C for 5 min followed by another wash in 0.12× SSC at room temperature for 5 min. The slides were then dried by spinning in a centrifuge before immediate scanning.

The hybridized microarray chips were scanned on a *LuxScan 10K*, a double channel scanner (*CapitalBio*). The *SpotData* image analysis software (*CapitalBio*) was used to analyze the images and convert them into numerical data. *Lowess* (Workman *et al.* 2002) normalization method was used to normalize the data. Differential gene expression was identified by significant analysis of microarray (SAM) method (Tusher *et al.* 2001).

Results

The results of transcriptomic analyses of over-expressing *GhRGL* and *Ghrgl Arabidopsis* for each genotype were highly reproducible, therefore the mean expression value for each transcript across replicates for each genotype was used for differential expression analysis.

To identify the effects on global gene expressions of *GhRGL* and *Ghrgl*, the differential levels of all the 28 964 transcripts in the array of the *Arabidopsis* genome *Oligo Set* were analyzed by significant analysis of microarray method (Tusher *et al.* 2001). Then the *KOBAS* system (for *KEGG* orthology based annotation system) was used to map the differentially expressed transcripts to metabolic pathways (Shi *et al.* 2006). We used semi-quantitative RT-PCR to validate the expression of 93 transcripts and found a high correlation (78 %) between cDNA microarray and semi-quantitative RT-PCR.

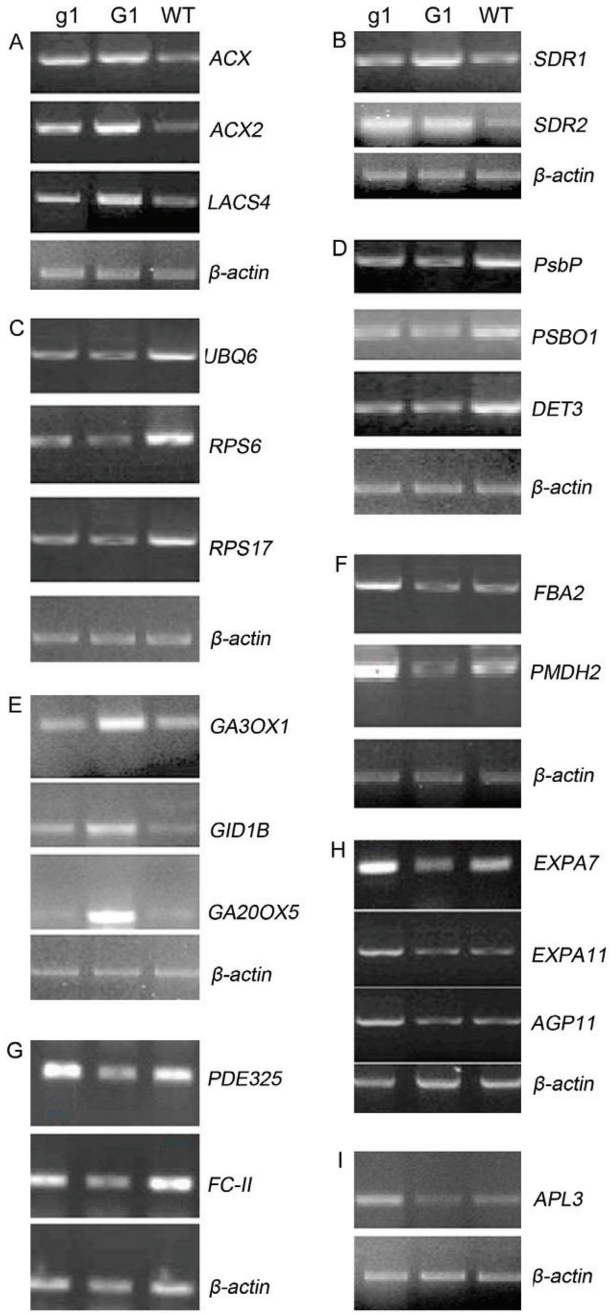

Fig. 1. Expression of genes in some key metabolism pathways confirmed by semi-quantitative RT-PCR. WT - wild type, g1 - *Ghrgl* transgenic lines, G1 - *GhRGL* transgenic lines. *Arabidopsis β-actin* gene was used as control. *A* - fatty acid metabolism, *B* - fatty acid biosynthesis, *C* - ribosomes, *D* - photosynthesis, *E* - gibberellic acid response, *F* - carbon fixation, *G* - porphyrin and chlorophylls, *H* - cell growth, *I* - starch and sucrose metabolism.

When compared to the WT plants, the *GhRGL* over-expression plants showed differential expression of genes playing a role in ribosome function, photosynthesis, fatty acid biosynthesis and metabolism, response to gibberellic acid, valine, leucine, and isoleucine biosynthesis, and arginine and proline metabolism (Table 1). Gene differently expressed in the *Ghrgl* and WT were mainly those concerning carbon fixation, cell growth, porphyrin and chlorophyll metabolism, pyruvate metabolism, alkaloid biosynthesis, starch and sucrose metabolism, and glyoxylate and dicarboxylate metabolism (Table 1).

The results from this experiment indicated that 8 genes in the fatty acid metabolism and 3 genes from the fatty acid biosynthesis pathway were up-regulated in the *GhRGL* and *Ghrgl* over-expressing plants (Table 2). Semi-quantitative RT PCR was performed to confirm the expression patterns of these genes (Fig. 1*A,B*) and all the results agreed with the microarray data. These genes are *acetyl-CoA oxidase 2* (*AtACX2*, At5g65110) and *acetyl-CoA oxidase* (*AtACX*, AT1G6440), both the genes encoding a long chain fatty acid CoA ligase, and a long-chain *acetyl-CoA synthetase 4* gene (*AtLACS4*, AT4G23850) representing a long chain acetyl-CoA synthetase. Further, some genes involved in fatty acid biosynthesis pathway have a higher expression in transgenic *Arabidopsis* compared to wild-type including a *FATTY ACID BIOSYNTHESIS 1* gene (*AtFAB1*, At1g74960) and a short-chain dehydrogenase/reductase family gene *AtSDR* (At4g13180; Fig. 1*B*).

The expression profiles of 21 genes in the ribosome metabolic pathways were differentially expressed in WT and transgenic plants (Table 2). When compared with expression in WT, these genes were less expressed in transgenic types. Semi-quantitative RT-PCR further confirmed this expression patterns for several genes, such as *ubiquitin 6* (*AtUBQ6*, At2g47110), *ribosomal protein S6* gene (*AtRPS6*, At4g31700), and *ribosomal protein S17* gene (*AtRPS17*, At1g79850) (Fig. 1*C*). These genes are the structural constituents of the ribosomes.

Similarly, 12 genes in the photosynthesis pathway were down-regulated in *Arabidopsis* over-expressing *GhRGL* or *Ghrgl* (Table 2, Fig. 1*D*). Two of them, *PHOTOSYSTEM II SUBUNIT P-1* (*AtPsbP*, At1g06680) and *PS II OXYGEN-EVOLVING COMPLEX 1* (*AtPSBO1*, At5g66570) are required for photosystem II function. The third gene *ARABIDOPSIS THALIANA VACUOLAR ATP SYNTHASE SUBUNIT C* (*AtDET3*, At1g12840) plays an important role in the control of cell elongation and the regulation of meristem activity.

Some genes concerning the response to gibberellic acid were up-regulated in over-expressing *GhRGL* plants but down-regulated in *Ghrgl* plants when compared to WT (Table 2). For example, *GIBBERELLIN 3-OXIDASE 1* gene (*AtGA3OX1*, AT1G15550) and *GIBBERELLIN 20-OXIDASE 5* gene (*AtGA20OX5*, AT1G44090) are involved in later steps of the gibberellic acid biosynthesis. The *RING-H2* zinc finger gene (*AtXERICO*,

Table 1. Representative metabolic pathways in which participate genes differentially expressed between transgenic *Arabidopsis* and WT plants.

Transgenics	KEGG pathways	Number of genes	*P* value	*Q* value
GhRGL	ribosome	21	0.0	0.0
	photosynthesis	12	0.0	0.0
	fatty acid metabolism	8	0.001	2.63E-4
	response to gibberellic acid	7	0.001	3.14E-4
	valine, leucine, and isoleucine biosynthesis	7	0.002	5.7E-4
	arginine and proline metabolism	8	0.006	0.001
	fatty acid biosynthesis	3	0.020	0.003
Ghrgl	carbon fixation	16	0.0	0.0
	cell growth	4	0.007	0.002
	porphyrin and chlorophyll metabolism	4	0.010	0.003
	pyruvate metabolism	6	0.011	0.003
	alkaloid biosynthesis	2	0.016	0.004
	starch and sucrose metabolism	6	0.036	0.008
	glyoxylate and dicarboxylate metabolism	3	0.051	0.051

Table 2. Fold changes of gene expression between transgenic *Arabidopsis* and WT plants. g1+WT - differential gene expression between *Ghrgl* transgenic line and WT, G1+WT - differential gene expression between *GhRGL* transgenic line and WT, g1+ G1 - differential gene expression between *Ghrgl* and *GhRGL* transgenic lines.

	AGI Locus	Description	g1+WT	G1+WT	g1+G1
Fatty acid metabolism	At4g16760	acyl-CoA oxidase	1.4509	3.4790	0.4170
	At1g54100	aldehyde dehydrogenase	1.7624	5.0204	0.3510
	At3g48000	aldehyde dehydrogenase 2	2.0499	2.6113	0.7850
	At3g51840	acyl-CoA oxidase 4	0.8574	2.7136	0.3160
	At5g63620	groES-like zinc-binding alcohol dehydrogenase family	0.8188	2.0416	0.4011
	At5g65110	acyl-CoA oxidase	1.3046	5.2110	0.2504
	At1g64400	long-chain acyl-CoA synthetase 3	3.6022	3.8772	0.9291
	At4g23850	long-chain acyl-CoA synthetase 4	2.2900	3.4778	0.6585
Fatty acid biosynthesis	AT1G74960	β-ketoacyl-ACP synthase II	1.5011	1.5198	0.9877
	AT2G38040	acetyl-CoA carboxylase	1.0110	1.3356	0.7570
	AT4G13180	NAD(P)-binding Rossmann-fold superfamily protein	1.4088	2.4532	0.5743
Gibberellic acid responses	At2g04240	xerico	0.8421	1.5210	0.5536
	At1g15550	gibberellin 3 beta-hydroxylase 1	0.7221	3.3245	0.2172
	At1g44090	gibberellin 20-oxidase	0.7788	2.4567	0.3170
	At5g67300	R2R3 factor MYB gene family	0.6871	2.0134	0.3412
	At3g63010	gibberellic acid insensitive dwarf1B	0.1983	4.1921	0.0473
	At5g02840	LHY/CCA1-LIKE 1	0.0576	1.7822	0.0323
	At5g07690	MYB29	0.2567	2.6791	0.0958
Ribosome pathway	At2g09990	ribosomal protein S5 domain 2-like superfamily protein	0.3786	0.2811	1.3469
	At4g31700	ribosomal protein S6	0.4480	0.3239	1.3831
	At5g39740	ribosomal protein RPL5B	0.8147	0.4463	1.8255
	At3g61110	ribosomal protein S27	0.4063	0.2437	1.6672
	At2g36160	ribosomal protein S11 family protein	0.3349	0.3957	0.8463
	At2g37190	ribosomal protein L11 family protein	0.6592	0.4905	1.3439
	At2g47110	UBIQUITIN 6	0.5182	0.4888	1.0601
	At3g04230	ribosomal S5 domain 2-like superfamily protein	0.3601	0.4014	0.8971
	At2g18020	embryo defective 2296	0.5255	0.2209	2.3789
	At1g77940	ribosomal L7Ae/L30e/S12e/Gadd45 family protein	0.6196	0.4492	1.3793
	At1g79850	ribosomal protein S17	0.4785	0.3118	1.5346
	At2g33800	ribosomal S5 family protein	0.5514	0.4250	1.2974
	At2g39460	60S ribosomal protein L23aA	0.5739	0.4660	1.2315
	At2g40205	ribosomal L41 family protein	0.4777	0.4953	0.9645
	At3g11120	ribosomal L41 familyprotein	0.2416	0.2700	0.8948
	At3g47370	ribosomal S10p/S20e family protein	0.3365	0.4543	0.7407

Table 2 cont.

	At3g54210	ribosomal L17 family protein	0.5017	0.4415	1.1364
	At3g14600	ribosomal L18ae/LX family protein	0.5086	0.4665	1.0902
	At3g22230	ribosomal L27e family protein	0.3993	0.2136	1.8694
	At3g60770	ribosomal protein S13/S15	0.4128	0.4211	0.9803
	At4g14320	zinc-binding ribosomal protein family protein	0.6242	0.4453	1.4018
Photosynthesis	At4g28750	quantum yield of photosystem II	0.2683	0.3728	0.7197
	At2g30790	photosystem II subunit P-2	0.2692	0.2620	1.0275
	At1g20340	DNA-damage resistance protein	0.2971	0.2006	1.4811
	At4g09650	chloroplast ATPase δ-subunit	0.3420	0.126	2.7143
	At1g20020	ferredoxin-NADP$^+$-oxidoreductase 2	0.4707	0.372	1.2653
	At1g06680	photosystem II subunit P-1	0.4383	0.3683	1.1901
	At1g03600	PSB27	0.6294	0.4703	1.3383
	At5g66570	PS II oxygen-evolving complex 1	0.3830	0.3545	1.0804
	At1g75630	vacuolar H+-pumping ATPase	0.3883	0.4588	0.8463
	At1g52230	photosystem i subunit H-2	0.6487	0.3571	1.8166
	At1g19910	vacuolar H+-pumping ATPase 2	0.3303	0.2165	1.5256
	At1g12840	photosystem i subunit H-2	0.5971	0.3626	1.6467
Carbon fixation	At4g38970	fructose-bisphosphate aldolase 2	2.8834	0.3176	9.0787
	At3g52930	aldolase superfamily protein	0.6281	0.3042	2.0648
	At3g04790	ribose 5-phosphate isomerase	0.7041	0.3358	2.0968
	At2g21330	fructose-bisphosphate aldolase 1	1.2382	0.0960	12.898
	At2g21170	plastidic triose phosphate isomerase	1.2880	0.5128	2.5117
	At2g01140	aldolase superfamily protein	0.8841	0.3412	2.5911
	At1g53240	mitochrondrial malate dehydrogenase	1.0320	0.5105	2.0215
	At5g58330	lactate/malate dehydrogenase family protein	3.5105	0.6163	5.6961
	At5g09660	microbody NAD-dependent malate dehydrogenase	2.6529	0.8305	3.1943
	At3g55800	sedoheptulose-1,7-bisphosphatase	2.6166	0.8993	2.9096
	At3g54050	chloroplastic fructose 1,6-bisphosphate phosphatase	1.7485	0.4031	4.3376
	At2g01290	ribose-5-phosphate isomerase	1.1345	0.1913	5.9305
	At1g79550	phosphoglycerate kinase	1.3604	0.5983	2.2738
	At1g70580	glyoxylate aminotransferase 2	2.3587	0.5867	4.8384
	At1g43670	fructose-1,6-bisphosphatase	0.8811	0.4240	2.0781
	At1g32060	phosphoribulokinase	2.0314	0.6500	3.1252
Chlorophyll metabolism	At2g30390	ferrochelatase gene 2	1.9040	0.5526	3.4455
	At3g51820	pigment defective 325	1.5760	0.4794	3.2874
	At1g19670	chlorophyllase 1	0.5557	0.1274	4.3619
	At1g03630	protochlorophyllide oxidoreductase	1.0382	0.3197	3.2474
Unidimensional cell growth pathway	At3g01700	arabinogalactan protein 11	1.9134	0.9683	2.2113
	At2g20000	CDC27 subunit	2.3134	0.8683	2.7113
	At1g12560	expansin A7	2.6322	0.8031	3.2324
	At1g20190	expansin 11	1.5623	1.0500	1.3078
Sucrose metabolism	At5g17310	UDP-glucose pyrophosphorylase 2	1.6322	0.8031	2.0324
	At4g39210	ADP-glucose pyrophosphorylase	6.3623	1.45	4.3878
	At2g40840	DPE2	2.4134	0.6683	3.6113
	At2g19860	hexokinase 2	2.3067	0.8926	2.5842
	At1g32900	UDP-glycosyltransferase superfamily protein	17.491	1.0497	16.663
	At1g20960	emb1507	2.4056	1.1911	2.0196

AT2G04240), which encodes a small protein with an N-terminal trans-membrane domain and a RING-H2 zinc finger motif located at the C-terminus, and the gene *GA INSENSITIVE DWARF1B* (*AtGID1B*, AT3G63010), which encodes a gibberellin receptor, were also found in this category (Fig. 1*E*).

Some genes were down-regulated in over-expressing *GhRGL* but up-regulated in *Ghrgl.* Some genes involved in the carbon fixation pathways and in the porphyrin and chlorophyll metabolism pathways, for example, *fructose-bisphosphate aldolase* gene (*AtFBA2*, At4g38970) and *peroxisomal NAD-malate dehydrogenase 2* gene (*AtPMDH2*, At5g09660), fit in this pattern of gene expression (Fig. 1*F*). *FERROCHELATASE 2* (*AtFC-II*, At2g30390) gene, which encodes one of two ferrochelatases in *Arabidopsis*, showed very low transcription in *GhRGL* lines compared to WT whereas the expresssion was significantly higher in *Ghrgl. PIGMENT DEFECTIVE 325* (*AtPDE325*, At3g51820) gene encoding a protein with chlorophyll synthase activity (performing the esterification of chlorophyllides *a* and *b*) was down-regulated in *GhRGL* lines (Table 2 and Fig. 1*G*). Two genes taking part in the cell growth were also up-regulated in *Ghrgl* and down-regulated in the *GhRGL* expressing lines (Table 2, Fig. 1*H*). There are two expansin genes, *EXPANSIN A7* (*AtEXPA7*, AT1G12560) and *EXPANSIN 11* (*AtEXPA11*, At1G20190), both expressed specifically in root hair cells and involved in root hair elongation. *ARABINOGALACTAN PROTEIN 11* (*AtAGP11*, AT3G01700) gene, which encodes a protein involved in pollen tube growth, had also a higher expression in the *Ghrgl* than in *GhRGL* expressing lines.

We also used gene ontology (GO) analysis to study the differential gene expression between the *GhRGL* over-expressing lines and WT. The major differences in gene expression were in the following biological processes: actin nucleation, adventitious root development, sucrose biosynthesis, auxin metabolism and signaling pathway, responses to abscisic acid, gibberellic acid, jasmonic acid, ethylene, and other hormones, wax biosynthesis, regulation of transcription or translation, and response to radiation. All those results provided a good base for the next step to study the changes in the metabolic pathways influenced by *GhRGL* in transgenic cotton plants.

Discussion

Plant growth inhibition by DELLA proteins: Both *Arabidopsis* transgenic lines over-expressing *GhRGL* and mutant *Ghrgl* were dwarf. Our cDNA microarray study has shown that 21 genes in the ribosome pathway were down-regulated in these two types of transgenic *Arabidopsis* plants. This result suggested that the reduction in overall protein synthesis may be an important factor leading to the dwarf phenotype. In addition, many genes in the photosynthesis pathway were also down-regulated in both of these transgenic lines which may also contribute to the dwarf phenotype (Qin *et al.* 2007).

Compared to the *GhRGL* gene, over-expression of *Ghrgl* gene increased expression of some genes involved in carbon fixation, and porphyrin and chlorophyll metabolisms indicating that the deletion of the DELLA domain in DELLA protein can improve plant photosynthesis. This result helps explain the effects of the so called '*Green Revolution*' genes (Thomas and Sun 2004). In wheat, the *Rht1* allele is a dominant gain-of-function mutation in the coding sequence of a DELLA protein (Peng *et al.* 1999). Gain-of-function mutations in these genes cause a semi-dominant dwarf phenotype and better grain yield, while loss-of-function mutations are recessive and result in increased growth.

Cotton DELLA protein and root hair development in transgenic *Arabidopsis*: The precise mechanism of the cotton fiber formation is still not fully understood, some scientists think that it may be similar to the *Arabidopsis* trichome formation (Wang *et al.* 2004, Guan *et al.* 2011) and other scientists think that it may be similar to the *Arabidopsis* root hair development (Kim *et al.* 2004, Pang *et al.* 2010). We did not discover the differences between transgenic *Arabidopsis* and WT in trichome development. However, there are some genes involved in plant root hair development that have different expression patterns in transgenic *Arabidopsis* and WT, *e.g.*, *AtEXPA7* and *AtEXPA11*, both genes having higher expression in over-expressing *GhRGL* plants. And GO analysis also confirmed the results that some genes can affect the process of the plant root development. In *Arabidopsis*, root growth can be inhibited by enhancing accumulation of DELLA proteins (Achard *et al.* 2007). These results also agree with the results of Hou *et al.* (2008). They discovered that over-expressing *RGA* in *Arabidopsis* can regulate root hair development.

Cotton DELLA proteins and the gibberellic acid biosynthesis: Our results showed that some genes participating in the GA biosynthesis pathway that is up-regulated in transgenic plants, for example *AtGA3OX1* and *AtGA20OX5* (genes involved in later steps of the GA biosynthesis). Similarly, Zentella et al. (2007) discovered that some genes involved in GA biosynthesis pathway are regulated by DELLA protein RGA. Moreover, GA promotes cotton fiber development under *in vitro* cotton ovule culture (Liao *et al.* 2009) and from this point, cotton DELLA proteins can be involved in regulating cotton fiber development form GA pathway.

Cotton fiber development and DELLA gene: Qin *et al.* (2007) demonstrated that long chain saturated fatty acids promoted cotton fiber elongation under *in vitro* cultures and cotton fiber elongation was inhibited by the addition of (2-chloro-N-[ethoxymethyl]-N-[2-ethyl-6-methylphenyl]-acetamide; ACE) an inhibitor of the long chain saturated fatty acid biosynthesis and this inhibition was rescued after addition of lignoceric acid. These results supported that fatty acid is an important factor that regulate cotton fiber development. In our results, many genes in fatty acid biosynthesis or metabolism were up-regulated in transgenic *Arabidopsis* plants over-expressing *GhRGL* or *Ghrgl* which suggests an increase in fatty acid turn-over.

Starch and sucrose metabolism genes were generally up-regulated in the *Ghrgl* but not in *GhRGL* over-expressing plants (Table 2) indicting that DELLA domain deletion leads to improvement in sucrose metabolism. Among the genes with confirmed expression there was *AtAPL3* coding the large subunit of ADP-glucose pyrophosphorylase, which catalyzes the rate limiting step in starch biosynthesis (Fig. 1*I*). Ruan *et al.* (2004) discovered that genes involved in sucrose biosynthesis have a role in cotton fiber elongation. Furthermore, *Sucrose synthase* (*SuSy*) transcripts were found to play a role in fiber cell differentiation (Ruan and Chourey 1998, Ruan *et al.* 2001, Lee *et al.* 2007). These results indicated that DELLA domain deletion may improve sucrose metabolism and hence promote cotton fiber development.

Conclusion: Comparative transcriptome profiling was performed on *Arabidopsis* transgenic lines over-expressing both *GhRGL* and *Ghrgl*. *KEGG* pathway analysis indicated that the differential genes participated in many important pathways. Fatty acid metabolism and biosynthesis pathways were up-regulated in both *GhRGL* and *Ghrgl* over-expressing plants, ribosome metabolic pathway and photosynthesis pathway were down-regulated in both *GhRGL* and *Ghrgl* transgenic *Arabidopsis* plants. Response to gibberellic acid stimulus pathway and unidimensional cell growth pathway were up-regulated in over-expressing GhRGL but down-regulated in *Ghrgl.* Carbon fixation pathway and porphyrin and chlorophyll biosynthesis were down-regulated in over-expressing *GhRGL* but up-regulated in *Ghrgl*. All these results give a clue to understand cotton fiber development.

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