

Effects of different planting dates and low light on cotton fibre length formation

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Abstract Declining temperature and low light often appear together to affect cotton (*Gossypium hirsutum* L.) growth and development. To investigate the interaction on fibre elongation, two cultivars were grown in fields in 2010 and 2011 and in pots in 2011 under three shading levels for three planting dates, and the differences of environmental conditions between different planting dates were primarily on temperature. Fibre length in the late planting date 25 May was the longest instead of the normal planting date. Late planting prolonged fibre elongation period and the effect of late planting on fibre length formation was greater than low light. In the normal planting date, shading increased fibre length through delaying the peak of β -1,3-glucanase gene expression and bringing the peak of β -1,3-glucan synthase gene expression forward, leading to a longer duration of plasmodesmata (PD) closure to increase fibre length, instead of changing sucrose contents or relate enzyme activities. However, in the late planting dates, the difference of the duration of PD closure between shading treatments was not obvious, but low light had a negative impact on sucrose contents, sucrose synthase (SuSy) and vacuolar invertase (VIN) activities during fibre rapid elongation period, leading to the decline of fibre length. Due to late planting and low light, the decreased extent of fibre length of Sumian 15 was larger than Kemian 1. Under the combined condition, Sumian 15 had a shorter gene expression of Expansin, and more sensitive sucrose

content, VIN and SuSy activity during fibre rapid elongation period. This resulted in the length formation of Sumian 15 which was more sensitive than Kemian 1, when the cotton suffered the combined effects.

Keywords Cotton (*Gossypium hirsutum* L.) · Fibre length · Planting date · Low light · Sucrose content · Enzyme activity · Gene expression

Introduction

Cotton fibre length is an important fibre quality index, which is related to both the loan value of a bale of cotton and the quality of yarns (Bradow and Davidonis 2000). Upland cotton (*Gossypium hirsutum* L.) is a model system to study the biochemical and physiological processes of cell elongation (Kim and Triplett 2001). As the fibre cell elongates to 2.5–3.0 cm within 16 days post anthesis (DPA), it is the longest single-cell structure with the fastest elongation rate in the world (Tiwari and Wilkins 1995). Fibre development is restricted by declining temperature or low light in many cotton-grown areas (Dong et al. 2006; Wang et al. 2005, 2006; Yeates et al. 2010), and the two climatic conditions often appear as a combined factor during the periods of flowering and boll development of cotton, e.g., in the Yangtze River Valley, cotton often suffers from rainy and overcast weather during the early stages of flowering and boll formation, and is also affected by the declining temperatures accompanying the cloudy and overcast weather at the later stages. The sub-optimal environmental conditions during the fibre elongation phase may decrease the rate of elongation per day or shorten the duration of the elongation period, so that the genotype fibre length potential is not fully realised (Hearn 1976; Bradow

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and Davidonis 2010; Zhao et al. 2012). Therefore, it is important to understand the mechanisms of the interactive effects of declining temperature and low light on cotton fibre elongation.

As a unidirectional cell expansion process, cotton fibre elongation is a result of a complex interplay between cell turgor and cell wall extensibility (Smart et al. 1998; Ruan et al. 2001; Wang and Ruan 2010). Previous studies showed a temporary closure of fibre plasmodesmata (PD), probably due to the deposition of callose, at the rapid phase of elongation, to generate and maintain a higher turgor to drive the extensive elongation, and the duration of the PD closure correlates positively with the final fibre length attained (Ruan et al. 2001, 2004, 2005; Ruan 2007). The high turgor maintained by osmotically active solutes, including malate, potassium and soluble sugars (mainly sucrose), driving the elongation by attracting water (Dhindsa et al. 1975; Cosgrove 1997; Ruan et al. 1997; Pflüger and Zambryski 2001) and the cell wall is expandable through the expression of expansin genes (Cosgrove 2000; Ruan et al. 2000; Harmer et al. 2002). Callose accumulation is controlled by the joint action of β -1,3-glucanase (β -1,3-gluc) and β -1,3-glucan synthase (β -1,3-glucS), which degrade and synthesise callose, respectively (Bucher et al. 2001; Ruan et al. 2004). Finally, after 16 DPA, the hydrolysis of callose led to the reopening of the PD, which correlated with the decrease of estimated fibre turgor and the termination of the elongation process (Ruan et al. 2001, 2004).

Sucrose transported into fibres may play multiple roles in fibre development, from contributing to the generation of turgor potential to the supply of substrate for cellulose biosynthesis (Ruan 2005; Haigler et al. 2001) and sucrose is degraded by sucrose synthase (SuSy, E.C. 2.4.1.13), which has been proved to play a critical role in cotton fibre elongation and cellulose biosynthesis, through osmotic regulation and supplying UDPglucose as substrate for cellulose production (Ruan et al. 1997, 2003; Wang and Ruan 2010). In addition to SuSy, high activity of invertase (E.C. 3.2.1.26) inside the vacuole (VIN) also played as a key role in early fibre elongation likely through osmotic regulation of vacuole enlargement (Wang et al. 2010), and a genotype with faster fibre elongation had significantly higher fibre VIN activity and hexose level than a slow-elongating genotype, by contrast, cell wall or cytoplasmic invertase activities did not show correlation with fibre elongation (Wang et al. 2010).

The enzymology of fibre elongation and cellulose synthesis metabolism were conducted in plants using only a single factor of cool temperature or low light (Martin and Haigler 2004; Bian et al. 2008; Shu et al. 2009); there is limited research about the response of key enzymes in cotton fibre elongation to the combined effects. This

research was designed to study the interaction of late planting and low light on related enzyme activities and gene expressions of expansin, β -1,3-Gluc, β -1,3-glucS, SuSy and VIN in fibre elongation and sucrose metabolism. The study aimed at elucidating the physiological and biochemical mechanism of cotton fibre elongation in response to adverse environmental conditions of declining temperature and low light. The research results should provide a theoretical basis for the breeding research and the quality cotton cultivation, to adapt to the changing climatic conditions.

Materials and methods

Experimental design

Field experiments were conducted in 2010 and 2011 and a pot experiment was conducted next to the field exposed to the environment in 2011 at Pailou experimental station of Nanjing Agricultural University at Nanjing, China (32°02'N, 118°50'E), in the Yangtze River Valley. The soil of the pot experiment was collected from 0 to 20 cm topsoil layers from the experimental field without cotton plants. The soil at the experimental site was clay, mixed, thermic, Typic alfisols (udalfs; FAO luvisol) with 18.3 and 18.1 g kg⁻¹ organic matter, 1.1 and 1.0 g kg⁻¹ total N, 64.5 and 70.2 mg kg⁻¹ available N, 17.9 and 20.3 mg kg⁻¹ available P, and 102.3 and 111.1 mg kg⁻¹ available K contained in 20 cm depth of the soil profile before sowing cotton of 2 years, respectively.

Based on the report of Wang et al. (2008, 2009) who divided cotton (*Gossypium hirsutum* L.) into temperature-sensitive groups, two contrasting cultivars, Kemian 1 (cool temperature-tolerant) and Sumian 15 (cool temperature-sensitive) were selected for this study. Different environmental conditions during fibre developing periods were provided by sowing cotton on different dates, 25 April, 25 May and 10 June in 2010 and 2011. The normal planting date in the Yangtze River Valley is 25 April, and 25 May and 10 June are late planting dates. Cotton was sown in a nursery bed, and seedlings with three true leaves were transplanted to the field at a spacing of 80 cm × 25 cm and to the pots. When flowers in the first fruiting position of the sixth main-stem sympodial branches of plants of each planting date bloomed, we imposed three shading treatments for the plots of every planting date, shading cloths were removed after the bolls in the first fruiting position of the 12th sympodial branches at maturity. Shading treatments included an unshaded control [crop relative light rate (CRLR) = 100 %], light shade (CRLR 80 %), severe shade (CRLR 60 %) achieved with white nylon cloth (12 m long, 7 m wide, 2 m high for the field experiment and 6 m long, 5 m wide, 2 m high for the pot

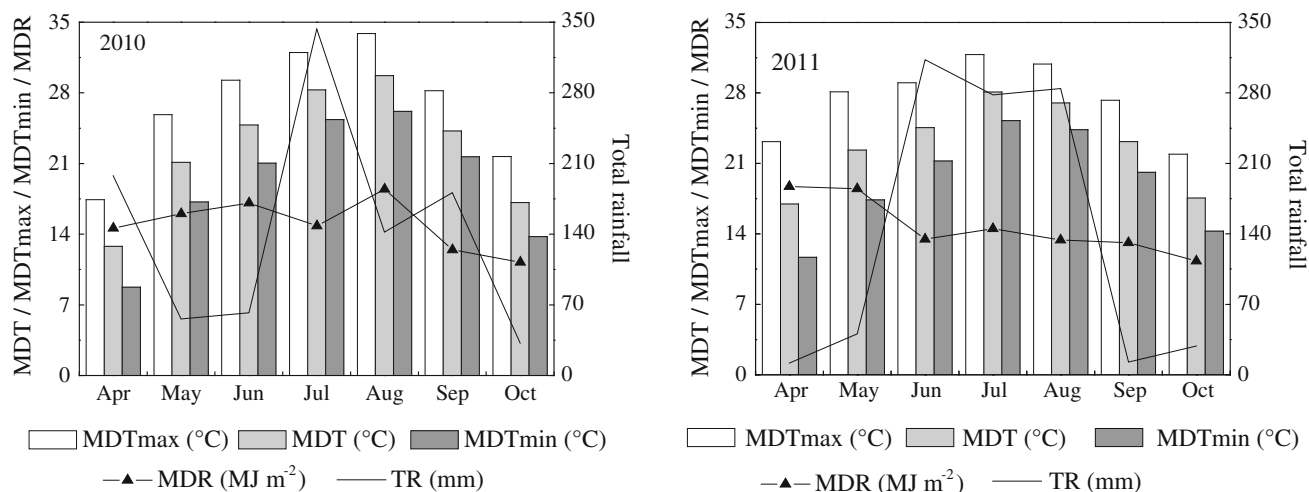


Fig. 1 Changes of environmental conditions during cotton growing period (April–October) in 2010 and 2011. MDT, MDT_{max}, MDT_{min}, MDR and TR stand for mean daily temperature, mean daily

maximum temperature, mean daily minimum temperature, mean daily radiation and total rainfall

experiment), and two different kinds of cloth, respectively, reduced 20 and 40 % photosynthetically active radiation (PAR) of the cotton canopy. In the pot experiment, each treatment had 33 pots (diameter 60 cm, height 55 cm), each pot was filled with 40 kg soil; in the field experiment, each plot was 6 m wide and 10.5 m long. Both pot and field experiments were arranged as a split-plot experimental design with three replications. Furrow-irrigation was applied as needed to minimise the moisture stress during both seasons, and conventional weed and insect control measures were utilised as needed.

Sampling and processing

Cotton flowers in the first or the second fruiting positions on the 6–7th sympodial fruiting branches with the same anthesis date were labelled with a tag listing the date. Bolls with the same anthesis date (about 6–8 bolls in the similar size for each treatment) were collected at 10, 17 and 24 DPA, which represent fast elongation, gentler elongation with secondary cell wall synthesis, and late elongation, respectively (31 DPA was also chosen in the late planting dates for the delaying of fibre development by late planting). Cotton boll samples were harvested at 9:00–11:00 am, and fibres were excised from the bolls with a scalpel and were immediately put into liquid nitrogen for subsequent measurement.

Weather data

Weather data were collected from the Nanjing weather station located about 6 km from the plot area. The monthly weather data consisting of mean daily temperature (MDT), mean daily maximum temperature (MDT_{max}), mean daily

minimum temperature (MDT_{min}), total rainfall (TR) and mean daily solar radiation (MDR) from April to October during the cotton growing period for 2010 and 2011 are given in Fig. 1. Weather data during fibre elongation period (FEP) in different planting dates are also shown in Table 1. The date of flowering was considered as the starting time of FEP, and the date when fibres reached a length greater than or equal to the length at boll opening was considered as the end of the FEP. The FEP was expressed by the number of days post anthesis (Braden and Smith 2004; Fig. 2).

Microclimate measurement

Canopy air temperature, relative humidity and PAR of each planting date under the shaded plot and control treatments were measured as soon as 15 days post anthesis for the first or the second fruiting positions on the 6–7th main-stem sympodial branches (15 days after shading). Microclimate was measured every two hours from 06:00 to 18:00, using a Hygro-Thermometer Psychrometer (DT-8892, CEM, Shenzhen, China) to measure air temperature and relative humidity at the 6–7th main-stem sympodial branches, and PAR was measured about 0.2 m above the canopy by a Decagon AccuPAR LP-80 Ceptometer (Decagon Devices, Logan, UT, USA). Measurements were made only when the direct sunlight was not blocked by clouds.

Sucrose content quantification

Sucrose was extracted and quantified by a modified method of Pettigrew (2001). About 0.3 g dry weight (DW) fibre samples were extracted with three successive 5 ml washes of 80 % ethanol (Shu et al. 2009). The ethanol samples

Table 1 Weather factors during the cotton fibre elongation period (FEP) of different planting dates

Year	Planting date	Flowering date	Termination date of fibre elongation	Duration of fibre elongation (days)	Weather factors during FEP				
					MDT (°C)	MDT _{max} (°C)	MDT _{min} (°C)	DRI _{max} (W m ⁻²)	TSH (h)
2010	25-Apr	28-Jul	13-Aug	17	31.7	36.1	27.8	977.4	183.1
	25-May	19-Aug	11-Sep	24	27.0	30.9	24.3	860.0	143.9
	10-Jun	4-Sep	4-Oct	31	23.8	27.2	20.3	766.2	154.7
				CV (%)	14.54	14.30	15.48	12.20	12.61
2011	25-Apr	27-Jul	19-Aug	24	28.7	33.3	25.7	957.1	154.2
	25-May	25-Aug	24-Sep	31	23.9	27.8	20.9	818.6	160.3
	10-Jun	10-Sep	10-Oct	31	21.3	25.7	18.1	808.6	175.3
				CV (%)	15.24	13.52	17.85	9.64	6.65

MDT, MDT_{max}, MDT_{min}, DRI_{max} and TSH stand for mean daily temperature, mean daily maximum temperature, mean daily minimum temperature, maximum daily total radiation irradiation and total sunshine hours, respectively

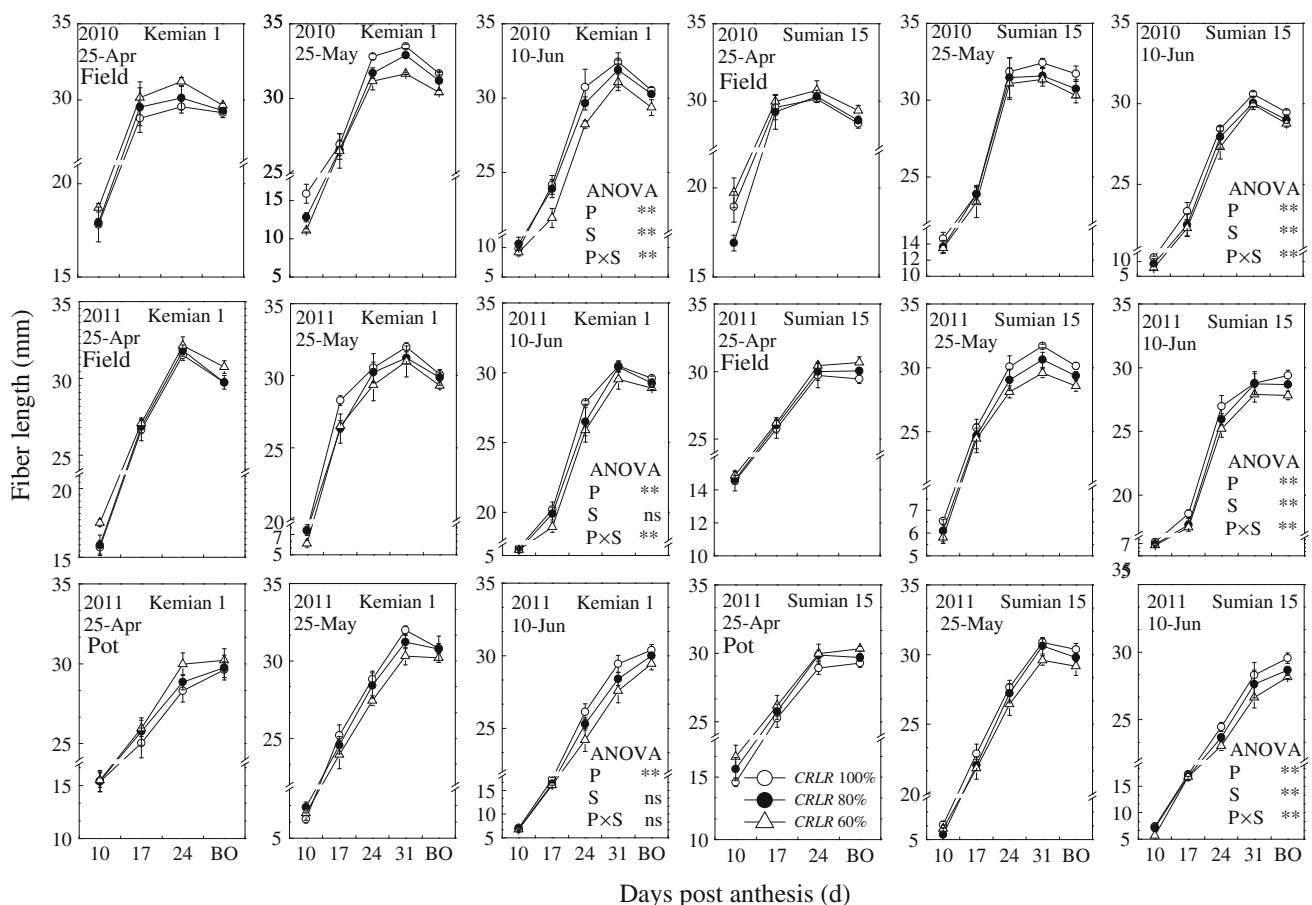


Fig. 2 Changes of cotton fibre length for Kemian 1 and Sumian 15 for combinations of different planting dates and shading treatments in 2010 and 2011. *BO* boll opened day, *P* planting date, *S* shading, *P* × *S* planting date × shading. The values are mean ± SD (*n* = 6).

Two-way ANOVA results embedded in the figure were calculated from the data of final fibre length. *ns* not significant; **P* < 0.05; ***P* < 0.01 (*n* = 6)

were incubated in an 80 °C water bath for 30 min. Then, the samples were centrifuged at 10,000*g* for 10 min, and three aliquots of supernatant were collected together for

sucrose measurement (Shu et al. 2009). The sucrose assay was carried out according to the method described by Hendeix (1993).

Fibre length measurement

Cotton fibre length (mm) of the harvested bolls within 30 DPA was measured using the water washing method (Thaker et al. 1989), and the length after 30 DPA was measured with a Y-146 cotton fibre photodosimeter (Tancang Electron Apparatus Co. Ltd, China).

Enzymatic analyses

Enzyme extraction and assay were performed according to King et al. (1997) with minor modifications. Fibre cell samples, about 0.5 g fresh weight (FW), were ground into a fine powder in liquid nitrogen followed by grinding in cold extraction buffer (5:1 v/w), which contained 50 mM *N*-(2-hydroxyethyl) piperazine-*N'*-(2-ethanesulfonic acid)-NaOH (Hepes-NaOH) (pH 7.5), 10 mM MgCl₂, 1 mM ethylene diamine tetraacetic acid (EDTA), 1 mM ethyleneglycol bis-(2-aminoethyl ether)-tetraacetic acid (EGTA), 0.5 % (w/v) bovine serum albumin (BSA), 2 % (w/v) polyvinylpyrrolidone (PVP), 0.1 % (v/v) Triton X-100, 2 mM dithiothreitol (DTT), and 1 mM phenylmethylsulfonyl fluoride (PMSF) as described in the previous research (Shu et al. 2009). The resulting homogenate was centrifuged at 15,000g for 20 min, and the supernatant was stored at 4 °C for analysis (Shu et al. 2009). All extraction procedures were carried out at 0–4 °C.

SuSy activity was assayed by measuring the cleavage of sucrose (King et al. 1997). Each reaction contained 20 mM piperazine-*N,N'*-bis (2-ethanesulfonic acid)-KOH (Pipes-KOH) (pH 6.5), 100 mM sucrose, 2 mM UDP, and 200 µl of extract in a total volume of 650 µl as described previously (Shu et al. 2009). Reactions were started by incubating at 30 °C for 30 min. The reactions were stopped with 250 µl of 0.5 M *N*-tric-(hydroxy-methyl) methylglycine-KOH (Tricine-KOH) (pH 8.3), which were heated for 10 min in boiling water. The amount of fructose in SuSy reactions was determined as described by Shu et al. (2009).

VIN activity (soluble acid invertase activity) was measured by incubation of 100 µl of extract with 1 M sucrose in 200 mM acetic acid–NaOH (pH 5.0), in a total volume of 2.5 ml (Shu et al. 2009). Reactions were started by incubating at 30 °C for 30 min (King et al. 1997; Shu et al. 2009). The reactions were stopped with 1 ml of 3,5-dinitro salicylic acid (DNS), and boiling for 5 min. Glucose content was determined by relating the spectrophotometer metrically at 540 nm.

Semiquantitative RT-PCR analyses

Fibres sampled from the pot experiment were used in the semiquantitative RT-PCR analyses. Total RNA was isolated from cotton fibre according to Jiang and Zhang (2003). For each reaction, 2 µg of RNA was reverse

transcribed to cDNA with oligo(dT)₁₅. PCR was performed in a final volume of 25 µl containing 2 U of *Taq* DNA polymerase. The gene-specific primers of Expansin (*GhEXP1*, Shu et al. 2008), β-1,3-glucanase (*GhGluc1*, Ruan et al. 2004) and β-1,3-glucan synthase (*GhGlucS*, Ruan et al. 2004) were designed to unique regions of each cDNA (Table 2); the cotton 18srRNA gene (*Gh18srR*, Wang et al. 2010) was used as an internal control. PCR reaction was initially denatured at 94 °C for 5 min and 23–30 cycles at 94 °C for 30 s, proper annealing temperature for 30 s and 72 °C for extending 30–40 s (Table 2), a final extension of RT-PCR products at 72 °C for 10 min. PCR products were size-separated by electrophoresis in a 1.8 % agarose gel. All photographs were statistically analysed with the software Quantity One version 4.62.

Data analysis

OriginPro 8.0 was used for data processing and drawing of figures. A two-way analysis of variance (ANOVA) was performed using the SPSS statistical package version 17.0. The means were separated using the least significant difference (LSD) test at 5 % of probability level. The coefficient of variation (CV) was calculated as the ratio of the standard deviation (including all planting dates and shading treatments) to the mean. Changing amplitude ($\Delta\%$) = (Treatment–Control)/Control × 100 %. Control is the plot of CRLR 100 % plus 25 April.

Results

Environmental condition and cotton canopy microclimate

The planting date of 25 April is considered typical for the Yangtze River Valley. Environmental conditions around this time of the year are favourable for cotton development. Delaying the planting date increased the period of cotton fibre elongation from 17 to 24 days in 2010 and from 24 to 31 days in 2011 (Table 1). In the two experimental years, the average climatic conditions were close to the long-term average as described before (Liu et al. 2013). Though the MDT, MDT_{max}, MDT_{min} and DRI_{max} of FEP decreased as planting dates was delayed, the coefficients of variance of MDT, MDT_{max} and MDT_{min} for the three planting dates were higher than that of other indices, and MDT_{min} of FEP (MDT_{minFEP}) was the greatest index affected by planting dates, and the range of MDT_{minFEP} was reduced from 27.8 to 18.1 °C (Table 1). These results indicated that the differences in environmental conditions during FEP for different planting dates were primarily influenced by temperature, especially MDT_{min}.

Table 2 Primers, T_m , No. of cycles, extension time and length of amplified DNA in RT-PCR program

Gene	Accession No.	Primer	T_m (°C)	No. of cycles	Extension time (s)	Length of amplified DNA (bp)
<i>GhEXPI</i>	AF043284	Forward: 5'-AGTCGAACCATAA CCGTGACAGCC-3' Reverse: 5'-CCCAATTTCTGGAC ATAGGTAGCC-3'	56	23	30	328
<i>GhGluc1</i>	D88416	Forward: 5'-ATATGGGTTTTAATCTCAG CAATGG-3' Reverse: 5'-AGGGACGATGTTGGT GTAAACCC-3'	58	30	30	303
<i>GhGlucS</i>	AI730469	Forward: 5'-TGGAAGGGCTTATTTGGCAC-3' Reverse: 5'-GTCCGCCTCTGAAAATAGC-3'	58	30	40	603
<i>Gh18srR</i>	U42827	Forward: 5'-CTGAGAAACGGCTACCACAT-3' Reverse: 5'-CTATGAAATACGAATGCCCC-3'	53	25	30	500

Table 3 Air temperature, relative humidity and photosynthetically active radiation in the cotton field for combinations of different planting dates and shading treatments in 2010 and 2011

Planting date	CRLR (%)	Air temperature (°C)			Relative humidity (%)			PAR ($\mu\text{mol m}^{-2} \text{s}^{-1}$)		
		2010 field	2011 field	2011 pot	2010 field	2011 field	2011 pot	2010 field	2011 field	2011 pot
25-Apr	100	34.2 a	33.4 a	33.4 a	64.2 a	68.1 a	60.3 a	734 a	654 a	679 a
	80	34.0 a	33.3 a	33.5 a	64.1 a	66.4 a	60.3 a	585 b	501 b	528 b
	60	34.0 a	33.2 a	33.5 a	67.7 a	67.2 a	61.1 a	486 c	422 c	425 c
25-May	100	30.5 a	30.9 a	30.6 a	62.0 a	54.7 b	60.2 a	639 a	687 a	621 a
	80	30.2 a	31.8 a	31.2 a	64.5 a	55.5 b	59.5 a	517 b	535 b	512 b
	60	30.3 a	31.1 a	31.2 a	64.5 a	59.2 a	61.0 a	394 c	438 c	402 c
10-Jun	100	23.2 a	25.8 a	26.4 a	58.8 b	46.9 a	55.7 a	534 a	568 a	568 a
	80	23.5 a	26.1 a	26.3 a	57.1 b	47.6 a	55.2 a	407 b	437 b	435 b
	60	22.6 a	26.2 a	26.5 a	64.5 a	48.7 a	56.0 a	311 c	336 c	349 c

Data in Table 3 were averaged by measurement data from 6:00 to 18:00 of 15 DPA

CRLR and PAR stand for crop relative light rates and photosynthetically active radiation

Values followed by a different small letter within the same column in the same planting date are significantly different at 0.05 probability levels ($n = 6$)

All canopy microclimate data were expressed as the mean of measurements from 6:00 to 18:00 at 15 DPA (15 days after shading). PAR of cotton canopy significantly; CRLR 80 % and CRLR 60 % observed an 18–26 % and a 34–39 % reduction in PAR, respectively (Table 3), which was consistent with the design of the test. In the field and pot experiments, canopy air temperature and canopy air relative humidity were not significantly different between different shading treatments (Table 3), so we considered that the effects of shading treatments of the experiments were mainly on the decline of PAR.

Changes in cotton fibre length

Fibre length steadily increased during the FEP (10–24 DPA or 10–31 DPA), and differences were observed among different combined treatments. At normal planting conditions,

fibres reached final length at 17 DPA (2010) or 24 DPA (2011), and at late planting conditions, the time that fibres reached final length delayed to 24 DPA (2010) or 31 DPA (2011) (Fig. 2). Under normal light condition, the fibre length in the planting date 25 May was the longest and that in the planting date 25 April was the shortest over the 2 years (Table 4). Shading affected fibre length formation differently at different planting dates. The fibre length of CRLR 80 % and CRLR 60 % in the normal planting date 25 April were about 1.2 and 3.4 % longer than that of CRLR 100 % over genotypes and years (Table 4). Shading reduced fibre length as planting date delayed, and the length was about 1.6 and 3.4 % shorter for CRLR 80 % and CRLR 60 % than for CRLR 100 % in the planting date 25 May, while the length was about 1.4 and 3.1 % shorter for CRLR 80 % and CRLR 60 % than for CRLR 100 % in the planting date 10 June over genotypes and years (Table 4). Shading

Table 4 Average sucrose content, average sucrose synthase (SuSy) and vacuole invertase (VIN) activity in cotton fibres during fibre elongation period and final fibre length and their analysis of variance for combinations of different planting dates and shading treatments in 2010 and 2011

Planting date	CRLR (%)	Sucrose content (mg g ⁻¹ DW)		VIN activity (mg glucose g ⁻¹ FW h ⁻¹)		SuSy activity (mg sucrose g ⁻¹ FW h ⁻¹)		Fibre length (mm)					
		2010 field	2011 field	2011 pot	2010 field	2011 field	2011 pot	2010 field	2011 field	2011 pot			
The cultivar of Kemian 1													
25-Apr	100	10.1 a	12.3 a	12.3 a	91.7 a	92.4 a	66.3 a	79.2 a	86.4 a	89.5 a	29.1 b	29.7 b	29.6 b
	80	9.4 b	10.8 b	10.8 b	89.7 ab	89.4 b	63.8 b	77.5 b	78.6 b	80.9 b	29.3 ab	29.7 b	29.7 ab
	60	8.8 c	9.8 b	10.3 b	86.6 b	88.5 b	63.3 b	74.4 c	77.8 b	79.1 b	29.6 a	30.7 a	30.2 a
25-May	100	16.6 a	11.6 a	13.5 a	114.0 a	112.1 a	78.0 a	81.1 a	82.5 a	86.8 a	31.7 a	30.0 a	30.8 a
	80	14.0 b	10.0 b	12.5 b	111.1 b	105.7 b	75.0 b	80.3 a	80.0 b	79.6 b	31.2 a	29.9 a	30.7 a
	60	14.2 b	9.5 b	12.0 b	104.8 c	101.2 c	76.0 b	76.5 b	75.6 c	75.6 b	30.4 b	29.3 b	30.2 b
10-Jun	100	19.8 a	17.7 a	17.0 a	131.3 a	130.9 a	108.0 a	102.1 a	95.2 a	108.5 a	30.5 a	29.3 a	30.3 a
	80	20.2 a	16.5 ab	16.6 b	125.7 b	127.8 b	103.2 b	97.8 b	91.5 b	100.1 b	30.3 a	29.3 a	29.9 a
	60	20.1 a	15.5 b	16.1 b	120.9 c	120.3 c	101.2 b	92.4 c	90.1 b	94.8 c	29.4 b	28.9 b	29.4 b
	CV _S (%)	6.90	11.47	9.55	2.88	2.27	2.53	3.16	5.87	6.66	0.86	1.92	1.08
	CV _P (%)	31.89	24.07	16.98	17.67	17.22	25.56	14.53	7.39	12.45	4.28	1.18	1.99
	CV _{P × s} (%)	31.49	24.74	18.75	15.07	14.60	21.73	11.98	8.19	12.46	2.96	1.78	1.60
The cultivar of Sumian 15													
25-Apr	100	9.3 a	9.6 a	10.4 a	94.4 a	103.6 a	67.0 a	82.6 a	89.5 a	81.2 a	28.6 b	29.4 c	29.2 b
	80	8.9 ab	9.2 a	10.0 a	92.7 b	95.0 b	64.7 b	78.2 b	82.4 b	74.3 b	28.8 b	30.1 b	29.6 b
	60	8.2 b	8.1 a	9.0 b	88.4 c	93.4 b	62.8 c	77.5 b	77.2 c	68.4 c	29.5 a	30.7 a	30.3 a
25-May	100	15.6 a	10.9 a	12.0 a	116.1 a	120.0 a	83.8 a	82.3 a	88.2 a	98.5 a	31.7 a	29.4 a	30.4 a
	80	15.2 a	10.3 a	11.1 b	111.4 b	111.5 b	79.8 b	80.7 b	81.3 b	87.5 b	30.7 ab	29.1 ab	29.8 ab
	60	13.3 b	9.4 b	10.2 c	99.9 c	111.2 b	75.8 c	81.5 b	78.0 b	90.7 b	30.3 b	28.6 b	29.2 b
10-Jun	100	21.9 a	16.7 a	16.5 a	137.5 a	149.2 a	114.0 a	109.7 a	102.1 a	109.0 a	29.5 a	28.9 a	29.5 a
	80	21.4 b	15.6 b	16.0 b	136.6 a	142.5 b	110.9 b	106.5 b	99.2 a	98.0 b	29.0 ab	28.7 a	28.7 b
	60	21.3 b	15.0 b	15.7 b	134.5 b	140.8 b	108.7 b	101.7 c	93.8 b	96.7 b	28.8 b	27.8 b	28.2 c
	CV _S (%)	6.33	8.66	7.62	3.37	5.64	3.21	3.48	7.46	8.59	1.63	2.16	1.87
	CV _P (%)	40.38	30.49	24.37	18.58	18.59	26.98	17.19	8.22	14.55	5.33	0.99	2.10
	CV _{P × s} (%)	37.05	27.59	23.87	17.69	17.70	24.22	14.63	10.27	14.44	3.53	2.92	2.41

Values in the same planting date followed by the different letters within a column are significantly different at 0.05 probability level ($n = 6$)
 CV_S was calculated from the data of three shading treatments in normal planting date 25 April, CV_P was calculated from the data of three planting dates and CV_{P × s} was calculated from the data of all treatments

DW dry weight, FW fresh weight

Table 5 The changing amplitude (Δ) of average sucrose content, average vacuole invertase (VIN) activity, average sucrose synthase (SuSy) activity and final length in cotton fibres of Kemian 1 and Sumian 15 for combinations of different planting dates and shading treatments in 2010 and 2011

Treatment	Sucrose content			VIN activity			SuSy activity			Fibre length		
	2010 field	2011 field	2011 pot	2010 field	2011 field	2011 pot	2010 field	2011 field	2011 pot	2010 field	2011 field	2011 pot
The cultivar of Kemian 1												
CRLR 80 %	-6.9	-12.2	-12.1	-2.2	-3.2	-3.8	-2.1	-9.0	-9.6	0.7	0.0	0.3
CRLR 60 %	-12.9	-20.3	-16.7	-5.6	-4.2	-4.6	-6.1	-10.0	-11.6	1.7	3.4	2.0
25-May	64.4	-5.7	9.7	24.3	21.3	17.6	2.4	-4.5	-3.0	8.9	1.0	4.1
25-May plus CRLR 80 %	38.6	-18.7	1.6	21.2	14.4	13.1	1.4	-7.4	-11.1	7.2	0.7	3.7
25-May plus CRLR 60 %	40.6	-22.8	-2.9	14.3	9.5	14.6	-3.4	-12.5	-15.6	4.5	-1.4	2.0
10-Jun	96.0	43.9	37.9	43.2	41.7	62.8	28.9	10.2	21.2	4.8	-1.4	2.4
10-Jun plus CRLR 80 %	100.0	34.2	35.0	37.1	34.0	55.5	23.5	5.9	11.8	4.1	-1.4	1.0
10-Jun plus CRLR 60 %	99.0	26.0	30.5	31.8	30.2	52.5	16.7	4.3	5.9	1.0	-2.7	-0.7
The cultivar of Sumian 15												
CRLR 80 %	-4.3	-4.2	-4.1	-1.8	-8.3	-3.4	-5.3	-8.0	-8.5	0.7	2.4	1.4
CRLR 60 %	-11.8	-15.6	-13.9	-6.4	-9.8	-6.2	-6.2	-13.8	-15.8	3.2	4.4	3.8
25-May	67.7	13.5	14.8	23.0	15.8	25.2	-0.4	-1.5	21.2	10.8	0.0	4.1
25-May plus CRLR 80 %	63.4	7.3	6.0	18.0	7.6	19.1	-2.3	-9.2	7.7	7.3	-1.0	2.1
25-May plus CRLR 60 %	43.0	-2.1	-2.5	5.8	7.3	13.2	-1.3	-12.9	11.7	5.9	-2.7	0.0
10-Jun	135.5	74.0	58.3	45.7	44.0	70.2	32.8	14.0	34.1	3.2	-1.7	1.0
10-Jun plus CRLR 80 %	130.1	62.5	53.3	44.7	37.5	65.6	28.9	10.8	20.7	1.4	-2.4	-1.7
10-Jun plus CRLR 60 %	129.0	56.3	50.8	42.5	35.9	62.3	23.1	4.8	19.1	0.7	-5.4	-3.4

Δ (%) = (Treatment–Control)/Control \times 100 %, control is the plot of 25 April plus CRLR 100 %

OriginPro 8.0 was used for drawing of figures

and late planting had significant interactive effects on fibre length (Fig. 2). The changing amplitude (Δ) of fibre length of the two cultivars increased under late planting or shading, which indicated the increasing fibre length, but the increasing amplitude dropped under the combined stress of late planting plus shading and the Δ even became negative under the combined condition in 2011 (Table 5). Over the 2 years, the coefficient of variation (CV) due to late planting were 2.48 % for Kemian 1 and 2.81 % for Sumian 15, the CV due to shading were 1.29 % for Kemian 1 and 1.89 % for Sumian 15 and the CV due to late planting plus shading were 2.11 % for Kemian 1 and 2.95 % for Sumian 15 (Table 4). For Kemian 1, late planting had greater effects on fibre length than shading or the combined stress of late planting plus shading, for Sumian 15, the combined stress of late planting plus shading had greater effects than either stress alone.

Sucrose content in cotton fibre

During fibre development period of the normal planting date 25 April, sucrose contents in cotton fibres declined from 10 DPA, but in the late planting dates 25 May and 10 June, the peak value delayed to 17 DPA, even 24 DPA, and

late planting also enhanced sucrose contents compared to normal planting. Shading reduced fibre sucrose contents of all planting dates, and reduced even more if the shading became heavier (Fig. 3; Table 4). Over the 2 years, the CV due to late planting were 24.28 % for Kemian 1 and 31.75 % for Sumian 15. The CV due to shading were 9.31 % for Kemian 1 and 7.54 % for Sumian 15. The CV due to late planting plus shading were 24.99 % for Kemian 1 and 29.50 % for Sumian 15 (Table 4). The CV due to late planting and the combined stress were much higher than the CV due to late planting or shading, indicating that late planting and the combined stress had greater effects on sucrose contents than shading, while late planting and the combined stress also had greater effects on Sumian 15 than Kemian 1. Under late planting plus shading, the Δ of average sucrose content during FEP of Kemian 1 was less than that of Sumian 15, and the difference was even larger as the level of late planting plus shading became heavier (Table 5). Late planting or shading had significant effects on sucrose contents but significant interactive effects did not appear in all treatments (Fig. 3). Due to late planting plus shading, the CV and Δ of sucrose contents of Sumian 15 were larger than that of Kemian 1 (Tables 4, 5), indicating that Sumian 15 was more sensitive to the combined

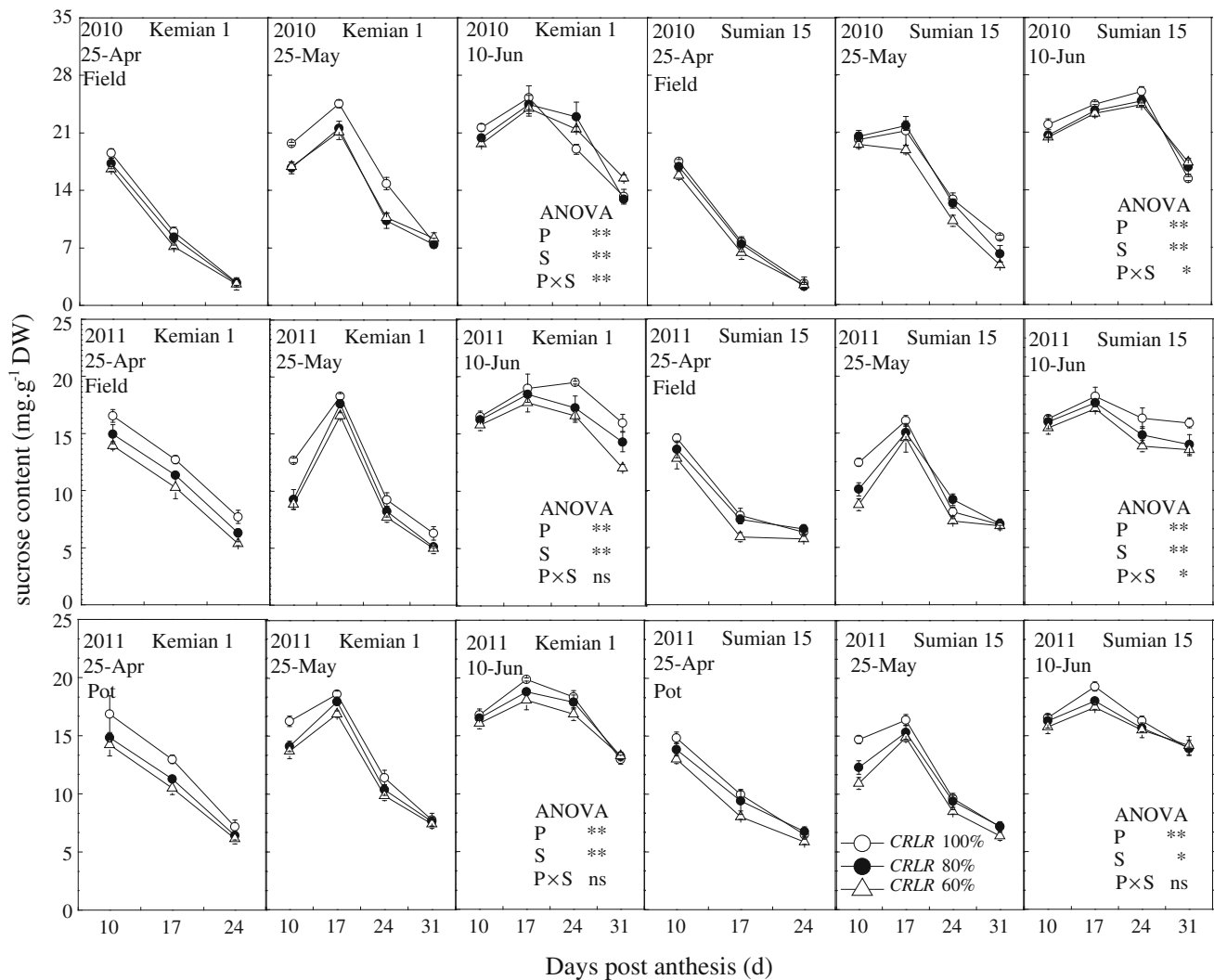


Fig. 3 Changes of cotton fibre sucrose content for Kemian 1 and Sumian 15 for combinations of different planting dates and shading treatments in 2010 and 2011. *P* planting date, *S* shading, *P* × *S* planting date × shading. The values are mean ± SD (*n* = 6). Two-way

ANOVA results embedded in the figure were calculated from the data of average fibre sucrose content. *ns* not significant; **P* < 0.05; ***P* < 0.01 (*n* = 6)

stress of late planting plus shading than Kemian 1 in fibre sucrose metabolism.

Changes in activities and expressions of related enzymes of fibre elongation

In cotton fibres, vacuolar invertase (VIN) activity declined with fibre elongation (Fig. 4). Compared to the normal planting date 25 April, the VIN activities of the late planting dates were higher, and kept at a high level until the end of elongation. Shading in all planting dates reduced VIN activities significantly and reduced even greater as the shading became heavier (Fig. 4; Table 4). Compared to normal planting cottons, the VIN activities of late planting plus shading were higher (The corresponding Δ of VIN

activity was plus), although they were still lower than the activities of the corresponding late planting treatments without shading (Tables 4, 5). Late planting and shading had significant interactive effects on VIN activities of the two cultivars in 2010 and of Kemian 1 in 2011 but there were no significant interactive effects on VIN activities of Sumian 15 in 2011 (Fig. 4).

Sucrose synthase (SuSy) activity of the normal planting date declined with fibre elongation, but as the planting date delayed, the peak values of SuSy activities were delayed to 17 or 24 DPA (Fig. 5). Compared to the planting date 25 April, the SuSy activities of the late planting dates were higher, and kept at a high level until the end of elongation. Shading in all planting dates reduced SuSy activities significantly and reduced even more as the shading became

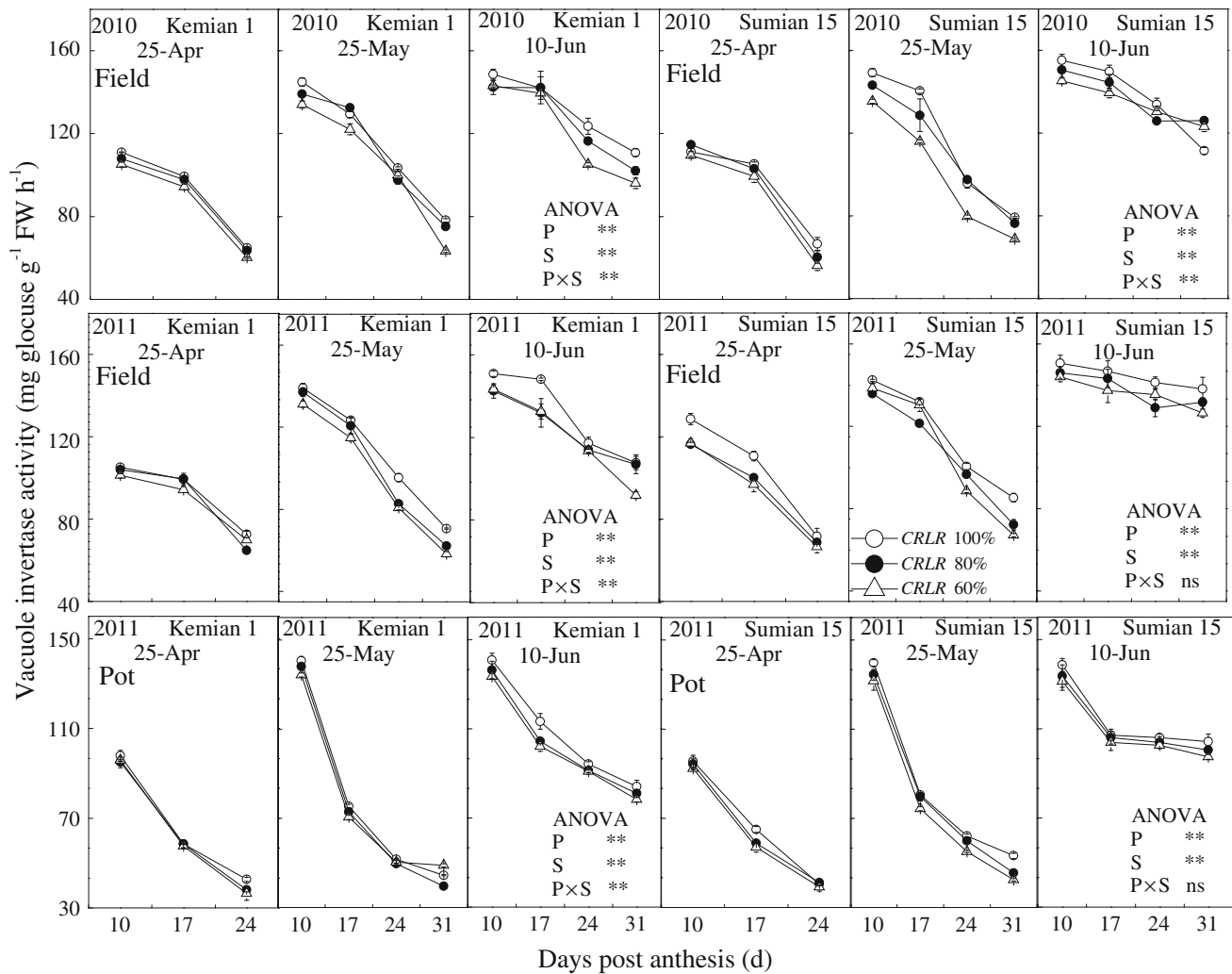


Fig. 4 Changes of cotton fibre vacuole invertase activity for Kemian 1 and Sumian 15 for combinations of different planting dates and shading treatments in 2010 and 2011. *P* planting date, *S* shading, *P* × *S* planting date × shading. The values are mean ± SD (*n* = 6).

heavier (Fig. 5; Table 4). SuSy activities reduced by shading in the late planting dates were close to or lower (the corresponding Δ of SuSy activity dropped or became negative) than the activities of normal planting cotton (Table 5). Late planting and shading had significant interactive effects on SuSy activities of the two cultivars in 2010 and 2011 (Fig. 5).

In the point of view of CV value, late planting had greater effects on VIN and SuSy activity than shading, and the effects on Sumian 15 were greater than Kemian 1. Due to late planting plus shading, the Δ of VIN and SuSy activity in Sumian 15 were also larger than that of Kemian 1 (Table 5), indicating that compared to Kemian 1, the fibre sucrose degrading enzymes of Sumian 15 were more sensitive to the combined stress.

The expression of *GhEXPI* was prolonged by late planting, which matched the prolonging of fibre

Two-way ANOVA results embedded in the figure were calculated from the data of average fibre vacuole invertase activity. *ns* not significant; **P* < 0.05; ***P* < 0.01 (*n* = 6)

elongation. The peak of *GhEXPI* expression appeared at 10–17 DPA in the normal planting date 25 April, and it was postponed to 17–24 DPA by late planting (Fig. 6). The *GhEXPI* of Kemian 1 expressed greater than Sumian 15 in 24 DPA (in the normal planting date 25 April) or 31 DPA (in the late planting date 25 May), indicating that the expression of *GhEXPI* of Kemian 1 was longer than Sumian 15. In 10 DPA, shading did not reduce the *GhEXPI* expression of Kemian 1, however, from 17 DPA (in the normal planting date 25 April) or 24 DPA (in the late planting dates 25 May and 10 June) to the end of fibre elongation, the expression under shading was lower than normal light. Shading also reduced the *GhEXPI* expression of Sumian 15 from 24 DPA to the end of fibre elongation in the late planting dates 25 May and 10 June, but increased the expression in the normal planting date 25 April (Fig. 7a, b).

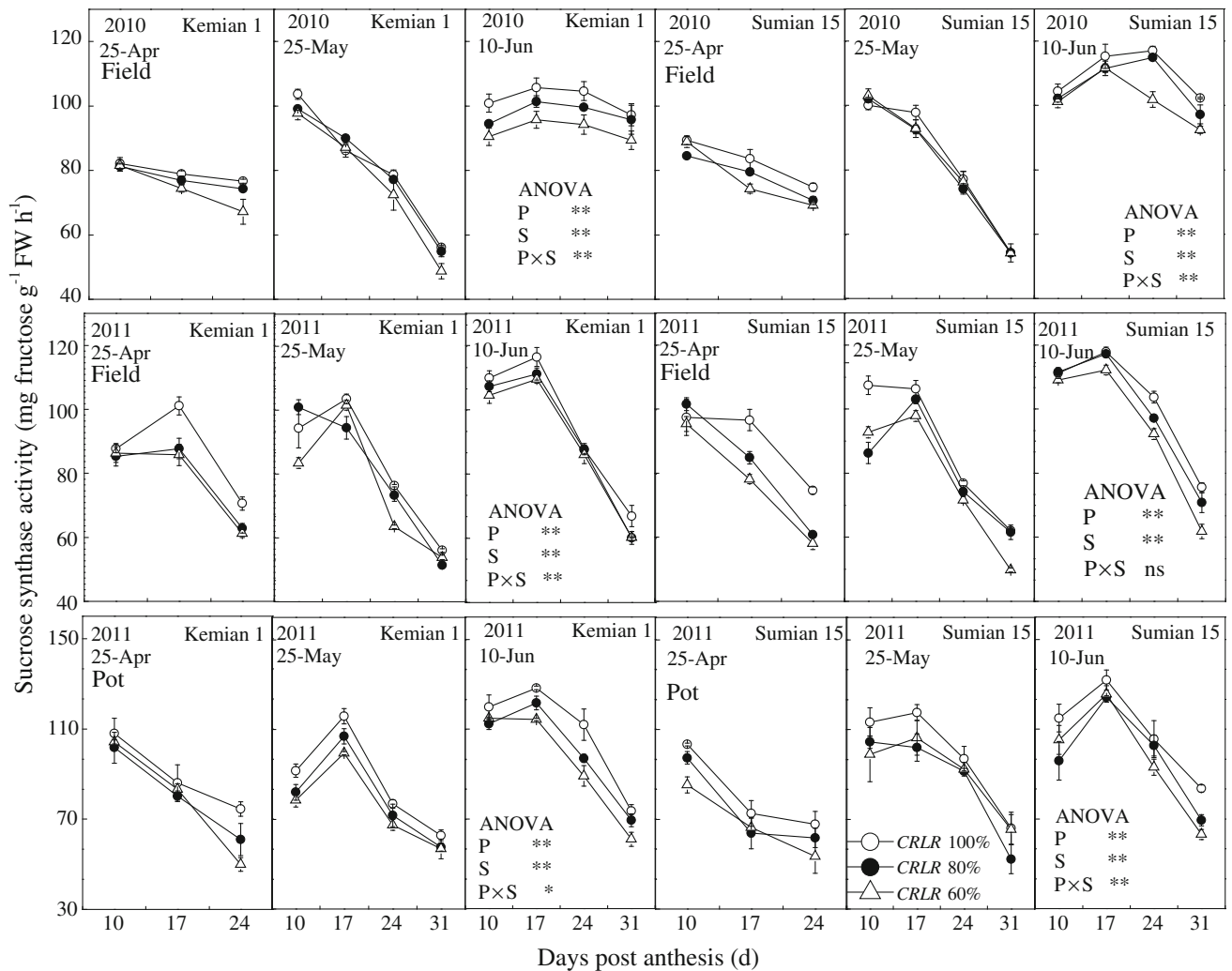


Fig. 5 Changes of cotton fibre sucrose synthase activity for Kemian 1 and Sumian 15 for combinations of different planting dates and shading treatments in 2010 and 2011. *P* planting date, *S* shading, *P* × *S* planting date × shading. The values are mean ± SD (*n* = 6).

Two-way ANOVA results embedded in the figure were calculated from the data of average fibre sucrose synthase activity. *ns* not significant; **P* < 0.05; ***P* < 0.01 (*n* = 6)

The peak of *GhGluc1* and *GhGlucS* expression appeared around 10 DPA in the normal planting date 25 April, and late planting delayed it to 17 DPA or 24 DPA (Fig. 6). In the normal planting date 25 April, shading made *GhGluc1* expression lower than normal light at the beginning of fibre elongation (10 DPA), and shading made the expression higher than normal light at the end of elongation (17 DPA for Sumian 15 and 24 DPA for Kemian 1). The results indicated that the peak appearing time of *GhGluc1* expression under shading was later than that of under normal light, and the appearing time of Kemian 1 was later than Sumian 15. Peak delaying caused by shading also appeared in the late planting date 10 June, but was not significant in the late planting date 25 May (Fig. 7c, d). In contrast, shading in the normal planting date 25 April finished the expression of *GhGlucS* at 17 or 24 DPA, which

was earlier than normal light, indicating that the peak appearing time of *GhGlucS* expression under shading was earlier than that of under normal light. In the planting dates 25 May and 10 June, the *GhGlucS* expression under shading was lower than normal light at 17 DPA, but was higher than normal light at 24 DPA, indicating that shading delayed the peak appearing time of the *GhGlucS* expression in the planting dates 25 May and 10 June (Fig. 7e, f).

Discussion

Many studies have shown that environmental factors can affect fibre development (Eaton and Ergle 1953; Pettigrew 1995, 2001; Liaktas et al. 1998; Dong et al. 2006), and temperature and light often appeared as a compounding

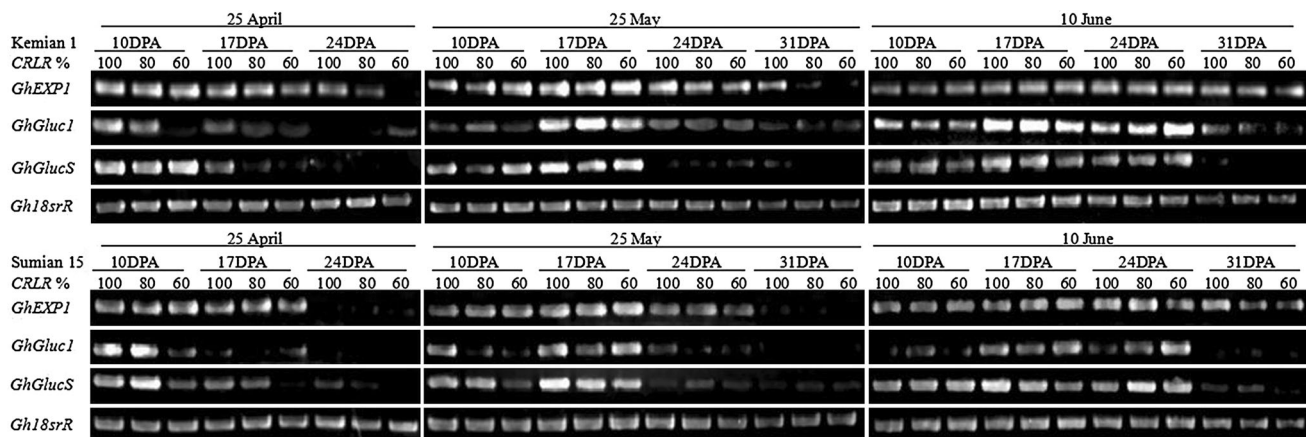


Fig. 6 Gene expressions of cotton fibre elongation relative enzymes for Kemian 1 and Sumian 15 for combinations of different planting dates and shading treatments of the pot experiment in 2011

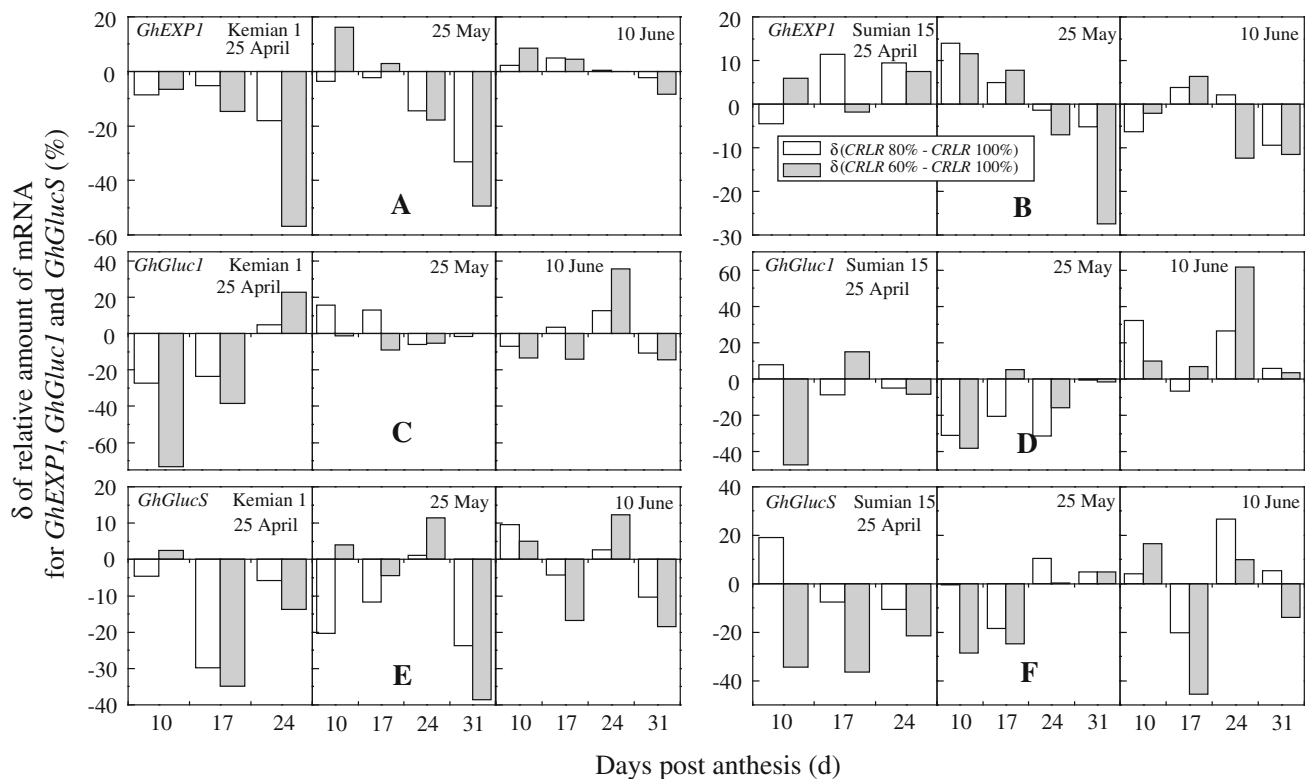


Fig. 7 δ of relative amount of mRNA for *GhEXP1*, *GhGluc1* and *GhGlucS* in cotton fibre for Kemian 1 and Sumian 15 for combinations of different planting dates and shading treatments of the pot

experiment in 2011; $\delta = (\text{CRLR } 80\% - \text{CRLR } 100\%)$ or $(\text{CRLR } 60\% - \text{CRLR } 100\%)$

factor in cotton production. This experiment utilised late planting plus shading to create different combinations of temperature and light in the period of cotton fibre elongation. Due to late planting, $\text{MDT}_{\text{minFEP}}$ declined from 27.8 to 20.3 °C in 2010 and 25.7 to 18.1 °C in 2011 (Table 1), and because of two kinds of shade cloth, PAR at cotton canopy were reduced 20 and 40 %, respectively (Table 3). The

adverse conditions resulted in the extended period of fibre elongation and affected fibre length formation.

In the Yangtze River Valley of China, planting in April is suitable for upland cotton yield. Fibre quality formation and the boll formation of late planting cotton usually suffer from declining temperatures accompanying the cloudy and overcast weather. However, 25 April was not

the most suitable planting date for fibre length formation in this experiment; the longest fibre length was formed by the late planting date 25 May (MDT_{minFEP} was around 20.9–24.3 °C, Table 4). In the planting date 25 April, shading was advantageous to fibre length but disadvantageous to fibre strength, as reported before (Lv et al. 2013). As planting date delayed, shading was also disadvantageous to the length formation (Table 4), and the physiological reasons were discussed in the following sections.

Cotton fibre elongation is a result of a complex interplay between cell turgor and cell wall extensibility which is a unidirectional cell expansion process (Smart et al. 1998; Ruan et al. 2001; Wang and Ruan 2010). During growth, plant cells secrete a protein called expansin which plays an important role in fibre elongation through unlocking the network of wall polysaccharides and permitting turgor-driven cell enlargement (Shimizu et al. 1997; Cosgrove 2000; Shu et al. 2008). The expressing duration of *GhEXPI*, which displayed expansin sequence features, was prolonged as MDT_{minFEP} declined (Fig. 6), corresponding with the prolonging of fibre elongation by the declining temperature. Low light caused by shading inhibited the *GhEXPI* expression at the later period of fibre elongation. The expression was prolonged under the condition of late planting plus low light, but was still shorter than that of late planting plus normal light, which further had an adverse effect on the fibre secondary wall relaxation and the cellulose accumulation. Two cotton cultivars in this study had been found to have different sensitivities to cool temperature, with Sumian 15 being more sensitive than Kemian 1 (Wang et al. 2008, 2009). Compared to Sumian 15, Kemian 1 with a longer expression of *GhEXPI* would be beneficial to fibre length formation.

Fibre cell turgor is controlled by osmotic active solutes and duration of PD closure (Ruan et al. 2004, 2005). Sucrose is not only a major part of osmotic active solutes, but also a source of energy for elongation (Ruan et al. 1997; Wang and Ruan 2010), and it was affected by reducing environmental condition (Pettigrew 2001). Plant adapting to low (nonfreezing) temperatures often accompanied with abundant accumulation of sucrose, which was a self-regulating phenomenon (Guy et al. 1992; Bhowmik et al. 2001), and the high sucrose content might cause a large turgor pressure, leading to a longer fibre. In this research, as the MDT_{minFEP} declined, sucrose contents of fibres increased, and they were reduced by low light (Fig. 3; Table 4). The decreasing trend of sucrose content caused by shading was the same as the decreasing trend of fibre length in the late planting dates (MDT_{minFEP} was around 18.1–24.3 °C) instead of the normal planting dates (MDT_{minFEP} was around 25.7–27.8 °C), so under the

condition of late planting plus low light, the amount of available sucrose in the fibres increased, which might be beneficial for the rising of turgor pressure and physiological metabolism, and finally conducive to fibre elongation. However, the sucrose contents under the conditions of late planting plus low light were still lower than that of corresponding late planting plus normal light treatments, leading to a relatively shorter length (Table 4). In contrast, the cause of the increasing fibre length accompanying with the decline of sucrose content under shading in the normal planting dates needed further analysis.

Relative enzymes of sucrose metabolism play an important role in the controlling of cell turgor. SuSy plays roles in both fibre elongation and cellulose biosynthesis, through osmotic regulation and supplying UDPglucose as substrate for cellulose production; SuSy also supplies hexoses for generation of ATP as an energy source for a variety of solutes transport and metabolic processes (Ruan et al. 1997, 2003; Wang and Ruan 2010). VIN appears to be more specifically required for the formation and enlargement of the central vacuole, pivotal for early fibre elongation (Wang et al. 2010; Wang and Ruan 2010). However, in this experiment, the changing trend of VIN and SuSy activities under treatments did not entirely consistent with fibre length. The decreasing trend of enzyme activities caused by shading was the same as fibre length in the late planting dates instead of the normal planting dates (Table 4). The decline of relative enzyme activities of sucrose metabolism might lead to the decline of fibre length caused by low light in the late planting conditions, but in the normal planting conditions, the decline of enzyme activities caused by low light had little effect on the length formation. Under the conditions of late planting plus low light, the CVs of the sucrose contents, VIN and SuSy activities of Sumian 15 were higher than that of Kemian 1 (Table 4), which indicated that Sumian 15 had more sensitive fibre sucrose content, VIN and SuSy activity to the combined stress than Kemian 1 during fibre rapid elongation period. This would probably explain why the decreased extent of fibre length of Sumian 15 was larger than that of Kemian 1 under the conditions of late planting plus low light (Table 4).

The duration of the PD closure is determined by the synthesizing and degrading of callose (Ruan et al. 2001, 2004, 2005; Ruan 2007), which is controlled by the joint action of β -1,3-glucan synthase and β -1,3-glucanase (Bucher et al. 2001; Ruan et al. 2004). In this research, due to the decline of MDT_{minFEP} caused by late planting, the peak of *GhGluc1* and *GhGlucS* expression was delayed (Fig. 6), which might cause the delay of the PD closure, leading to the delay of fibre rapid elongation period. In the normal planting date 25 April (MDT_{minFEP} was around 24.3–

25.7 °C), shading delayed the peak of *GhGluc1* expression (the peak appearing time of Kemian 1 delayed more than Sumian 15) and brought the peak of *GhGlucS* expression forward (Figs. 6, 7), leading to a longer duration of PD closure, and the duration of PD closure of Kemian 1 was longer than Sumian 15, as a result, the length would be longer. However, this tendency was not obvious in the planting date 25 May (the $MDT_{\min FEP}$ dropped to 20.9–24.3 °C). When the $MDT_{\min FEP}$ dropped lower (around 18.1–20.3 °C) in the planting date 10 June, shading delayed both the peak of *GhGlucS* and *GhGluc1* expression (Figs. 6, 7), however, whether the duration of PD closure was prolonged by shading could not be certain.

In this study, sucrose content, VIN and SuSy activities and the duration of the PD closure all played important roles in fibre elongation, but under the combined conditions of different planting dates and low light, there might exist a temperature reversal range in the fibre length formation. Above the temperature range, the duration of the PD closure contributed more to fibre elongation than the sucrose content, VIN and SuSy activities under low light. Below the temperature range, the sucrose content, VIN and SuSy activities contributed more to fibre elongation than the duration of the PD closure under low light.

In conclusion, the longest fibre length was formed when planting was done on 25 May ($MDT_{\min FEP}$ was around 20.9–24.3 °C) instead of the normal planting date 25 April in our research. Low light had diverse effects on fibre length when cotton was grown under different planting dates. For the cotton grown in the normal planting date, low light increased fibre length by prolonging the duration of PD closure instead of changing sucrose contents or enzyme activities of sucrose metabolism. However, when cotton suffered the decline of temperature caused by late planting, the difference of the PD closure duration between shading treatments were not obvious, but low light had a negative impact on fibre sucrose content, VIN and SuSy activities during fibre rapid elongation period, leading to a decline of fibre length. There was variability between two cultivars in the decreased extent of fibre length under the condition of late planting plus low light. The probable reason was that compared to cool temperature-tolerant cultivar Kemian 1, cool temperature-sensitive cultivar Sumian 15 had a shorter *GhEXPI* expression, and the sucrose content, VIN and SuSy activities of Sumian 15 during fibre rapid elongation period were more sensitive than Kemian 1 under the combined condition.

Author contribution Ji Chen, Fengjuan Lv, Jingran Liu, Yina Ma, Binglin Chen and Zhiguo Zhou had designed the research; Ji Chen, Fengjuan Lv, Jingran Liu, Yina Ma, Youhua Wang and Yali Meng had performed the experiments; Ji Chen analysed the data and wrote the paper;

Zhiguo Zhou had supervised the work. All authors have read and approved the final manuscript.

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