

Full Length Research Paper

Effect of different light quality on DNA methylation variation for brown cotton (*Gossypium hirsutum*)

Tingchun Li^{1,2#}, Honghong Fan^{1#}, Zhengpeng Li¹, Jun Wei¹, Yongping Cai¹ and Yi Lin^{1*}

¹Department of life science, Anhui Agricultural University, Hefei, 230036, People's Republic of China.

²Anhui Academy of Agricultural Sciences, Hefei, 230031, People's Republic of China.

Accepted 14 March, 2011

DNA methylation plays an important role in regulating gene expression during plant development. We studied the effects of different light quality on DNA methylation patterns of brown cotton (*Gossypium hirsutum*) by using the methylation sensitive amplified polymorphism (MSAP). We selected 66 pairs of MSAP selective amplification primers to assess the status of cytosine methylation, with diverse patterns and percentages of DNA methylation under different light quality. When the brown cotton was treated with blue, white + ultraviolet-B, red, white and yellow lights, respectively, frequency of total and full methylation were 24.15/13.64, 25.68/15.23, 27.09/16.35, 19.29/7.58 and 21.92/11.08%, respectively. In addition, 4 monomorphic fragments and 1 polymorphic fragment that appeared in the electrophoretogram were sequenced and analyzed. The sequences alignment revealed that both coding and non-coding regions could be methylated or demethylated by differing light quality, suggesting an epigenetic response of plants to light.

Key words: DNA methylation, methylation sensitive amplified polymorphism (MSAP), light response, light quality, cotton.

INTRODUCTION

Light is the main source of energy for plant growth and has profound impacts on plant development. It induces complicated gene-expression networks leading to physiological and biochemical responses including phototropism, photomorphogenesis, chloroplast differentiation,

germination, flowering and fructification (Jiao et al., 2007). In order to respond to light, plants have adapted their abilities to sense complex parameters of light signals, including light period, light direction, light quality and quantity. They have also evolved complicated and sophisticated systems for responding to a broad spectrum of light, ranging from far-red light to ultraviolet B (UV-B), possessing distinct photoreceptors sensing UV-B, ultraviolet A (UV-A), blue, green, red and far-red (Chen et al., 2004; Jiao et al., 2007; Hu and Desai, 2008; Castillon et al., 2009). Four distinct families of photoreceptors are known: Phytochromes, cytochromes, phototropins and unidentified ultraviolet B photoreceptors. The phytochromes are dimeric chromoproteins and they predominately absorb the far-red and red lights. The cytochromes and phototropins absorb blue light and UV-A and the unidentified photoreceptors absorb UV-B.

Light response is an intricate process for plants, involving many genes and regulating pathways of plant metabolism systems. In recent years, extensive progress has been made towards characterizing the organization of light-regulated transcriptional networks and various regulators downstream of photoreceptors have been identified (Jiao

*Corresponding author. E-mail: yjsc01@ahau.edu.cn. Tel: +86-551-5786406. Fax: +86-551-5786256.

Abbreviations: UV-B, Ultraviolet B; *CPRF1*, common plant regulatory factor 1; *CPRF2*, common plant regulatory factor 2; *LAF1*, long after far-red light 1; *bHLH*, basic helix-loop-helix protein; *CTAB*, cetyl trimethyl ammonium bromide; MIP, major intrinsic proteins; *TIP*, tonoplast intrinsic protein; *PIF*, proteolysis-inducing factor; *phy*, phytochrome; *GBFs*, G-box-binding transcription factors; *MSAP*, methylation-sensitive amplified polymorphism; *PCR*, polymerase chain reaction; *EDTA*, ethylenediaminetetraacetic acid; *BLAST*, Basic Local Alignment Search Tool; *NCBI*, National Center for Biotechnology Information; *MIP*, major intrinsic proteins; *BAC*, bacterial artificial chromosome; *PEP*, phosphoenolpyruvate.

#These Authors contributed equally to this work.

et al., 2007; Hu and Desai, 2008; Castillon et al., 2009). Many of them encode transcription factors, phosphatases and kinases, such as common plant regulatory factor 1 (*CPRF1*), common plant regulatory factor 2 (*CPRF2*), long after far-red light 1 (*LAF1*), basic helix-loop-helix protein (*bHLH*) and others. *CPRF1* and *CPRF2* are G-box-binding transcription factors (*GBFs*). *LAF1* functions as a positive regulator of gene expression downstream of far-red light. However, a *bHLH* transcription factor is a repressor of seedling de-etiolation which was mediated by blue and far-red light (Jiao et al., 2007). Some of these genes are specifically expressed, others as regulators of signal transduction networks in response to different kinds of light signals. Large number of gene transcripts is reprogrammed to express activated proteins when induced by light. Light effects are so profound and perplexing that most of the biochemical pathways are involved and coordinately regulated.

DNA methylation has been hypothesized as an underlying mechanism of temporary changes in the phenotype (Suzuki et al., 2008). It is associated with regulation of gene expression, cell differentiation, genomic imprinting and so on. It plays a fundamental role in epigenetic regulation in the whole process of plant development (Jaligot et al., 2008). For instance, it is closely correlated with the response mechanism when plants suffer various environmental changes such as salt stress, drought menace and virus infection (Zhong et al., 2009; Dalakouras et al., 2010; Pan et al., 2009; Mason et al., 2008). However, reports on relationships between DNA methylation and light response mechanism for plant are few. *Stellaria longipes* treated with low R/FR light ratios showed a lower level of methylation which was a crucial factor in controlling the stem elongation response (Tatra et al., 2000).

Methylation-sensitive amplified polymorphism (MSAP) technique is a powerful tool for the analysis of genome methylation status (Reyna et al., 1997; Jaligot et al., 2004). It has been successfully applied to study epigenetic variation in many plants, such as the effect of short day photoperiod on DNA methylation in rice, the DNA-methylation changes in wheat induced by salt stress and variations of DNA methylation during tomato fruit development and ripening (Zhong et al., 2009; Thanananta et al., 2006; Teyssier et al., 2008). Light response is a complex process for plants. To our knowledge, research reports on the changes of DNA methylation status of plants under different light quality are few. In this study, the MSAP technique was used to test the effect of light quality on the pattern and extent of cytosine methylation in cotton cultivar.

MATERIALS AND METHODS

Plant materials and treatment conditions

The experimental cotton cultivar (*Gossypium hirsutum*) was ZONGCAIXUAN No.1, bred by Life Science Department of Anhui

Agricultural University, People's Republic of China. Seeds were germinated on a filter paper soaked in water at 28°C in the dark for 5 days. 15-day-old seedlings were cultivated in Hoagland solution in a growth chamber and separately supplied with white (W), white + UV-B (C), red (R), yellow (Y) and blue (B) with a photosynthetic photon flux density of 200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ by LED light source for 7 days with time period 10/14 (day/night), the treating temperature 30/25°C (day/night) and relative humidity 65 to 67%. The plants under treatment of white + UV-B were selected as controls, which were additionally illuminated with UV-B with 600 $\mu\text{W}\cdot\text{cm}^{-2}$ intensity for 15 min everyday.

DNA extraction

Genomic DNA was extracted from cotton leaves which were collected from the second leaf from top using a modified cetyl trimethyl ammonium bromide (CTAB) method as described by Murray and Thompson (1980).

Methylation-sensitive amplified polymorphism (MSAP) analysis

To detect MSAP, 200 ng of cotton genomic DNA was double-digested with two combinations of restriction enzymes concurrently. In the first reaction, the DNA was digested with 20 units *EcoRI* (Takara, People's Republic of China) and 20 units *HpaII* (Takara, People's Republic of China) in 20 μl of reaction mixture at 37°C for 2 h. The second digestion reaction was carried out in the same way, except that *MspI* (Takara, People's Republic of China) was used instead of *HpaII*. The reaction was terminated by incubation at 65°C for 10 min. Subsequently, the ligation reaction was carried out for additional 6 h at 20°C in a final volume of 40 μl , which contains 1 unit T4 DNA ligase, 0.2 mM ATP, 5 pM *EcoRI* adapters (5'-CTCGTAGACTGCGTACC-3', 5'-AATTGGTACGCAGTC-3') and 50 pM *HpaII/MspI* adapters (5'-GATCATGAGTCCTGCT-3', 5'-CGAGCAGGACTCATGA-3').

The pre-amplification was performed by using the ligation mixture as template DNA with E+A primer (5'-GACTGCGTACCAATTC+A-3') and HM+T primer (5'-ATCATGAGTCCTGCTCGG+T-3'). The polymerase chain reaction (PCR) reaction was performed in a 25 μl of a reaction mixture with 1 μl of ligation reaction mixture, 50 ng of E+A, 50 ng of HM +T, 0.5 unit Taq DNA polymerase (Biocentury transgene, People's Republic of China), 0.2 mM dNTP (Biocentury transgene, People's Republic of China) and 2.5 μl of 10 × polymerase buffer (Biocentury transgene, People's Republic of China) for 21 cycles with denaturation at 94°C for 1 min, annealing at 56°C for 1 min and extension at 72°C for 1 min.

For selective amplification, the product of pre-amplification was selected as DNA template and six *EcoRI* primers in combination with eleven *HpaII/MspI* primers were synthesized based on the principles that its sequence was identical to the pre-amplification primers in addition to two nucleotides at the 3' terminus: E+A+NN primer (5'-GACTGCGTACCAATTC+A+NN-3') and HM+T+NN primer (5'-ATCATGAGTCCTGCTCGG+T+NN-3'). The PCR reactions were performed in volumes of 25 μl , containing 0.3 μl of pre-amplification product, 50 ng of *EcoRI* primer, 50 ng of *HpaII/MspI* primer, 1 unit Taq polymerase, 0.5 mM dNTP and 2.5 μl of 10×PCR buffer. The PCR procedure was carried out according to the standard amplified fragment polymorphism touchdown protocol (Vos et al., 1995). The adapters, the pre-amplified primers and the selective primers of *EcoRI* and *HpaII/MspI* are listed in Table 1.

The samples of selective amplification were mixed with 8 μl of denaturing buffer (98% formamide, 10 mM ethylenediamine-tetraacetic acid (EDTA), 0.1% bromophenol blue and 0.1% xylene cyanol) (Sangon, People's Republic of China), then denatured at 95°C for 5 min and separated on 6% polyacrylamide gel (6% polyacrylamide, 8 M urea) (Sangon, People's Republic of China), in

Table 1. Adaptors and primers used for MSAP analysis.

Adaptor/primer	Enzyme	
	<i>EcoRI</i> (E) (5' to 3')	<i>HpaII/MspI</i> (H/M) (5' to 3')
Adaptors	CTCGTAGACTGCGTACC AATTGGTACGCAGTC	GATCATGAGTCCTGCT CGAGCAGGACTCATGA
Pre-amplification primers	GACTGCGTACCAATTCA	ATCATGAGTCCTGCTCGGT
Selective amplification primers	GACTGCGTACCAATTCAAC(E1)	ATCATGAGTCCTGCTCGGTGCG(H1)
	GACTGCGTACCAATTCAAG(E2)	ATCATGAGTCCTGCTCGGTGCG(H2)
	GACTGCGTACCAATTCAC(T)(E3)	ATCATGAGTCCTGCTCGGTGCG(H3)
	GACTGCGTACCAATTCATC(E4)	ATCATGAGTCCTGCTCGGTGCG(H4)
	GACTGCGTACCAATTCACC(E5)	ATCATGAGTCCTGCTCGGTGCG(H5)
	GACTGCGTACCAATTCACG(E6)	ATCATGAGTCCTGCTCGGTGCG(H6)
	GACTGCGTACCAATTCAGG(E7)	
	GACTGCGTACCAATTCAGA(E8)	
	GACTGCGTACCAATTCAGT(E9)	
	GACTGCGTACCAATTCAGC(E10)	
	GACTGCGTACCAATTCACA(E11)	

Table 2. DNA methylation patterns of enzymes sites of *HpaII* and *MspI*.

Enzyme	Sites cut			Sites not cut	
	Type I	Type II	Type III	Type IV	
<i>HpaII</i>	CCGG C ^m CGG	^m CCGG	-	^m C ^m C G G	^m CCGG
	GGCC G GCC	GGCC		G G ^m C ^m C	GGCC ^m
<i>MspI</i>	CCGG C ^m CGG	-	C ^m CGG	^m C ^m C G G	^m CCGG
	GGCC G GCC		GG ^m CC	G G ^m C ^m C	GGCC ^m

1×TBE buffer at 80 watts for 1 h. Gels were stained according to the silver staining method (Bassam et al., 1991).

ncbi.nlm.nih.gov).

Analysis of electrophoretogram

After staining, the bands appeared in the electrophoretogram were detected and counted by using Genescope software of gel imaging system Biosens SC645 (Biotop, People's Republic of China). The scored MSAP bands were transformed into a binary character matrix, using "0" to define the absence of a band and "1" to define the presence of a band, respectively.

Cloning and sequencing of MSAP fragments

The differential bands were excised from gels and eluted with TE buffer (pH 8.0), then boiled in water for 5 min. For re-amplification of fragments, 2 µl of eluted solution was used as template. The same primers were selected for PCR by using the selective PCR procedure in total 25 µl of reaction volume. The PCR products were verified by 0.1% agarose gel and purified with the SanPrep gel extraction kit (Sangon, People's Republic of China). The fragments were cloned and sequenced at Sangon (People's Republic of China). The homology of sequences was analyzed by using the Basic Local Alignment Search Tool (BLAST) at the public database National Center for Biotechnology Information (NCBI) (<http://www>.

RESULTS

DNA methylation patterns of cotton under different quality of light

Methylation-sensitive amplified polymorphism (MSAP) introduces two isoschizomers *HpaII* and *MspI* to recognize the 5'-CCGG-3' sequence and cut unmethylated or methylated sites of cytosine. However, two isoschizomers have different sensitivity to methylation. *HpaII* cleaves hemimethylated sequence, whereas *MspI* cuts methylated sites at the internal cytosine C^mCGG. Thus, the methylation status of the plant genome can be understood through digesting genome with *HpaII* and *MspI*.

Based on the absence or presence of a band in the MSAP experiment, DNA methylation patterns could be classified into four types (Table 2, Figure 1). Type I was identified when both enzymes *HpaII* and *MspI* were used. Type II was identified according to the presence of a band produced by *MspI*, but its absence when *HpaII* were

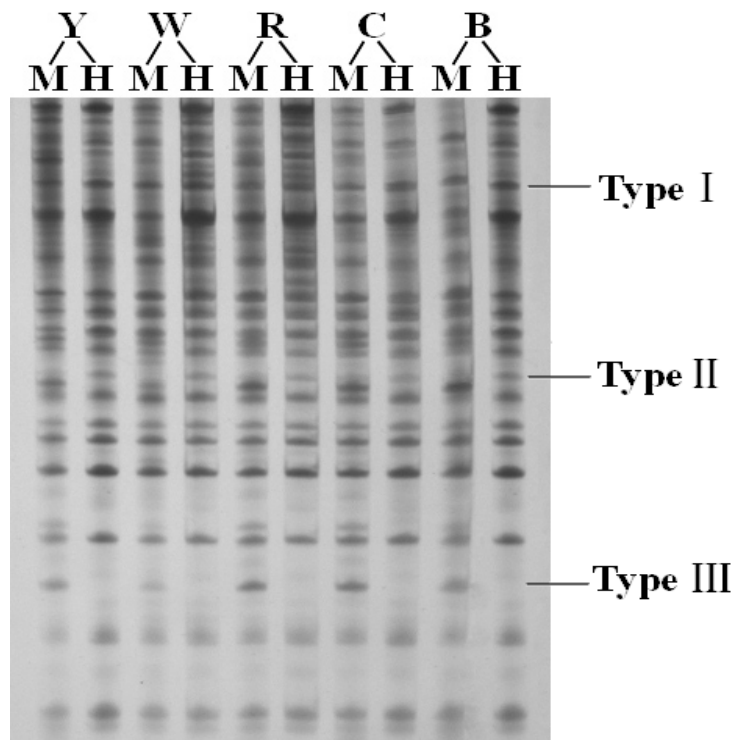


Figure 1. DNA methylation patterns of enzymes sites of *HpaII* and *MspI*. Note: The result of MSAP which amplified using primer H6/E10. Labels: B, C, R, W, Y were the abbreviation of blue light, control (white light+UV), red light, white light and yellow light. The letter M showed that, the enzyme sites were cleaved with *EcoRI* and *MspI*, H indicated the sites cut with *EcoRI* and *HpaII*.

Table 3. Bands of different patterns obtained by MASP.

Light quality	Numbers of bands			Total number of bands
	Type I	Type II	Type III	
B	895	124	161	1180
C	851	103	191	1145
R	880	122	205	1207
W	887	117	95	1099
Y	887	110	139	1136

Labels of light quality in Table B, C, R, W, Y are the abbreviations for blue light, control (white light+UV), red light, white light and yellow light.

used. Type III was recognized when the inverse pattern of type II was observed. Type IV was identified by the absence of bands when both enzymes *HpaII* and *MspI* could not cut the methylated sites because of fully methylated or hyper-methylated of the outside cytosine.

In the present study, 66 pairs of MSAP selective amplification primers were used. For each lane, 13 to 33 fragments were obtained. Total amounts of bands from cotton under different light quality were different from each other (Table 3, Figure 2). As shown in Table 3, the total bands numbers of type I and II from cotton under

blue, red, white and yellow lights were more than that in the control group, but the total bands numbers of type III under blue, white and yellow lights were relatively less than that in the control group. The summed bands of type III under red light were larger than that in the control group.

Levels of DNA methylation for cotton under different quality of light

As shown in Table 4, there are obvious differences of the status and percentage of DNA methylation among all light

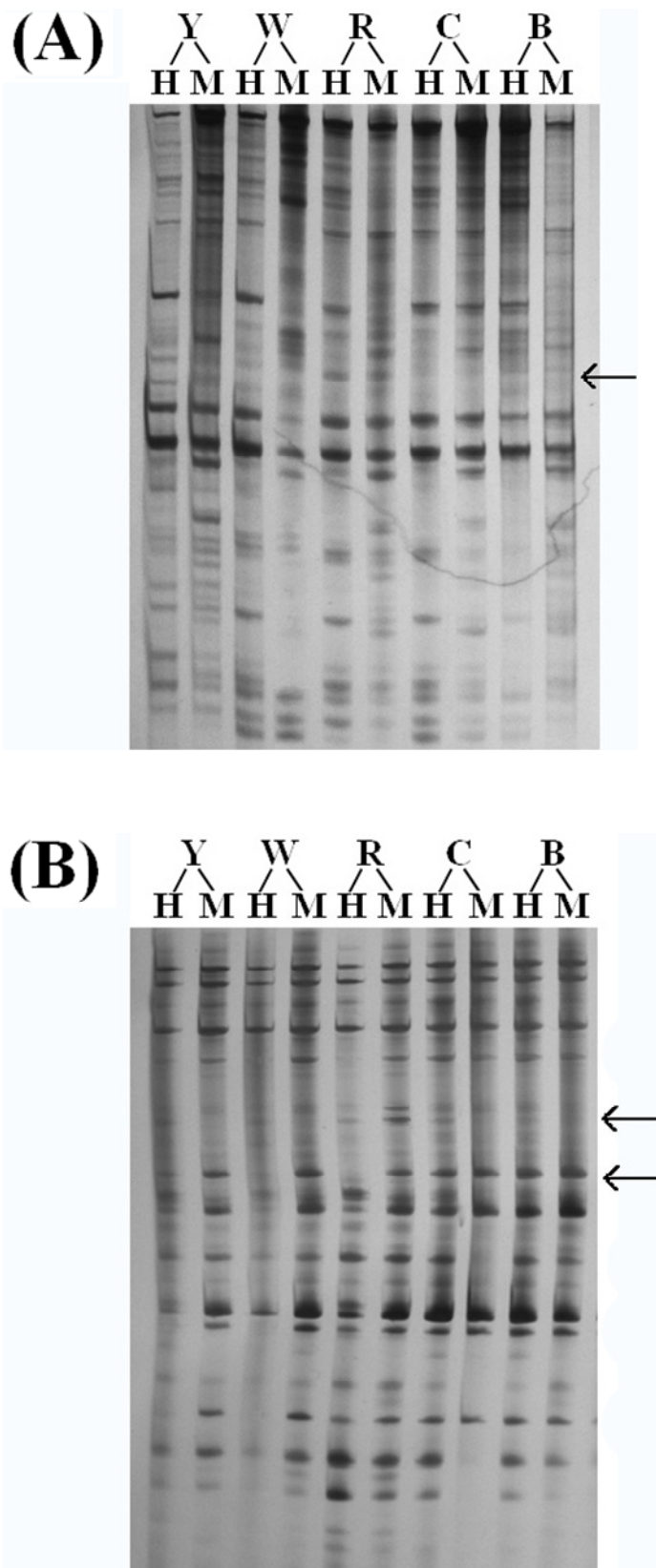


Figure 2. The result of MSAP using primers H6-E8 (A) and primers H4-E8 (B). Note: The bands pointed with arrows were monomorphic or polymorphic fragments. Polymorphic bands are variable across treatments with different quality light.

quality treatments. Compared with control group, the percentage of total methylation was increased when the cotton was illuminated with red light but decreased when the cotton was treated with blue, white and yellow lights. The percentage of hemi-methylation was increased when the cotton was set under blue, red, white and yellow lights. In contrast, the internal full methylation of cotton under blue, white and yellow lights were reduced if compared with control.

Cloning and sequencing of differential MSAP fragments

In order to make clear the effects of cotton under different light quality on DNA methylation in the present study, 4 monomorphic fragments and 1 polymorphic fragment appeared in the electrophoretogram were selected and excised from the gel, then re-amplified and sequenced after purification (Figure 2). After blasted at the website NCBI, the homologies of sequences were analyzed. The sequences were uploaded to the public database at NCBI website (Table 5). Among these sequences, Sequence 1 was demethylated by blue light appeared to be identical to the pyruvate kinase, Sequence 2, demethylated by red light had higher homology with shotgun sequence of *Vitis vinifera*, Sequence 3 showed higher identity to *Gossypium arboreum* clone bacterial artificial chromosome (BAC), Sequence 4 shared homology with aquaporin form major intrinsic proteins (MIP) family/ tonoplast intrinsic protein (TIP) subfamily, and Sequence 5 showed higher similarity to simple sequence repeat (SSR) marker for *Gossypium hirsutum*. Light with white, white+UV-B, red, yellow and blue quality has different effects on methylated or demethylated at the cytosine nucleotide, respectively. Both coding and non-coding regions could be methylated or demethylated at the cytosine nucleotide induced by different light quality.

DISCUSSION

Previously, in order to explain light response reaction mechanism, many functional genes and transcription factors were cloned and identified to be correlated with the light response. For instance, proteolysis-inducing factors (*PIFs*) are nuclear *bHLH* transcription factors which function as negative regulators of photomorphogenesis both in the dark and light in *Arabidopsis*. Under continuous blue light, *PIF1* interacted with phytochrome A (*phyA*) and B (*phyB*) which negatively regulate photomorphogenesis. In response to both red and far-red light, the rapid phosphorylation and degradation of *PIFs* are induced by the *phy* family of photoreceptors to promote photomorphogenesis. Based on the genetic and genomic studies, a growing list of light-controlled genes and transcription factors have been identified, which offer a general understanding of basic framework of light-

Table 4. Percentage of different methylation profiles.

Light quality	Percentage of hemi-methylation (%)	Percentage of internal full methylation (%)	Percentage of total methylation (%)
B	10.51	13.64	24.15
C	8.21	15.23	25.68
R	9.73	16.35	27.09
W	9.33	7.58	19.29
Y	8.77	11.08	21.92

regulated signal networks for photomorphogenesis (Chen et al., 2004; Jiao et al., 2007; Hu and Desai, 2008; Castillon et al., 2009). However, it is still unclear how plants distinguish different light quality.

Methylation of DNA, which has been proposed as an essential mechanism for temporary changes in plant phenotype, is correlated with cell differentiation (Chen et al., 2009), response to short day photoperiod (Thanananta et al., 2006), control of plant development (Teyssier et al., 2008), answer to salt stress (Zhong et al., 2009) and so on. Methylation of DNA is verified to be an important role in epigenetic regulation in the whole process of plant development. In the present study, effects of light quality on DNA methylation patterns and levels for cotton were detected by MSAP technique. As a result, different light quality showed variously specific impact on cytosine methylation patterns and methylation extent. Based on the presence or absence of a band, the methylation patterns could be divided into four types: Demethylation (type I), hemi-methylation (type II), internal full methylation (type III) and outside fully methylated or hyper-methylated (type IV). The frequencies of total methylation, internally full methylation and hemi-methylation were changed when cotton were illuminated with different quality light. Under blue, white+UV-B, red, white and yellow light, the frequencies of total methylation accounted respectively for 24.15, 25.68, 27.09, 19.29 and 21.92%; the ratios of internally full methylation reached respectively to 13.64, 15.32, 16.35, 7.58 and 11.08%; the proportions of hemi-methylation were separately up to 10.51, 8.21, 9.73, 9.33 and 8.77%. It is well known that, the status of DNA methylation for plant is sensitive to the environmental conditions. NaCl treatment may induce some CCGG sites demethylation and some hypermethylation in wheat (Zhong et al., 2009). Under short day period, differences in DNA methylation pattern were found during the transition from vegetative to reproductive phase in rice (Thanananta et al., 2006). In this article, different effects of blue, white+UV-B, red, white and yellow light quality on DNA methylation level were detected. Red light enhanced DNA internally, full methylation and hemi-methylation. Blue, white and yellow lights reduced DNA internally full methylation, but increased percentage of DNA hemi-methylation. These results might suggest an epigenetic mechanism of plants' response to light quality.

Although, many phenotype changes are found to be closely related to the cytosine methylation (Suzuki et al., 2008), the underlying mechanism of DNA methylation in plant are still unknown. Previously, many candidate genes were found similar to functional genes and transcription factors which were involved with gene regulation and expression by the MSAP technique (Suzuki et al., 2008; Inagaki and Kakutani, 2010; Hanai et al., 2010). In the present study, 4 monomorphic fragments and 1 polymorphic fragment were purified and sequenced to study the response mechanism to light quality for cotton. By alignment at the NCBI database, the Sequence 2, 3 and 5 were found to show higher similarity to non-coding regions of some plants; Sequence 1 was identical to the pyruvate kinase of *Ricinus communis*, Sequence 4 shared homology with aquaporin from MIP family/TIP subfamily. Pyruvate kinase is an important enzyme of glycolytic pathway, which accompanies phosphoenolpyruvate phosphatase. It catalyzes the transfer of a phosphate group from phosphoenolpyruvate (PEP) to ADP and yields one molecule of pyruvate and one molecule of ATP. It is related to the regulation of downstream of carbohydrate metabolism. Aquaporins are proteins embedded in the cell membrane that regulate the flow of water. Some transport other small uncharged solutes, such as glycerol, CO₂, ammonia and so on. Taken together, these may imply that response mechanisms to light quality for cotton are correlated with carbohydrate metabolism and photosynthetic reaction.

In conclusion, different light qualities had diverse impacts on DNA methylation patterns and levels. In the response process to light quality, dramatic methylation status changed in both coding and non-coding regions for cotton were detected. These variations were also accompanied by changes in gene expression, which were possibly controlled by regulation of methylation status in promoter region or coding region of genes. These may suggest an epigenetic mechanism for light response of brown cotton.

ACKNOWLEDGEMENTS

The present investigation is financially supported by a grant from the Doctoral Programs Foundation of the

Table 5. Homology analysis of 5 fragments by blasting in Genebank database.

Fragment	Primers	Accession number	Homology	E value	Methylation pattern									
					B		C		R		W		Y	
					H	M	H	M	H	M	H	M	H	M
Seq1	H6/E11	HQ008720	<i>R. communis</i> pyruvate kinase, putative (XM_002523350)	9.00E-19	1	1	0	1	0	1	0	1	1	1
Seq2	H4/E8	HQ008721	<i>V. vinifera</i> contig VV78X181060.3, whole genome shotgun sequence (AM425991)	3.00E-17	0	1	0	1	1	1	0	1	0	1
Seq3	H4/E8	HQ008722	<i>G. arboreum</i> clone BAC 271C22, complete sequence (EU626444)	2.00E-40	1	1	1	1	1	0	1	0	1	0
Seq4	H3/E7	HQ008723	<i>P. trichocarpa</i> aquaporin, MIP family, TIP subfamily (XM_002326421)	5.00E-17	1	1	1	0	0	0	0	0	0	0
Seq5	H4/E11	HQ008724	<i>G. hirsutum</i> cultivar Deltapine 33 B clone MONCS1193 SSR marker CGR6783 genomic sequence (GQ394228)	2.00E-61	0	1	0	1	1	1	0	1	0	1

Note: Accession numbers were provided by the GenBank submissions staff for the uploaded sequences (Seq1, Seq2, Seq3, Seq4 and Seq5). E value according to information from BLASTX search of the non-redundant database at NCBI. The number "0" was defined as the band is absent and "1" was defined as the band appeared.

Ministry of Education of People's Republic of China (No. 200803640002) and Eleventh Five-Year Plan of Anhui Province (No. 07010302133).

REFERENCES

- Bassam BJ, Caetano-Anolles G, Gresshoff PM (1991). Fast and sensitive silver staining of DNA in polyacrylamide gels. *Anal. Biochem.* 196: 80-83.
- Castillon A, Shen H, Huq E (2009). Blue light induces degradation of the negative regulator Phytochrome Interacting Factor 1 to promote photomorphogenic development of *Arabidopsis* seedlings. *Genet.* 182: 161-171.
- Chen M, Chory J, Fankhauser C (2004). Light signal transduction in higher plants. *Annu. Rev. Genet.* 38: 87-117
- Chen XQ, Ma Y, Chen F, Song WQ, Zhang L (2009). Analysis of DNA methylation patterns of PLBs derived from *Cymbidium hybridum* based on MSAP. *Plant Cell Tiss. Organ Cult.* 98: 67-77.
- Dalakouras A, Moser M, Krczal G, Wassenegger M (2010). A chimeric satellite transgene sequence is inefficiently targeted by viroid-induced DNA methylation in tobacco. *Plant Mol. Biol.* 73: 439-447.
- Hanai LR, Floh EIS, Fungaro MHP, Santa-Catarina C, De Paula FM, Viana AM, Vieira MLC (2010). Methylation patterns revealed by MSAP profiling in genetically stable somatic embryogenic cultures of *Ocotea catharinensis* (Lauraceae). *In Vitro Cell Dev-Plant.* 46: 368-377.
- Hu JP, Desai M (2008). Light control of peroxisome proliferation during arabidopsis photomorphogenesis. *Plant Signal and Behav.* 3: 801-803.
- Inagaki S, Kakutani T (2010). Control of genic DNA methylation in *Arabidopsis*. *J. Plant Res.* 123: 299-302
- Jalligot E, Beule T, Baurens FC, Billotte N, Rival A (2004). Search for methylation-sensitive amplification polymorphisms associated with the "mantled" variant phenotype in oil palm (*Elaeis guineensis* Jacq.). *Genome.* 47: 224-228.
- Jiao YL, Lau OS, Deng XW (2007). Light-regulated transcriptional networks in higher plants. *Nat. Rev. Genet.* 8: 218-230.
- Mason G, Noris E, Lanteri S, Acquadro A, Accotto GP, Portis E (2008). Potentiality of Methylation-sensitive Amplification Polymorphism (MSAP) in Identifying Genes Involved in Tomato Response to Tomato Yellow Leaf Curl Sardinia Virus. *Plant Mol. Biol. Rep.* 26: 156-173.
- Murray HG, Thompson WF (1980). Rapid isolation of high molecular weight DNA. *Nucleic Acids Res.* 8: 4321-4325.
- Pan YJ, Fu BY, Wang D, Zhu LH, Li ZK (2009). Spatial and temporal profiling of DNA methylation induced by drought stress in rice. (in Chinese with English abstract). *Sci. Agric. Sinica.* 42: 3009-3018.
- Reyna Lopez GE, Simpson J, RuizHeresesa J (1997). Differences in DNA methylation patterns are detectable during the dimorphic transition of fungi by amplification of restriction polymorphisms. *Mol. Gen. Genet.* 253: 703-710.
- Suzuki MM, Bird A (2008). DNA methylation landscapes: provocative insights from epigenomics. *Nature Review Genet.* 9: 465-476.
- Tatra GS, Miranda J, Chinnappa CC, Reid DM (2000). Effect of light quality and 5-azacytidine on genomic methylation and stem elongation in two ecotypes of *Stellaria longipes*. *Physiol. Planta.* 109: 313-321.
- Teyssier E, Bernacchia G, Maury S, How. Kit A, Stammitti-Bert L, Rolin D, Gallusci P (2008). Tissue dependent variations of DNA methylation and endoreduplication levels during tomato fruit development and ripening. *Planta.* 228: 391-399.
- Thanananta T, Pongtongkam P, Thongpan A, Kaveeta L, Peyachoknagul S (2006). Effect of short day photoperiod on DNA methylation and expression of a gene in rice KDML105. *Afr. J. Biotechnol.* 5: 1375-1382.
- Vos P, Hogers R, Bleeker M, Reijans M, Van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M(1995). AFLP: A new technique for DNA fingerprinting. *Nucleic Acids Res.* 23: 4407-4414
- Zhong L, Xu YH, Wang JB (2009). DNA-methylation changes induced by salt stress in wheat *Triticum aestivum*. *Afr. J. Biotechnol.* 8: 6201-6207.