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SHORT COMMUNICATION

Exogenous Jasmonic Acid Inhibits Cotton Fiber Elongation

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Abstract Jasmonic acid (JA) is a well-characterized phytohormone that acts in various ways to influence plant development. Its role in cotton fiber development, however, has not yet been thoroughly explored. In this study, JA was proven to be an inhibitor of ovule and fiber development in vitro. Continuous exogenous JA application inhibited fiber elongation. This effect was dependent on development stage and dosage. Fibers and ovules at three different stages of development and different JA dosages were compared. The most serious suppression was detected when ovules 1 day before anthesis (–1 DPA) were cultured in medium with 2.5 μM JA. Genes related to trichome and fiber development responded differently to JA treatment between –1 DPA and 1 day post anthesis (1 DPA). JAs (JA and JA-Ile) quantification showed that JAs content was sharply decreased from –1 DPA to 5 DPA ovules, which indicated that JA was negatively associated with fiber elongation in vivo. In addition, gene expression analysis showed the same trend. Our results demonstrate that there was a negative relationship of JA with fiber elongation in vitro and in vivo. These results are meaningful for uncovering the mechanism of fiber elongation in cotton.

Keywords Jasmonic acid · Fiber elongation · Ovule culture · Gene expression

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Introduction

Cotton fiber, commonly known as cotton lint, is one of the longest plant cells. Fiber development is divided into several distinctive but overlapping phases. The fate of fiber cells is determined before the day of anthesis (−1 day post anthesis [DPA]). The fiber cells initiate on the day of anthesis and elongate until 20 DPA. The most active elongation period is from 5 to 10 DPA (Shi and others 2006). Study of in vitro cultures of cotton ovules has verified that hormones are extremely important to fiber development (Beasley 1971). Auxin and gibberellin promote fiber development (Beasley and Ting 1973), and abscisic acid and cytokinin are inhibitors (Beasley 1973; Gokani and others 1998). Recent studies revealed that brassinosteroids (BRs) and ethylene also promote fiber development (Shi and others 2006; Sun and others 2005). A previous study indicated that endogenous hormones seem to temporally regulate fiber development (Chen and others 1996).

Cotton fibers are seed trichomes, and their development has many similarities with *Arabidopsis* leaf trichome development (Lee and others 2007). Jasmonates are positive factors in the development of *Arabidopsis* trichomes. Exogenous treatment with JA increases the density of leaf trichomes in *Arabidopsis* (Traw and Bergelson 2003). Further study indicated that JA controls patterning of trichomes by regulating GL3, a key transcription factor involved in wound-induced trichome formation (Yoshida and others 2009). Although jasmonates increase trichome density in *Arabidopsis*, JA inhibits cell elongation as an antagonist of gibberellic acid (GA) and indole-3-acetic acid (IAA) (Tung and others 1996; Ueda and others 1994). We speculated that JA might also be involved in fiber development in cotton. Our data indicate that JA is negatively associated with fiber development.

Materials and Methods

Plant Materials

Plants of upland cotton *G. hirsutum* cv. Xuzhou142 were grown in the field. Bolls were tagged and the day of anthesis was defined as 0 DPA. The flower bud that was longer than the bracts and would open the next day was defined as –1 DPA boll. Ovules were collected at various intervals (−1 to 16 DPA) in the morning. The materials were frozen in liquid nitrogen and stored at −70°C before use.

Cotton Ovule Culture

Flowers were harvested at –1, 0, and 1 DPA, from 8 to 9 o'clock in the morning. Ovule culture was performed as described previously (Beasley and Ting 1973). JA was added to the liquid BT media in concentrations of 0.1, 0.5, and 2.5 μM. All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). The ovules were collected after 2 days of culture for electron microscopical scanning, gene expression analysis, and hormone measurement. Fiber length and ovule volume were measured after 20 days of incubation. Fiber measurement was performed as previously described (Sun and others 2005). The volumes of ovules, whose fibers were stripped, were measured by the displacement method. Three replicates were done in each assay. For each replicate five random ovules were grouped together and then dipped into a measuring cylinder with a fixed volume of water. The increase in volume was statistically analyzed. More than four groups were tested in each replicate.

RNA Extraction and qRT-PCR Assays

Total RNA was isolated using a modified guanidine thiocyanate method (Zhu and others 2005). Total RNA quantitation, cDNA synthesis, and qRT-PCR were performed according to a method previously described by Tu and others (2007). Three micrograms of RNA and 200 U of Superscript III Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA) were added to each reaction following the procedure. The cDNA was diluted to 300 μl and stored at –20°C until use. Real-time PCR was performed on an ABI Prism 7000 (Applied Biosystems, Foster City, CA, USA). The JA-related genes were acquired from the DFCI Cotton Gene Index (Table 1). Genes related to fiber development were selected from previous publications (Lee and others 2007; Qin and others 2007). All the genes were named by referring to their closest *Arabidopsis* homologs without the *Gh* prefix. Each sample was assayed three times; error bars represent standard deviation (SD). *GhUB7* was considered an internal control (Tu and others 2007). Related primers are listed in Table 1.

Scanning Electron Microscopy (SEM)

Scanning electron microscopy of cultured cotton ovules was performed according to a method from a previous study (Sun and others 2005). The samples were viewed and photographed with a JSM-6390/LV SEM (Jeol, Tokyo, Japan).

Extraction and Quantification of JAs

Jasmonic acid were extracted according to the method described by Shindy and Smith (1975). Samples (200 mg) were ground to powder and extracted twice with 80% cold methanol (v/v) (containing 0.01% ascorbic acid as an antioxidant) overnight at 4°C. The supernatant was evaporated to the aqueous phase with N_2, adjusted to pH 3.0 with 0.4 M citric acid, and partitioned twice with equal volumes of ethyl acetate. The combined acidic ethyl acetate phase was evaporated until dry with N_2, then dissolved in 0.4 ml methanol and stored at –20°C before measurement. JA (which we applied on ovule culture) and JA-Ile (the most active jasmonate) were quantified using an Applied Biosystems 4000Q-TRAR HPLC-MS system, with JA (Sigma) and JA-Ile (OlChemIm, Olomouc, Czech Republic) as the external standards. Three independent assays were carried out. The quantifications for each assay were repeated three times per ovule sample and five times per fiber sample. Error bars represent SD.

Results and Discussion

Phytohormones are very important regulators of fiber development in cotton. Although many meaningful studies have been performed, none have reported on the effect of JA on ovule and fiber development. Additionally, JA has been shown to affect cell elongation and trichome formation (Staswick and others 1992; Traw and Bergelson 2003), which spurred us to investigate whether JA participates in fiber development.

To confirm the effect of JA on fiber initiation, –1 DPA ovules were treated with JA in vitro. After 2 days of culture, the number of initiated fiber cells was reduced with JA treatment (Fig. 1a). Many initiated fibers were broken in the ovules after treatment with 0.1 and 0.5 μM JA [Fig. 1a (6), (9)]. All the ovules treated with JA exhibited fewer fiber initiations than the controls (Fig. 1a). The fewest fiber initiations were detected with the highest concentration of JA (2.5 μM) [Fig. 1a (11), (12)]. Unlike leaf trichome formation in *Arabidopsis*, fiber initiation was obviously suppressed by exogenous JA application.

A previous study also indicated that JA is involved in cell elongation (Tung and others 1996; Ueda and others

Table 1 JA- and fiber development-related genes and primers used for real-time PCR

Gene	Full name	Accession No.	Primer sequence: Forward (5'–3'), Reverse (5'–3')
OPCL1	Fatty-acyl-CoA synthase	DT566506	Forward-AGCATGGAGGCTAAGGTTGTG
			Reverse-CCATCCGTCCGAGTCAAGTGT
ST2A	12-hydroxyjasmonate sulfotransferase	ES838316	Forward-TTCTCTGCCAAGCTCCGAGAC
			Reverse-GGGTTTTCTTGGCTTGCCTTC
JMT	JA carboxyl methyltransferase	DW497937	Forward-CTAGCACAAGTCCACCAAACG
			Reverse-AACCATACGCCCTCCATCCA
JAR1	Jasmonyl-isoleucine synthetase	ES824991	Forward-AGCCGCATCACTTTCCCATC
			Reverse-TTAGGGAAAAGCTCCGGTATC
JAZ1	Jasmonate ZIM domain gene 1	TC190071	Forward-ACAAGGCGGAAAGCATCTTCAAACT
			Reverse-CCATTACGAGCTTCGAGGAGGTTTT
JAZ2	Jasmonate ZIM domain gene 2	DW240147	Forward-CTCAAAAAGGAAGACCTCAAACTTG
			Reverse-CGAAAGGATAGGATCACCGCAAAA
COI1	Coronatine-insensitive 1	ES801795	Forward-GACTCGGTTCGTTTCATTACTGG
			Reverse-TTCTTCGCCGTTGGTTATCATT
MYC2	MYC transcription factor	TC210013	Forward-ATTTCCCACCATCCCTACATTCT
			Reverse-GTCGACGCAAATCGATAGTCAGT
GL2	GLABRA2	TC181236	Forward-ATCGTCTGTGCTGTTTCTTCCG
			Reverse-GCATTTCCCCGATCCTTTCC
GL3	GLABRA 3	TC182676	Forward-GTGCAATCAGACTAAACTAACA
			Reverse-TGGTCCTAAAATCAACGGGTGT
EGL3	Enhancer of GLABRA 3	TC181771	Forward-ATGAACTTTGTAAAAGCCACGTCT
			Reverse-TTTCGTTTTAGTCTCAGATTCGGT
MYB109	MYB transcription factor	AJ549758	Forward-ACAGTGGATCAGAGTAACCAGCAG
			Reverse-ATGGTCAGGAATCCAGAAAGTGTT
EXP1	Alpha-expansin	AF512539	Forward-CAATCCCCCACGAGAACACT
			Reverse-GCAAAAGGTCAAACACCCAAC
GLP1	Germin-like protein 1	AF116537	Forward-CCGACTTCTGTGTTGGGGAC
			Reverse-CGAAAAGGCTGGTGTTATTGC
KCS12 [a]	3-Ketoacyl-CoA synthase 12	AJ608934	Forward-CGTTCTCTCCCAACTATCTCGCCCTT
			Reverse-GTGATCAGCACCATGGGTACGGAGG
GhUB7 [b]	Ubiquitin 7	DQ116441	Forward-GAAGGCATTCCACCTGACCAAC
			Reverse-CTTGACCTTCTTCTTCTTGTGCTTG

[a] Primers referenced from Qin and others (2007)

[b] Primers referenced from Shi and others (2006)

1994). To analyze the effect of JA on fiber elongation, –1, 0, and 1 DPA ovules treated with JA were cultured for 20 days, when the ovules and fibers were almost at maximum growth in the medium (Fig. 1b). The fiber length of the ovules was significantly reduced by JA application (Fig. 1c). Fiber elongation was reduced with 0.1 μM JA and a more severe effect was detected with 0.5 μM JA; fiber development was absolutely suppressed in –1 and 0 DPA ovules with 2.5 μM JA.

Additionally, –1 DPA ovules were more sensitive to exogenous JA application during in vitro culture. Significant differences between the ovules of three different developmental stages were found with JA at a dose of 0.5 μM. A more severe effect was observed with 2.5 μM JA, the unfertilized ovules (–1 and 0 DPA) showed no fiber development (Fig. 1c). The volumes of ovules were reduced with a low concentration of JA and they exhibited a significant difference in different development stages with the same dose of JA treatment (Fig. 1d). Ovules treated with 2.5 μM JA increased in volume because they were callused, and the younger ovules exhibited more sensitivity to JA application. These results indicate that JA inhibited ovules and fiber development in a dose- and development stage-dependent manner.

The –1 and 1 DPA ovules responded differently to JA application. The expressions of some well-known fiber development-related genes were analyzed in these ovules after 2 days of culture (Fig. 2). The gene expression

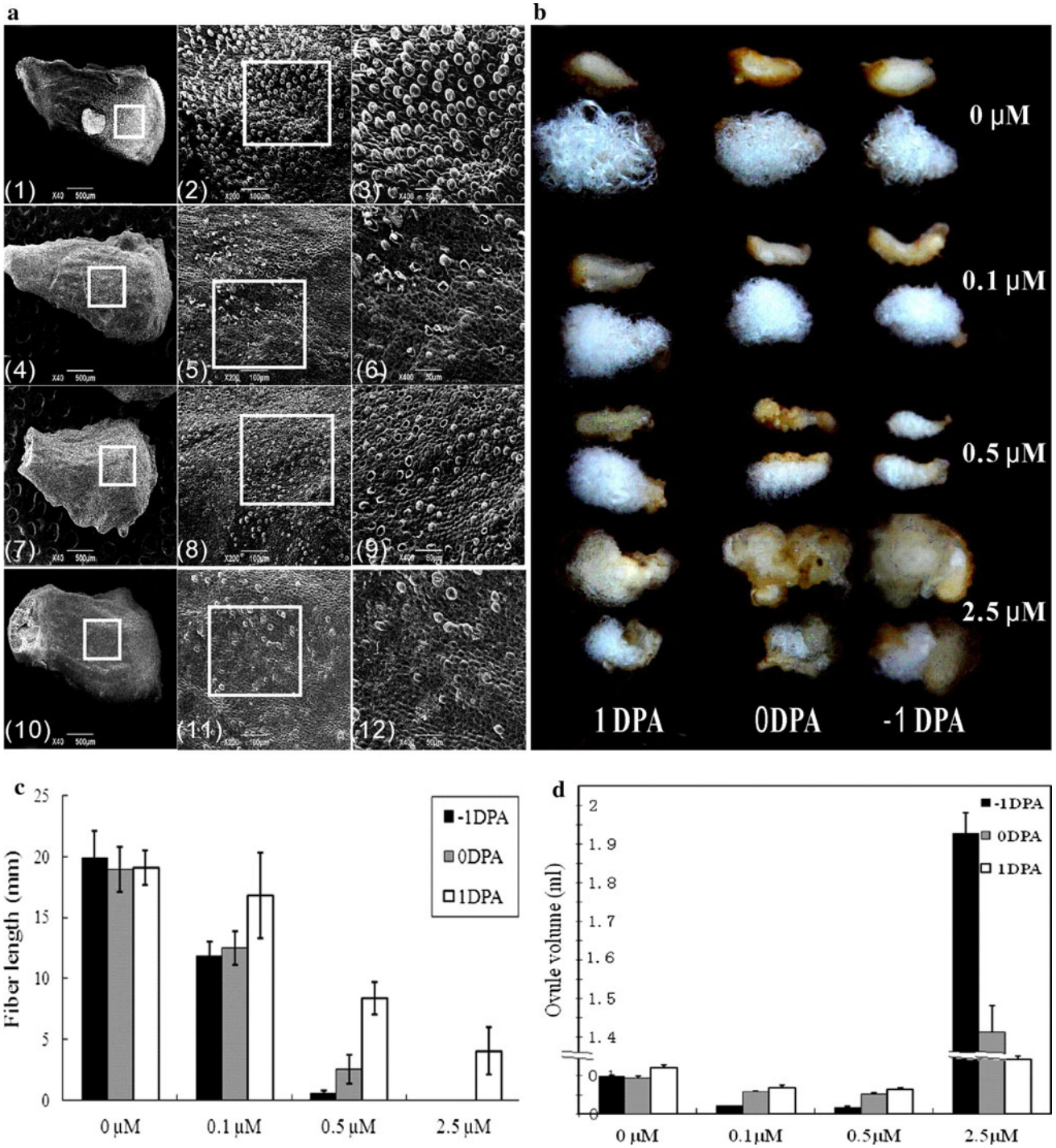

Fig. 1 Effects of exogenous JA application on cultured ovules. **a** SEM analysis of the fiber development of –1 DPA ovules after 2 days of culture. (*1*) Ovules were cultured with standard BT medium; (*4*) ovules treated with 0.1 μM JA; (*7*) ovules treated with 0.5 μM JA; and (*10*) ovules treated with 2.5 μM JA; scale bar = 500 μm. (*2*), (*5*), (*8*), and (*11*) are the enlarged views of the rectangles in (*1*), (*4*), (*7*), and (*10*), respectively; scale bar = 100 μm. (*3*), (*6*), (*9*), and (*12*) are the enlarged views of the rectangles in (*2*), (*5*), (*8*), and (*11*), respectively; scale bar = 50 μm. **b** Three kinds of ovules were cultured for 20 days. For each sample the upside is the delinted cultured ovule and the bottom is an intact cultured ovule. **c** Fiber length of cultured cotton ovules. **d** Volume of cultured ovules without fiber. The values are means of 20 randomly selected ovules for each treatment in three independent assays. Error bars represent the SD

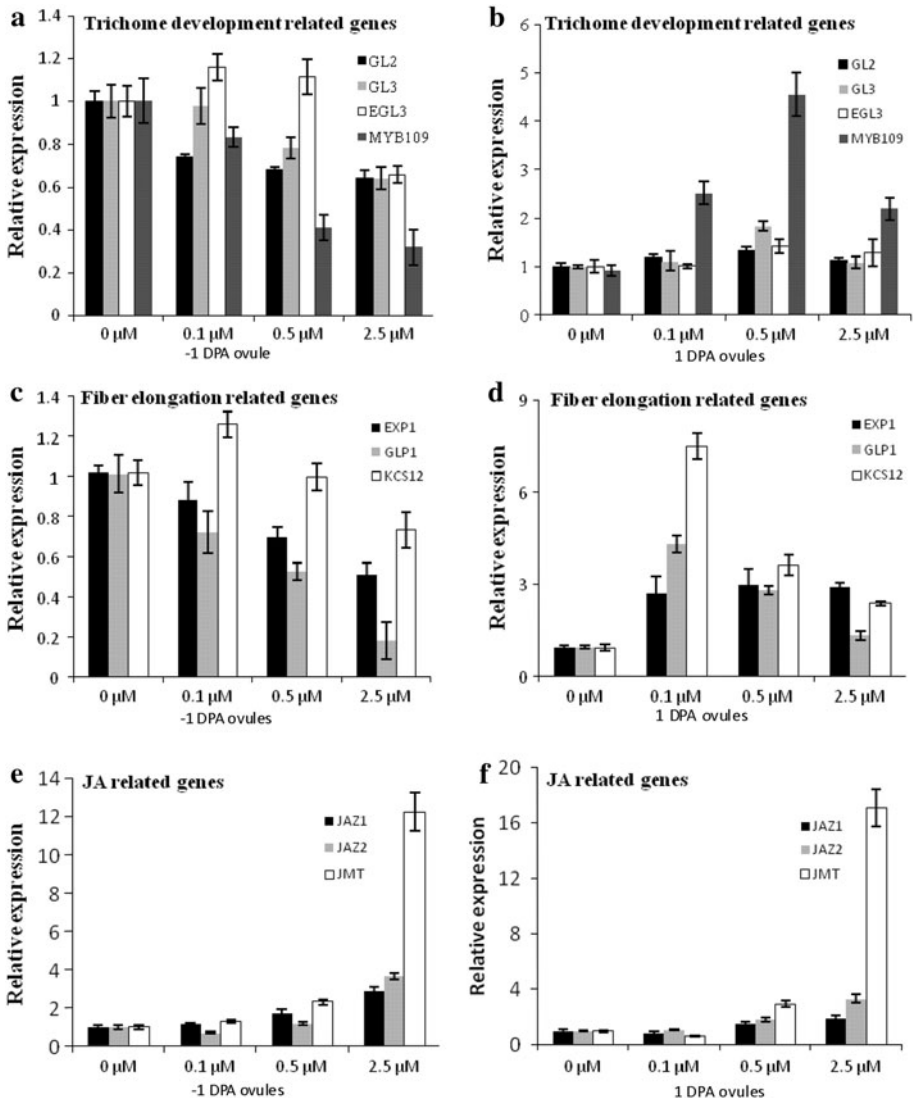

Fig. 2 JA-altered gene expression in ovules after 2 days of culture. The expression of three types of genes was analyzed in (**a**, **c**, **e**) –1 DPA and in (**b**, **d**, **f**) 1 DPA ovules after 2 days of culture. **a**, **b** The expression of trichome development-related genes. **c**, **d** The expression of fiber elongation-related genes. **e**, **f** The expression of JA-related genes. The concentrations of JA are on the *x* axis. The expression level with 0 μM JA treatment was referenced as 1. The cultured ovules for each treatment are indicated under the line

patterns were altered differently in –1 and 1 DPA ovules (Fig. 2a–d). After 2 days of culture, most genes in the –1 DPA ovules treated with JA were suppressed. The expression of *GL2*, *MYB109*, *EXP1*, and *GLP1* exhibited obviously negative correlations with JA treatment (Fig. 2a, c), and the expression of *GL3*, *EGL3*, and *KCS12* was also suppressed with high levels of JA (Fig. 2a, c). Gene expression was consistent with the suppression of development in the –1 DPA ovules (Fig. 1b). The expression pattern of *GL3* was different from that in *Arabidopsis* (Yoshida and others 2009), which indicates that the JA response in fiber cells is different from that in leaf trichomes. In contrast, the expression of all genes was upregulated in 1 DPA ovules. The expression of trichome development-related genes was highest in ovules treated with 0.5 μM JA (Fig. 2b), and the expression of fiber elongation-related genes peaked with 0.1 μM JA (Fig. 2d). These data confirm that ovules in different stages of development respond differently to JA treatment. We also noticed that JA promoted the expression of genes related to fiber development in 1 DPA cultured ovules but obviously inhibited fiber development in the cultured ovules. The increased transcription of these genes might not offset JA's inhibitor effect.

Ovules in different stages of development responded differently to JA application. JA-related genes were analyzed after 2 days of culture. Three JA-related genes, *JMT*, *JAZ1*, and *JAZ2*, were upregulated by an increased dosage of JA in both –1 and 1 DPA ovules. *JMT* was most markedly affected (Fig. 2a, f). These results indicate that the first response to JA application is similar in different types of ovules. The serious effect on fiber development might be caused by a mechanism downstream of the JA response.

Exogenous JA inhibited fiber development, but what about the content of JAs in vitro and in vivo? The JAs content in cultured ovules and the ovules from plants were measured (Fig. 3). The JA and JA-Ile levels were increased with the increased concentration of JA. We noticed that the content of these JAs in the cultured ovules treated with the highest level of JA (2.5 μM) was comparable to that of –1 DPA in vivo ovules, which means that the JA treatment level was not too extreme for ovule development. The JAs content was sharply decreased in –1 to 5 DPA ovules in plants (Fig. 3). The cultured ovules would be continuously encompassed with a relatively high level of JA, especially during the fiber elongation stage, and this would be the main reason that exogenous JA inhibits fiber development. JA was proven to be the antagonist of several fiber promoters such as GA, IAA, BRs, and ethylene. However, their content and related gene expression profiles (data not shown) had no obvious relevance to JA application.

The transcription pattern of JA-related genes was also analyzed. These genes were identified from the DFCI Cotton Gene Index database through homologous alignment with the *Arabidopsis* JA-related genes (Table 1). The transcripts of *OPCL1*, *JAR1*, *ST2A*, and *JMT*, which were upstream of JA biosynthesis (Fig. 4a), and of *JAZs*, *COI1*, and *MYC2*, which were downstream of JA biosynthesis (Fig. 4b), were all highly expressed in –1 DPA ovules, but they were expressed at a lower level after 0 DPA. The transcription patterns of these genes were consistent with

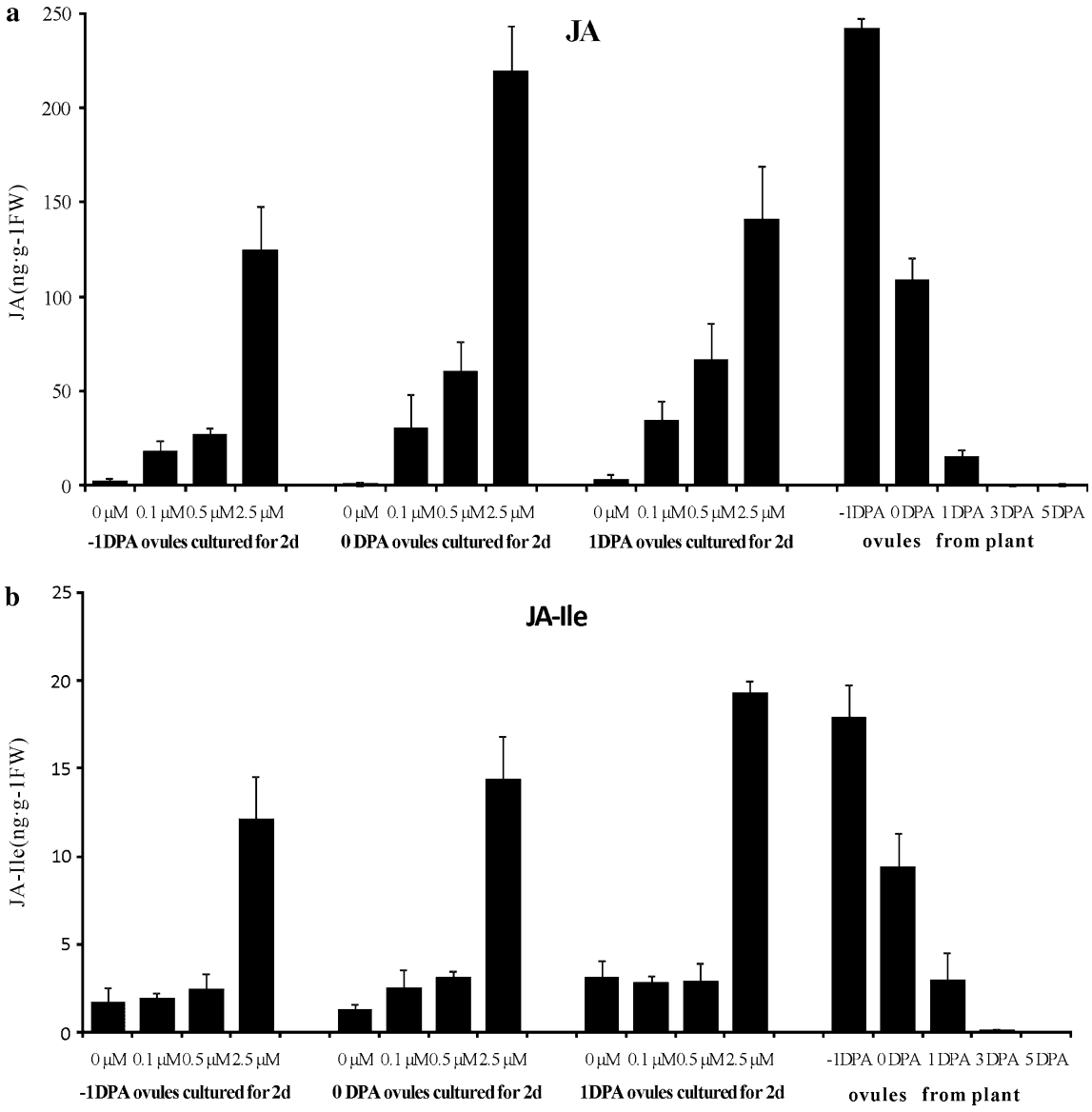

Fig. 3 JA content (**a**) and JA-Ile content (**b**) in cultured ovules and ovules from plants. Three independent experiments were performed. Bars represent the SD

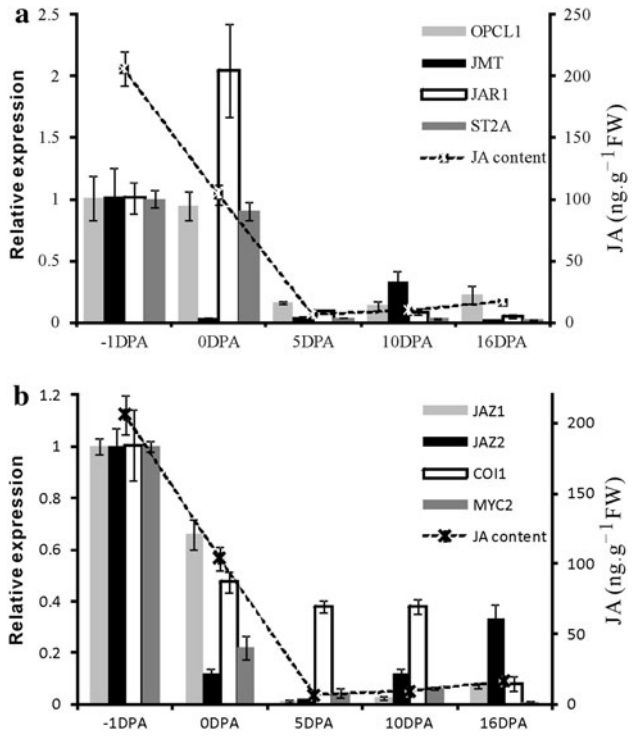

Fig. 4 In vivo analysis of JA in ovules and fibers. **a**, **b** Expression patterns of JA biosynthesis and responsiveness-related genes and the corresponding JA levels in vivo in developing ovules and fibers. For gene expression, error bars represent SD for three replicates. For endogenous JA levels, error bars represent SD for three independent experiments. Ovules (–1 to 5 DPA) and fibers (10 and 16 DPA) were employed for analysis

the decreasing JA levels in plants (Fig. 4). These genes were very important to JA responsiveness in vivo. The low transcription levels and low JAs content both indicate that JA responsiveness was weak in developing fibers after 0 DPA. These results show that JA is negatively associated with fiber elongation in vivo.

Our work presents the first view of the effect of JA on fiber development. JA was an inhibitor of fiber elongation in vitro, which was consistent with the increasing endogenous JAs content in cultured ovules. Continuous exogenous JA application would inhibit fiber initiation and elongation. Inhibition was dependent on development and dosage. Active JA responsiveness was found in –1 DPA ovules, but it sharply decreased after –1 DPA, showing that it was negatively related to fiber development. JA might be positively involved in fiber development in –1 DPA ovules but has a negative effect on fiber elongation. The high JA content in –1 DPA ovules and the functional aspects of JA need to be further investigated.

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References

Beasley CA (1971) *In vitro* culture of fertilized cotton ovules. Bioscience 21:906–907

Beasley CA (1973) Hormonal regulation of growth in unfertilized cotton ovules. Science 179:1003–1005

Beasley CA, Ting IP (1973) The effects of plant growth substances on *in vitro* fiber development from fertilized cotton ovules. Am J Bot 60:130–139

Chen J, Du X, Zhao H, Zhou X (1996) Fluctuation in levels of endogenous plant hormones in ovules of normal and mutant cotton during flowering and their relation to fiber development. J Plant Growth Regul 15:173–177

Gokani S, Kumar R, Thaker V (1998) Potential role of abscisic acid in cotton fiber and ovule development. J Plant Growth Regul 17:1–5

Lee JJ, Woodward AW, Chen Z (2007) Gene expression changes and early events in cotton fibre development. Ann Bot (Lond) 100:1391–1401

Qin Y, Hu C, Pang Y, Kastaniotis AJ, Hiltunen JK, Zhu Y (2007) Saturated very-long-chain fatty acids promote cotton fiber and *Arabidopsis* cell elongation by activating ethylene biosynthesis. Plant Cell 19:3692–3704

Shi Y, Zhu S, Mao X, Feng J, Qin Y, Zhang L, Cheng J, Wei L, Wang Z, Zhu Y (2006) Transcriptome profiling, molecular biological, and physiological studies reveal a major role for ethylene in cotton fiber cell elongation. Plant Cell 18:651–664

Shindy WW, Smith OE (1975) Identification of plant hormones from cotton ovules. Plant Physiol 55:550–554

Staswick PE, Su W, Howell SH (1992) Methyl jasmonate inhibition of root growth and induction of a leaf protein are decreased in an *Arabidopsis thaliana* mutant. Proc Natl Acad Sci U S A 89: 6837–6840

Sun Y, Veerabomma S, Abdel-Mageed HA, Fokar M, Asami T, Yoshida S, Allen RD (2005) Brassinosteroid regulates fiber development on cultured cotton ovules. Plant Cell Physiol 46:1384–1391

Traw MB, Bergelson J (2003) Interactive effects of jasmonic acid, salicylic acid, and gibberellin on induction of trichomes in *Arabidopsis*. Plant Physiol 133:1367–1375

Tu L, Zhang X, Liu D, Jin S, Cao J, Zhu L, Deng F, Tan J, Zhang C (2007) Suitable internal control genes for qRT-PCR normalization in cotton fiber development and somatic embryogenesis. Chin Sci Bull 52:3110–3117

Tung P, Hooker TS, Tampe PA, Reid DM, Thorpe TA (1996) Jasmonic acid: effects on growth and development of isolated tomato roots cultured *in vitro*. Int J Plant Sci 157:713–721

Ueda J, Miyamoto K, Aoki M (1994) Jasmonic acid inhibits the IAA-induced elongation of oat coleoptile segments: a possible mechanism involving the metabolism of cell wall polysaccharides. Plant Cell Physiol 35:1065–1070

Yoshida Y, Sano R, Wada T, Takabayashi J, Okada K (2009) Jasmonic acid control of GLABRA3 links inducible defense and trichome patterning in *Arabidopsis*. Development 136:1039–1048

Zhu L, Tu L, Zeng F, Liu D, Zhang X (2005) An improved simple protocol for isolation of high quality RNA from *Gossypium* spp. suitable for cDNA library construction. Acta Agron Sin 31:1657–1666