Effects of Removing Basal Fruiting Branches on Cellulose Accumulation and Activities of Cellulose Synthesis-Related Enzymes in Cotton

W. J. Zhang[1], Z. L. Huang[1*], T, H. Zhou[1], L. Liu[1], and L. Mi[1]

ABSTRACT

Using cotton cultivar that undergoes premature senescence in the late stage of growth, we studied the effects of removing two basal fruiting branches of two cotton varieties, namely, Quanyin-2 and Jiza-999, on the leaf area index, the SPAD value of leaf subtending cotton boll, and the development of cotton fiber in the late stage of cotton growth. We focused on the differences in cotton cellulose accumulation and fiber-related enzyme activities after removal of the basal fruiting branches and the development of cotton fiber during premature senescence. The results showed that removing the basal fruiting branches can maintain the green leaf area of the cotton canopy and the SPAD value of the subtending leaf during the late stage of reproductive growth. The period of rapid accumulation of fiber cellulose lasted longer in plants from which the basal fruiting branches had been removed, and the beginning and end of the rapid accumulation period was later than in the control group with premature senescence. The activities of cellulose synthesis-related enzymes (sucrose phosphate synthase, sucrose synthase, β-1,3-glucanase, and invertase) were higher in plants in which the basal fruiting branches had been removed than in the control group after 10 days post-anthesis. Removing the basal fruiting branches can optimize the accumulation of cellulose in cotton boll during the late growth stage and mitigate the effects of premature senescence on cellulose synthesis. We found that the peak values for cotton fiber development-related enzyme activities in the control group occurred earlier, which tended to bring cotton fiber development forward and negatively impacted fiber yield.

Keywords: Cellulose synthesis, cv. Jiza-999, cv. Quanyin-2, Fiber-related enzyme, Premature senescence.

INTRODUCTION

Premature senescence in cotton refers to the premature cessation of photosynthesis during the effective growth period, with the result that cotton boll development and fiber filling are restricted or stopped (Liu *et al*., 2012). In the cotton-growing regions of the middle and lower reaches of the Yangtze River and the middle and lower reaches of the Yellow River, the rate of premature senescence of cotton is high. Several hypotheses have been proposed to explain the causes of premature senescence in cotton, and, at present, it is generally accepted that the source-sink ratio imbalance may be an important physiological reason for premature senescence (Stewart, 2010; Wright, 1999). Previous studies have shown that defoliant (Gormus *et al.*, 2017) or removal of fruit branches (Zhang *et al.*, 2013) is beneficial for the production of cotton fibers. Currently, research into premature senescence in cotton focuses on the physiological characteristics of the functional leaves, carbohydrate distribution, and other aspects (Bowling *et al.*, 2011; Li *et al.*, 2012). The impacts of premature senescence on cotton fiber development have not been reported.

[1] Department of Agronomy, Anhui Agricultural University, Anhui Province, P. R. China.
* Corresponding author; e-mail: zhangwenjing79@126.com

The cellulose content of the mature cotton fiber is > 95% of the dry weight, and it is now widely recognized that sucrose is the starting substrate required for cellulose synthesis in cotton (Pettigrew, 1999; Doblin *et al*., 2002), Uridine DiPhosphate Glucose (UDPG) is a direct substrate for cellulose synthesis. Sucrose levels in the developing fiber cells are mainly determined by invertase that provides energy, Sucrose Synthase (SuS) that catalyzes the formation of UDPG from sucrose, and Sucrose Phosphate Synthase (SPS) that can synthesize sucrose (Saxena and Brown, 2005; Wang *et al*., 2011). SuS can synthesize the cellulose precursor UDPG in the catalytic reaction, and the main function of the enzyme in cotton fiber cells is to break down sucrose to generate UDPG (Doblin *et al*., 2002; Bowling *et al*., 2011). SuS is associated with the plasma membrane where it acts as a carbon channel that can directly transport carbon in the form of sucrose to cellulose synthase (Amor *et al.*, 1995; Brill *et al*., 2011). In fiber cells, in addition to the UDPG produced by SuS catalysis, there is a pool of free UDPG that cannot be directly used as a cellulose precursor. However, sucrose phosphate synthase can use free UDPG to synthesize sucrose (Wang *et al*., 2011), and its relative activity can affect the rate of cellulose deposition (Michelle and Haigler, 2001; Endler and Persson, 2011). Invertase hydrolyzes sucrose to form fructose and glucose, which can provide both a carbon source and energy for the rapid accumulation of cellulose (Ruan *et al*., 2009. When cotton fibers enter the developmental stage of secondary wall thickening, the relative expression of the *β*-1,3-glucanase gene is also increasing rapidly. High level of endo-1,3-*β*-glucanase activity is required during massive deposition of cellulose in cotton fibers, although direct evidence that *β*-1,3-glucan is an intermediate in biosynthesis of cellulose is lacking (Shimizu *et al*., 1997; Bucher *et al*., 2001).

In cotton, it is possible to adjust the source-sink relationship and carbon and nitrogen metabolism by removing the basal fruiting branches, thereby delaying the occurrence of premature senescence (Dong *et al*., 2009; Zhang *et al*., 2013). Thus, we can test the effect of adjusting the source-sink ratio by delaying or reducing the occurrence of premature senescence on cellulose biosynthesis in cotton. In the research reported here, we aimed to adjust the source-sink ratio in cotton by removing the basal fruiting branches in early flowering to delay the occurrence of premature senescence, in order to study the effects of premature senescence on cellulose accumulation and the changes in cellulose synthesis-related enzyme activity.

MATERIALS AND METHODS

Field Experiments and Experimental Design

Experiments were conducted from April to November in 2013 and 2014 (the cotton growing season) in Dayangdian Test Farms, Anhui Agricultural University, Anhui Hefei, China (31.52 N, 117.17 E). The soil was a yellow-cinnamon soil, the pH of the 0-20 cm layer was 7.0, the organic matter content was 1.30%, total nitrogen was 0.1%, and there was 101.5 mg kg^{-1} of available nitrogen, 8.5 mg kg^{-1} of available phosphate, and 92.4 mg kg^{-1} of available potassium.

Based on the previous results of Zhang *et al*. (2013), premature senescence can be suppressed by removing the basal fruiting branches during early flowering in cotton cultivars Quanyin-2 (Anhui Quanyin Hefeng Seed Industry Co., Ltd.; the growth period is 123 days) and Jiza-999 (Institute of cotton research, Hebei Academy of Agriculture and Forestry Sciences; the growth period is 122 days). Seeds were sown in the field on April 18, 2013, and May 5, 2014, in 7.0m×7.5 m plots with wide-narrow row arrangement (75 cm+50 cm). Plant density was 24,000 ha^{-1}, and the spacing between the plots was 1 m. There were two experimental groups of plants for each cultivar: (1) The basal fruiting branches were removed during early flowering to suppress premature senescence (the treatment), and (2) The basal fruiting

branches were not removed and plants were allowed to undergo premature senescence in the late growth stage (the control). Two basal fruiting branches were removed at 10 Days Post-Anthesis (DPA) in the treatment, and the treatment and the control were replicated four times each. Field management was conducted based on the requirements for high yield cotton cultivation.

Because premature senescence is more apparent in the middle and later periods of growth and development in cotton plants, the white flowers that opened on September 1 in the middle and later periods of growth were selected and marked in both test years. Starting from 10 DPA, 15 cotton bolls of the same size were sampled every 5 days (sampling was carried out from 09:00 to 11:00) until a boll age of 40 days (prior to boll crack). Cotton fibers were removed from the bolls, flash frozen in liquid nitrogen, and stored at -80°C prior to testing.

Leaf Area Index (LAI)

We used the LAI-2000 Plant Canopy Analyzer (LI-COR) to measure LAI. Two measurements were taken on 1 August (mid-reproductive growth stage of cotton) and 10 September (late reproductive growth stage). In each plot, we chose three observation points. At every observation point, the probe was placed above the canopy, placed into the canopy (perpendicular to the line) and kept level. LAI values of the upper (from the top of the canopy of about 20 cm), middle, and lower canopy (from the ground to about 20 cm) were measured, and the average values were calculated.

Leaf SPAD Value

We used a SPAD-502 chlorophyll meter (Konica Minolta) to measure the SPAD value of the subtending leaf of the cotton boll. For the tagged white flowers, SPAD values of the subtending leaf of the cotton

bolls were measured every five days, starting at 10 DPA. Four loci per leaf and 10 pieces of leaf were measured randomly, and average values were calculated. The leaf main vein was avoided when taking SPAD measurements.

Cellulose Content

Cellulose content of cotton fibers was measured using anthrone reagent according to a modified method described by Updegraff (1969). Samples of cotton fiber that had been dried to a constant weight at 45°C (we used 0.2 g before 20 DPA, and 0.1 g after 25 DPA) were added to 100 mL 60% H_2SO_4 and allowed to digest for 30 minutes. The volume of the digested cellulose solution was adjusted with 60% H_2SO_4, shaken well, and filtered through a Buchner funnel. A 5-mL sample of the filtrate was transferred to a 100 mL volumetric flask and diluted with distilled water in a cold bath. For the assay, 0.5 mL 2% anthrone reagent and 5 mL concentrated H_2SO_4 were added to 2 mL of the digested cellulose solution, which was shaken and allowed to stand for 12 minutes, after which the absorbance was measured at 620 nm.

Enzyme Assays

Enzyme assays for invertase, sucrose synthase, sucrose phosphate synthase, and β-1,3-glucanase: ~1.0 g cotton fiber samples (fresh weight) were ground to a powder in a mortar with liquid nitrogen. Nine mL PBS buffer (pH= 7.3) was added in two portions, and the mixture was centrifuged at 3,500 rpm for 20 minutes. The supernatant was then used for ELISA (enzyme-linked immunosorbent assay) using a kit provided by Shanghai Ding Biological Technology Co., Ltd. Methods for determination of enzymes activities according to the instructions on the method.

Boll Weight:

After the cotton bolls matured and opened on 1 September, 10 to 20 cotton bolls of the same size per plot were collected, air-dried, and weighed.

Statistical Analysis

The Logistic regression model was used to describe cellulose accumulation:

$$Y = Y_m /(1 + a * e^{bt}) , \qquad (1)$$

Where, t is days post-anthesis (d), Y is cellulose content (%), Y_m is the maximum cellulose content, and a and b are parameters.

Several of the parameters could be derived from the equation. t_1: The start time of the period of rapid cellulose accumulation. t_2: The end time of the period of rapid cellulose accumulation. t_m: The time when the maximum rate of cellulose accumulation was reached. T: Time taken for cellulose rapid accumulation. The expressions are as follows:

$$t_1 = \frac{1}{b} \ln \frac{2+\sqrt{3}}{a} \qquad t_2 = \frac{1}{b} \ln \frac{2-\sqrt{3}}{a} \qquad t_m = -\frac{\ln a}{b}$$

$$T = t_2 - t_1 \qquad (2)$$

All data were analyzed with statistical software SPSS (version 16.0) and OriginPro (version 8.5).

RESULTS

LAI and SPAD Values

Table 1 shows the effects of removing the basal branches of cotton on the LAI values at a significant level of $P < 0.05$. The difference between the LAI of the treatment and the control group in the Jiza-999 canopy on 1 August was not significant. During the mid-reproductive growth stage of cotton (1 August), the LAI of the treatment group was less than that of the control group. However, until the late reproductive growth stage (1 September), the LAI of the treatment group remained high, and was greater than in the control group.

The nutrients in the boll are mainly derived from the subtending leaf of the boll (Constable and Rawson, 1980). Removal of the basal branches had an influence on the SPAD values of the subtending leaf during late reproductive growth (Table 2). In the control group, the SPAD value of the subtending leaves (flowering on 1 September) increased from flowering day one, reached a peak at a boll age of ~30 days, and then began to decrease. In the treatment group, the SPAD values remained relatively high from 10 to 40 DPA; especially after 30 DPA, SPAD values were significantly higher than in the control group

Table 1. Leaf area indices of the two cotton varieties for 2 years.

Year	Cultivar [a]	Treatment	LAI [d]	
			1 August	10 September
2013	Quanyin-2	Treatment [b]	2.40 b±0.07	1.62 a±0.02
		Control [c]	2.61 a±0.04	0.65 b±0.03
	Jiza-999	Treatment	1.85 a±0.04	1.17 a±0.04
		Control	1.92 a±0.01	0.62 b±0.02
2014	Quanyin-2	Treatment	1.97 b±0.04	1.34 a±0.03
		Control	2.05 a±0.01	0.91 b±0.02
	Jiza-999	Treatment	1.40 b±0.06	1.26 a±0.08
		Control	1.93 a±0.03	0.67 b±0.00

[a] Quanyin-2 and Jiza-999 are two cotton varieties, and the experiments were conducted in 2013 and 2014.
[b] Plants in which the two basal fruiting branches were removed during early flowering to delay the onset of senescence, [c] Plants in which the basal fruiting branches were not removed. [d] Values followed by the same lower case letter are not significantly different at the $P < 0.05$ level.

Table 2. Changes in chlorophyll content in the subtending leaf of the cotton boll.

Year	Cultivar	Treatment	10 DPA[b]	15 DPA	20 DPA	SPAD values[a] 25 DPA	30 DPA	35 DPA	40 DPA
2013	Quanyin-2	treatment	42.00a±2.35	43.23a±0.30	44.13a±0.48	45.37a±0.27	47.07a±0.44	48.80a±0.35	49.77a±0.32
		control	38.60a±2.86	39.90b±0.31	40.70b±0.54	41.63b±0.44	43.63b±0.61	41.93b±0.66	39.53b±0.19
	Jiza-999	treatment	35.07a±0.55	36.37a±0.56	38.00a±0.23	39.57a±0.44	41.47a±0.21	43.07a±0.50	44.30a±0.58
		control	35.47a±1.46	36.77a±0.44	37.10b±0.42	38.60a±0.35	39.83b±0.42	38.67b±0.93	36.93b±0.61
2014	Quanyin-2	treatment	41.43a±0.55	42.13a±0.44	43.53a±0.64	45.23a±0.58	46.27a±0.27	46.43a±0.43	44.67a±0.67
		control	32.60a±0.46	33.83b±0.37	34.80b±0.42	35.97b±0.24	37.87b±0.56	36.00b±0.64	37.10b±0.46
	Jiza-999	treatment	33.77a±0.44	35.17a±0.85	36.77a±0.44	38.23a±0.52	41.00a±0.50	42.17a±0.42	43.37a±0.44
		control	30.67a±0.19	31.80b±0.24	32.53b±0.44	33.27b±0.44	35.03b±0.55	34.43b±0.43	33.23b±0.91

[a] Values followed by the same lower case letter are not significantly different at the *P*< 0.05 level. [b] DPA: Days

(P< 0.05).

Cotton Cellulose Accumulation and Boll Weight

In the two test years, change of cellulose content in cotton bolls in experiment showed an S-shaped curve; the Logistic model was used to simulate the accumulation of cellulose, and the coefficient of determination (R^2) of the fitting equations was > 0.98 (Table 3). By analyzing the parameters and Eigen value of the model in Table 3, we found that the theoretical maximum for cotton cellulose accumulation in the treatment group was greater than that in the control with premature senescence, and there was also a difference in the start and end time, the duration, and the occurrence time of the maximum rate of cellulose accumulation between the treatment and the control. For the two test years and the two cultivars, the start and end times for the period of rapid cellulose accumulation occurred earlier in the control group than in the treatment. Also, the occurrence time of the maximum accumulation rate was earlier, and the duration of the rapid period of cellulose accumulation was shorter, in the control than in the treatment.

As shown in Table 4, after the removal of the fruiting branch at the base, the boll weight in the late reproductive growth stage was significantly higher than in the control (P< 0.05). The single boll weights for Quanyin-2 increased 4.85% and 9.44%, respectively, in 2013 and 2014, while the single boll weights for Jiza-999 increased 6.31% and 8.73% in the two years.

Sucrose Phosphate Synthase Activity

In the treatment group and the control group, fiber SPS activity showed a unimodal curve with respect to changes in boll age during fiber development, and the results were consistent for the two years (Figure 1).

Table 3. Kinetics of cellulose accumulation in the cotton fiber.

Year	Cultivar	Treatment	Model [a]	R^2	t_1 [b] (d)	t_2 [b] (d)	t_{max} [b] (d)	T [b] (d)
2013	Quanyin-2	Treatment	$Y=88.3814/(1+40.3024e^{-0.1688t})$	0.9926	14.0960	29.6981	21.8975	15.6021
		Control	$Y=86.2033/(1+51.6654e^{-0.1815t})$	0.9886	14.4752	28.9827	21.7294	14.5075
	Jiza-999	Treatment	$Y=94.0826/(1+21.5868e^{-0.1279t})$	0.9935	13.7197	34.3072	24.0142	20.5875
		Control	$Y=88.8907/(1+24.8194e^{-0.1366t})$	0.9939	13.8679	33.1451	23.5071	19.2771
2014	Quanyin-2	Treatment	$Y=95.8371/(1+34.6784e^{-0.1389t})$	0.9970	16.0434	34.9982	25.5214	18.9548
		Control	$Y=87.9103/(1+39.2321e^{-0.1512t})$	0.9975	15.5566	32.9724	24.2651	17.4158
	Jiza-999	Treatment	$Y=94.0498/(1+30.1382e^{-0.1337t})$	0.9953	15.6264	35.3289	25.4783	19.7025
		Control	$Y=81.9413/(1+31.1186e^{-0.1745t})$	0.9961	12.1552	27.2496	19.7029	15.0944

[a] Y: Cellulose content in the cotton fiber, t: Days post anthesis. ($n= 7$, $P_{0.05}=0.5693$, $P_{0.01}=0.7653$), [b] t_1, t_2: Beginning and ending dates of the period of rapid cellulose accumulation the cotton fiber, respectively, t_{max}: Date of the maximal rate of cellulose accumulation in the cotton fiber. T: Time in days of the rapid cellulose accumulation period in the cotton fiber.

SPS activity began to rise rapidly at 10 DPA and peaked at 25-30 DPA, then declined rapidly. It can be seen from the comparison between the control and treatment that SPS activity in cotton fibers in the treatment group was higher than in the control group during fiber development, especially in the middle and later periods of cotton fiber development. In addition, there was a retrusive tendency in the peak value of SPS activity in the treatment group.

Sucrose Synthase Activity

SuS activity showed a unimodal curve during fiber development; it increased rapidly when the fibers entered into the period of secondary wall thickening (at 15 DPA), and reached a peak at 25-30 DPA before declining rapidly (Figure 2). The difference between the premature senescence control group and the treatment group was mainly observed in the relative timing of the peak of SuS activity and the level of enzymatic activity. In the control, the peak of SuS activity in fibers of the two cotton cultivars occurred at about 25 DPA, after which it decreased rapidly. In the treatment group, the activity peak in fibers of the two cotton cultivars occurred at about 30 DPA, and the activity was significantly higher than in the control group at the same time.

Beta-1,3-glucanase Activity

Beta-1,3-glucanase activity in cotton fibers showed an overall downward trend starting at 15 DPA (Figure 3). The differences between the control group and the treatment group were in β-1,3-glucanase activity, mainly the relative levels of enzyme activity and the timing of peak activity. Under the experimental conditions used in our study, β-1,3-glucanase activity in cotton fibers in the two cotton cultivars showed a downward trend from 10-40 DPA in the controls. However, enzyme activity in the treatment

Table 4. Single boll weight for two cotton cultivars over two years.

Year	Cultivar	Treatment	Single boll weight (g)[a]
2013	Quanyin-2	Treatment	5.36a±0.05
		Control	5.11b±0.04
	Jiza-999	Treatment	5.54a±0.07
		Control	5.21b±0.04
2014	Quanyin-2	Treatment	5.53a±0.07
		Control	5.05b±0.08
	Jiza-999	Treatment	5.88a±0.04
		Control	5.40b±0.02

[a] Values followed by the same lower case letter are not significantly different at the *P*<0.05 level.
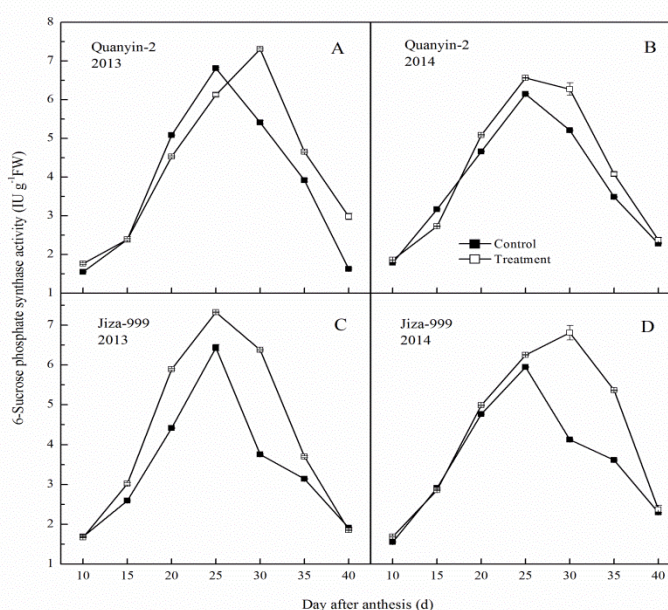
Figure 1. Dynamic changes of sucrose phosphate synthetase activity in fiber of two cotton varieties. Treatment: Plants in which the two basal fruiting branches were removed during early flowering to delay the onset of senescence; Control: Plants in which the basal fruiting branches were not removed. Quanyin-2 and Jiza-999 are two varieties of cotton, and the experiments were conducted in 2013 (A, C) and 2014 (B, D). FW: Fresh Weight. Data are presented as mean±*SD* (n= 3).

group increased and peaked at about 15 DPA (entering into the period of cotton fiber secondary wall thickening), followed by a continuous decline. After 15 DPA, *β*-1,3-glucanase activity in fibers in the treatment group was higher than in the control.
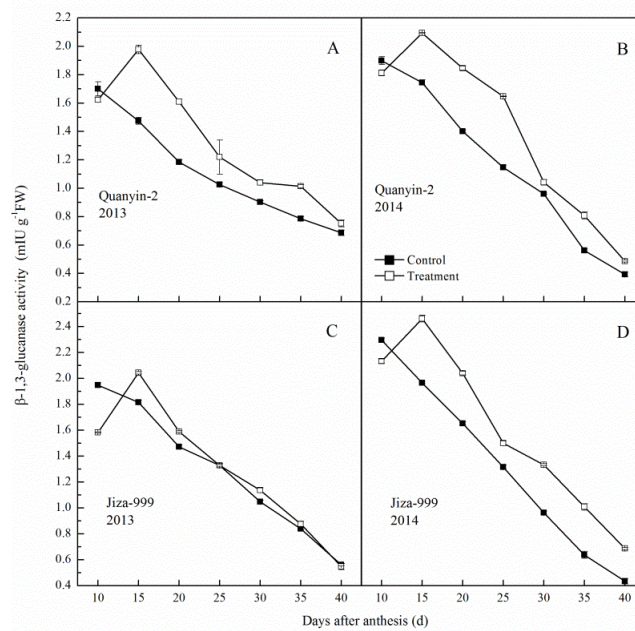
Invertase Activity

Similar to *β*-1, 3-glucanase, invertase activity in cotton fibers showed a continuous downward trend beginning at 10 DPA, and the results for the two test years were consistent (Figure 4). The difference between the control group and the treatment group was mainly observed in the level of enzymatic activity. Invertase activity in cotton fibers of both cultivars in the control group declined steadily from 10 DPA, while fiber invertase activity in the treatment group remained at a relatively high level from 10-15 DPA. Invertase activity then declined dramatically, but it generally
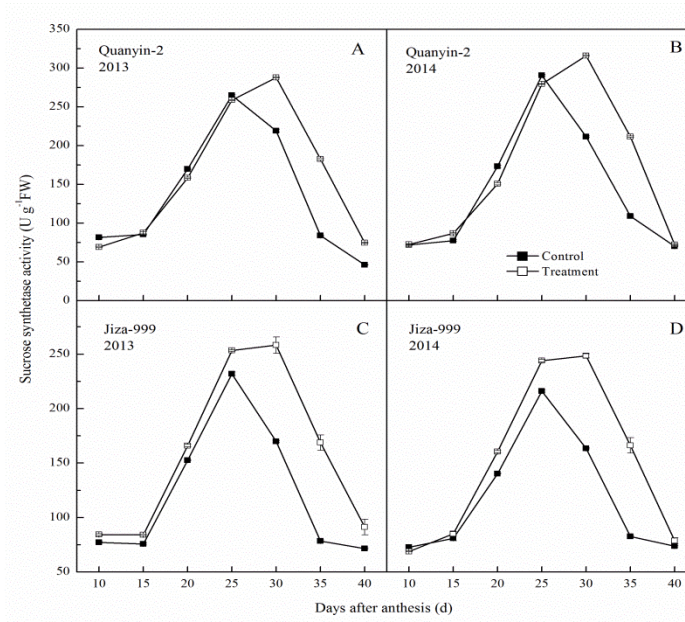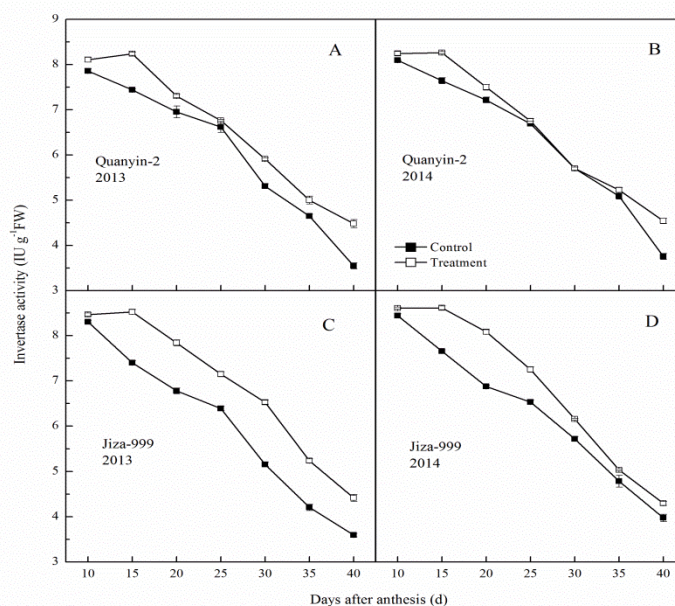
Figure 2. Dynamic changes of sucrose synthetase activity in fiber of two cotton varieties. Treatment and the control, as well as A, B, C, D. and FW are defined under Figure 1. Data are presented as mean±*SD* (n= 3).

Figure 3. Dynamic changes of *β*-1,3-glucanase activity in fiber of two cotton varieties. Treatment and the control, as well as A, B, C, D, and FW are defined under Figure 1. Data are presented as mean±*SD* (n= 3).

564

Figure 4. Dynamic changes of invertase activity in fiber of two cotton varieties.
Treatment and the control, as well as A, B, C, D, and FW are defined under Figure 1. Data are presented as
mean±*SD* (n= 3).

remained higher in the treatment than in the control group at each sampling time.

DISCUSSION

A previous study showed that removing the basal fruiting branches at the early flowering stage can delay the onset of premature senescence (Zhang *et al*., 2013). In this study, we investigated the effects of removing the basal fruiting branches on LAI, SPAD value of the subtending leaf of the cotton boll, growth of the cotton fiber, and boll weight in cotton that undergoes premature senescence.

The LAI is an important metric that reflects the ability of the plant canopy to intercept light and to construct a reasonable canopy structure (Plenet *et al*., 2000). Previous studies have shown that removal of the fruiting branch significantly increased the plant height and LAI (Dong *et al*., 2008). In our research, removing the basal fruiting branches caused a relatively high LAI in the

late stage of growth in the treatment group, which was significantly higher than in the control group, indicating that removing the basal fruiting branches early had a positive effect on maintaining photosynthetic area in the late stage of reproductive growth. The subtending leaf of the cotton boll provides 60-87% of the dry matter source of cotton boll, and its photosynthetic carbon fixation determines the quantity of cotton (Wullschleger and Oosterhuis, 1990). The SPAD was significantly affected by nitrogen nutrition (Cetin *et al*., 2015) and the development of leaf. In our experiment, we found that removing the basal fruiting branches improved the SPAD value of the subtending leaf of the cotton boll in late reproductive growth and reduced leaf senescence to a certain extent, thereby improving dry matter accumulation in the boll.

The main physiological activity in the developmental phase of the cotton fiber is the synthesis and deposition of cellulose, and almost all of the transported

carbohydrates are used for cellulose synthesis, except for those used for respiratory metabolism to provide energy (Haigler *et al.*, 2001). Pettigrew (1999) showed that the carbohydrate content of prematurely senescing cotton leaves and roots increased, leading to a reduction of photosynthetic products in sink organs, and ultimately reducing the yield and quality of the ginned cotton. In this study, the final theoretical values for cotton cellulose and mature boll weight in the treatment group in which the basal fruiting branches had been removed were higher than in the control group. Our results showed that removing the basal fruiting branches could provide enough photosynthate for the synthesis of cellulose and the accumulation of dry matter in the cotton boll. The parameters derived from the equation showed that the control group, which experienced premature senescence, entered the period of rapid cellulose accumulation earlier, and the duration of this period was shorter, than in plants from which the basal fruiting branches had been removed. Thus, removing the basal fruiting branches extended the period of rapid cellulose accumulation, resulting in an increase in cellulose synthesis. This may therefore be one of the reasons that the theoretical value for fiber cellulose production in the treatment group was higher than in the control.

Cotton cellulose biosynthesis is a complex physiological and biochemical process involving a cultivar of changes in materials and enzyme regulation (Tucker *et al.*, 2001; Ruan *et al.*, 2003; Ruan *et al.*, 2004). Sucrose is the initial substrate for cellulose synthesis in cotton, and sucrose metabolizing enzymes play an important regulatory role in this process (Haigler *et al.*, 2001; Delmer and Haigler, 2002). Removing the basal fruiting branches can result in increased levels of SuS and SPS activities in the cotton fiber in bolls that formed in the late period of cotton growth and development. A high level of SPS activity can utilize a large amount of free UDPG to synthesize sucrose, thus providing a supply

of initial synthetic substrates, and increased SuS activity can provide adequate UDPG for cellulose synthesis while ensuring a more complete conversion of sucrose in the fiber. However, during development, the activities of SuS and SPS in the fiber in the control (premature senescence) group was lower than that in the treatment group, and this was similar to previous findings that low temperature, shading, and other adverse conditions could lead to decreased SuS and SPS activities in cotton fibers and the subtending leaves of cotton bolls (Shu *et al.*, 2009; Lv *et al.*, 2013; Liu *et al.*, 2013; Chen *et al.*, 2014).

In addition to SuS and SPS, the activities of invertase and β-1,3-glucanase have important regulatory roles in cellulose synthesis (Ruan *et al.*, 2009; Tucker *et al.*, 2001). Removal of the basal fruiting branches can result in high levels of invertase and *β*-1,3-glucanase activities in fibers from cotton bolls that seeded in the late period of growth in premature senescing cotton. High levels of invertase activity can better provide a carbon source and energy for the rapid accumulation of cellulose, and high *β*-1,3-glucanase activity can be found in secondary wall thickening (Zhang *et al.*, 2009). When cotton plants are subjected to low temperatures, shade, and other adverse conditions, the activity of invertase and *β*-1,3-glucanase in the fiber increases to counter the negative effects on cellulose synthesis (Shu *et al.*, 2009; Lv *et al.*, 2013). During the normal growth in prematurely senescing cotton in the control group in our experiments, the activity of invertase and *β*-1,3-glucanase during fiber development was lower than in the treatment group, indicating that the activity of cotton cellulose synthesis-related enzymes in the bolls was inhibited when cotton plants senesced prematurely. In the treatment group in which the basal fruiting branches had been removed, the activity of cellulose synthesis-related enzymes remained high during cotton fiber development, particularly the activity peaks, and the rate of decline was significantly lower than in the control group

(there was premature senescence during normal growth), indicating that manipulating the source-sink ratio by removing the basal fruiting branches in early flowering can mitigate the effects of premature senescence on cellulose synthesis in cotton. However, in our experiments, we only used cotton bolls from the late period of growth and development of cotton plants, so the effects on fiber development in the early and middle growth periods has yet to be investigated.

In addition, our study also indicated that, compared with plants in which the basal fruiting branches had been removed, the onset of the period of rapid cellulose accumulation and the timing of the peak values of cellulose synthesis-related enzyme activities occurred earlier in the control group. This strongly indicates that in plants that undergo premature senescence, cotton fiber growth is significantly inhibited during the middle and later stages of development, resulting in incomplete development of the cotton fibers, which negatively impacts yield and quality.

CONCLUSIONS

Removing the basal fruiting branches can optimize the synthesis and accumulation of cellulose in cotton bolls during the late stage of growth. By removing two basal fruiting branches, the period of rapid accumulation of fiber cellulose lasted longer, and the activities of cellulose synthesis-related enzymes were higher than in the control group, beginning 10 days post-anthesis. This could be conducive to the steady accumulation of cellulose and reducing the inhibitory effects of premature senescence on fiber growth.

ACKNOWLEDGEMENTS

REFERENCES

1. Amor, Y., Haigler, C. H. and Johnson, S. 1995. A Membrane-Associated Form of Sucrose Synthase and Its Potential Role in Synthesis of Cellulose and Callose in Plant. *Proc. Natl. Acad. Sci. USA*, **92**: 9353-9357.

2. Bowling, A. J., Vaughn, K. C. and Turley R. B. 2011. Polysaccharide and Glycoprotein Distribution in the Epidermis of Cotton Ovules during Early Fiber Initiation and Growth. *Protoplasma*, **248**: 579-90.

3. Brill, E., van Thournout, M., White, R. G., Llewellyn, D., Campbell, P. M., Engelen, S., Ruan, Y. L., Arioli, T. and Furbank, R. T. 2011. A Novel Isoform of Sucrose Synthase Is Targeted to the Cell Wall during Secondary Cell Wall Synthesis in Cotton fiber. *Plant Physiol.*, **157**: 40-54.

4. Bucher, G. L., Tarina, C., Heinlein, M., Serio, F., Jr Meins, F. and Iqlesias, V. A. 2001. Local Expression of β-1,3-glucanase Enhances Symptoms of TMV Infection in Tobacco. *Plant J.*, **28**: 361-369.

5. Cetin, O., Uzen, N. and Temiz M. G. 2015. Effect of N-Fertigation Frequency on the Lint Yield, Chlorophyll, and Photosynthesis Rate of Cotton. *J. Agr. Sci. Tech.* **17**: 909-920.

6. Chen, J., Lv, F. J., Liu, J. R., Ma, Y. N., Wang, Y. H., Chen, B. L., Meng, Y. L. and Zhou, Z. G. 2014. Effects of Different Planting Dates and Low Light on Cotton Fiber Length Formation. *Acta Physiol. Plant.*, **36**: 2581-2595.

7. Constable, G. and Rawson, H. 1980. Carbon Production and Utilization in Cotton: Inferences from a Carbon Budget. *Funct. Plant Biol.*, **5**: 539-553.

8. Delmer, D. P. and Haigler, C. H. 2002. The Regulation of Metabolic Flux to Cellulose, a Major Sink for Carbon in Plants. *Metab. Eng.*, **4(1)** :22-28.

9. Doblin, M. S., Kurek, I., Jacok-Wilk, D. and Delmer, D. P. 2002. Cellulose Biosynthesis in Plants: from Genes to Rosettes. *Plant Cell Physiol.*, **43**:1407-1420.

10. Dong, H. Z., Niu, Y. H., Kong, X. Q. and Luo, Z. 2009. Effects of Early-Fruit Removal on Endogenous Cytokinins and Abscisic Acid in Relation to Leaf Senescence in Cotton. *Plant Growth Regul.*, **59**: 93-101.

11. Dong, H. Z., Tang, W., Li, W. J., Li, Z. H., Niu, Y. H. and Zhang, D. M. 2008. Yield, Leaf Senescence, and *Cry1Ac* Expression in Response to Removal of Early Fruiting Branches in Transgenic Bt Cotton. *Agr. Sci. China*, **7**: 692-702.

12. Endler, A. and Persson, S. 2011. Cellulose Synthases and Synthesis in *Arabidopsis*. *Mol. Plant*, **42**: 199-211.

13. Gormus, O., Kurt, F. and Sabagh, A. E. 2017. Impact of Defoliation Timings and Leaf Pubescence on Yield and Fiber Quality of Cotton. *J. Agr. Sci. Tech.*, **19**: 903-915.

14. Haigler, C. H., Ivanova-Datcheva, M., Hogan, P. S., Salnikov, V. V., Hwang, S., Martin, K. and Delmer, D. P. 2001. Carbon Partitioning to Cellulose Synthesis. *Plant Mol. Biol.*, **47**: 29-51.

15. Li, B., Wang, Y., Zhang, Z. Y., Wang, B. M., Eneji, A. E., Duan, L. S., Li, Z. H. and Tian, X. L. 2012. Cotton Shoot Plays a Major Role in Mediating Senescence Induced by Potassium Deficiency. *J. Plant Physiol.*, **169**: 327-335.

16. Liu, J. R., Ma, Y. N., Lv, F. J., Chen, J., Zhou, Z. G., Wang, Y. H., Abudurezike, A. K. and Oosterhuis, D. M. 2013. Changes of Sucrose Metabolism in Leaf Subtending to Cotton Boll under Cool Temperature Due to Late Planting. *Field Crop. Res.*, **144**: 200-211.

17. Liu, Y. D., Yin, Z. J., Yu, J. W., Li, J., Wei, H. L., Han, X. L. and Shen, F. F. 2012. Improved Salt Tolerance and Delayed Leaf Senescence in Transgenic Cotton Expressing the *Agrobacterium IPT* Gene. *Biol. Plantarum*, **56**: 237-246.

18. Lv, F. J., Liu, J. R., Ma, Y. N., Chen, J., Abudurezikeke, A. K., Wang, Y. H., Chen, B. L., Meng, Y. L. and Zhou, Z. G. 2013. Effect of Shading on Cotton Yield and Quality on Different Fruiting Branches. *Crop Sci.*, **53**: 2670-2678.

19. Michelle, B. V. and Haigler, C. H. 2001. Sucrose-Phosphate Synthase Activity Rises in Correlation with High-Rate Cellulose Synthesis in Three Heterotrophic Systems. *Plant Physiol.*, **127**: 1234-1242.

20. Pettigrew, W. T. 1999. Potassium Deficiency Increase Specific Leaf Weights and Leaf Glucose Levels in Field-Grown Cotton. *Agron. J.*, **91**: 962-968.

21. Plenet, D., Mollier, A. and Pellerin, S. 2000. Growth Analysis of Maize Field Crops under Phosphorus Deficiency. II. Radiation-Use Efficiency, Biomass Accumulation and Yield Components. *Plant Soil.*, **2**: 259-272.

22. Ruan, Y. L., Llewellyn, D. J. and Furbank, R. T. 2003. Suppression of Sucrose Synthase Gene Expression Represses Cotton Fiber Cell Initiation, Elongation, and Seed Development. *Plant Cell*, **15**: 952-964.

23. Ruan, Y. L., Xu, S. M., White, R. and Furbank, R. T. 2004. Genotypic and Developmental Evidence for the Role of Plasmodesmatal Regulation in Cotton Fiber Elongation Mediated by Callose Turnover. *Plant Physiol.*, **136**: 4104-4113.

24. Ruan, Y. L., Jin, Y. and Huang, J. 2009. Capping Invertase Activity by Its Inhibitor: Roles and Implications in Sugar Signaling, Carbon Allocation, Senescence and Evolution. *Plant Signal. Behav.*, **4**: 983-985.

25. Saxena, I. M. and Brown, R. M. Jr. 2005. Cellulose Biosynthesis: Current Views and Evolving Concepts. *Ann. Bot.*, **96**: 9-21.

26. Shimizu, Y., Aotsuka, S., Hasegawa, O., Sakai, F. and Hayashi, T. 1997. Changes in Levels of mRNAs for Cell Wall-Related Enzymes in Growing Cotton Fiber Cells. *Plant Cell Physiol.*, **38**: 375.

27. Shu, H. M., Zhou, Z. G., Xu, N. Y., Wang, Y. H. and Zheng, M. 2009. Sucrose Metabolism in Cotton (*Gossypium hirsutum* L.) Fibre under Low Temperature during Fibre Development. *Eur. J. Agron.*, **31**: 61-68.

28. Stewart, J. McD., Oosterhuis, D., Heitholt, J. J. and Mauney, J. 2010. Physiology of Cotton. Springer Ebooks, p: 80-96.

29. Tucker, M. R., Paech, N. A., Willemse, M. T. and Koltunow, A. M. 2001. Dynamics of Callose Deposition and *β*-1,3-Glucanase Expression during Reproductive Events in Sexual and Apomictic *Hieracium*. *Planta*, **212**: 487-498.

30. Updegraff, D.M. 1969. Semimicro Determination of Cellulose in Biological Materials. *Anal. Biochem.*, 32: 420-424.

568

31. Wang, Q. H., Zhang, X., Li, F. G., Hou, Y. X., Liu, X. L. and Zhang, X. Y. 2011. Identification of a UDP-Glucose Pyrophosphorylase from Cotton (*Gossipium hirsutum L.*) Involved in Cellulose Biosynthesis in *Arabidopsis thaliana*. *Plant Cell Rep.*, **30**: 1303-1312.

32. Wright, P. R. 1999. Premature Senescence of Cotton (*Gossypium hirsutum* L.): Predominantly a Potassium Disorder Caused by an Imbalance of Source and Sink. *Plant Soil*, **211**: 231-239.

33. Wullschleger, S. D. and Oosterhuis, D. M. 1990. Photosynthetic Carbon Production and Use by Developing Cotton Leaves and Bolls. *Crop Sci.*, **30**: 1259-1264.

34. Zhang, W. J., Shu, H. M., Hu, H. B., Chen, B. L., Wang, Y. H. and Zhou, Z. G. 2009. Genotypic Differences in Some Physiological Characteristics during Cotton Fiber Thickening and Its Influence on Fiber Strength. *Acta Physiol. Plant.*, **3**: 927-935.

35. Zhang, W. J., Zhou, T. H., Huang, N. N., Chen, X. H., Yi, B. L. and He, J. Y. 2013. Effect of Removal of Basal Fruiting Branches on Some Premature Senescence Characters of Different Cotton Genotypes. *Acta Bot. Boreali-Occidentalia Sin.*, **33**: 1435-1441. (in Chinese, with English Abstract).

اثر بریدن شاخه های زایشی پایین بوته پنبه روی انباشت سلولز و فعالیت آنزیم های مرتبط با ساخت سلولز در پنبه

و. ج. ژانگ، ز. ل. هوانگ، ت. ه. ژو، و ل. می

چکیده

با استفاده از کولتیوارهایی از پنبه که دچار زوال زودرس (premature senescence) می شود، اثرات بریدن دو شاخه زایشی از دو کولتیوار پنبه به نام Quanyin-2 و Jiza-999 روی شاخص سطح برگ، و میزان SPAD برگ متناظر غوزه پنبه و تشکیل الیاف پنبه در مراحل آخر رشد بوته بررسی شد. تاکید این بررسی روی تغییرات انباشت سلولز و فعالیت آنزیم مرتبط با الیاف پنبه بعد از بریدن شاخه های زایشی پایین بوته و تشکیل الیاف پنبه در طی زوال زودرس بود. نتایج نشان داد که بریدن شاخه های زایشی پایین بوته می تواند به حفظ سطح برگ سبز و میزان SPAD برگ های متناظر غوزه در طی مراحل آخر رشد زایشی منجر شود. همچنین، دوره انباشت سریع الیاف سلولز در گیاهانی که شاخه زایشی آنها بریده شده بود طولانی تر بود و زمان شروع و اتمام دوره انباشت سریع در آنها از گیاهان شاهدی که دچار زوال زودرس بودند دیرتر بود. فعالیت های آنزیم های مرتبط با ساخت سلولز(سانتاز فسفات ساکارز، سانتاز ساکارز،-β-1,3 glucanaseو invertase) در گیاهانی که شاخه زایشی آنها بریده شده بود بیشتر از گیاهان شاهد ده روز بعد از گلدهی بود. بریدن شاخه های زایشی پایین بوته می تواند منجر به بهینه شدن انباشت سلولز در غوزه پنبه در طی مراحل آخر رشد و کاهش اثر زوال زودرس روی ساخت سلولز بود. این نتیجه به دست آمد که اوج فعالیت های آنزیمی مرتبط با تشکیل الیاف پنبه در گیاهان شاهد زودهنگام تر بود و در نتیجه تشکیل الیاف پنبه را جلو انداخت و اثری منفی روی عملکرد الیاف گذاشت.