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ORIGINAL PAPER

Stem girdling influences concentrations of endogenous cytokinins and abscisic acid in relation to leaf senescence in cotton

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Abstract Many studies have shown that root–shoot imbalance influences vegetative growth and development of cotton (*Gossypium hirsutum* L.), but few have examined changes in leaf senescence and endogenous hormones due to stem girdling. The objective of this study was to determine the correlation between some endogenous phytohormones, particularly cytokinins and abscisic acid (ABA), and leaf senescence following stem girdling. Field-grown cotton plants were girdled on the main stem 5 days after squaring (DAS), while the non-girdled plants served as control. Plant biomass, seed cotton yield, main-stem leaf photosynthetic (Pn) rate, chlorophyll (Chl) and malondialdehyde (MDA) concentrations, as well as levels of cytokinins and ABA in main-stem leaves and xylem sap were determined after girdling or at harvest. Main-stem girdling decreased the dry root weight and root/shoot ratio from 5 to 70 days after girdling (DAG) and reduced seed cotton yield at harvest. Main-stem leaf Pn and Chl concentration in girdled plants were significantly lower than in control plants. Much higher levels of MDA were observed in main-stem leaves from 5 to 70 DAG, suggesting that stem girdling accelerated leaf senescence. Girdled plants contained less *trans*-zeatin and its riboside (t-Z + t-ZR), dihydrozeatin and its riboside (DHZ + DHZR), and isopentenyladenine and its riboside (iP + iPA), but more ABA than control plants in both main-stem leaves and xylem sap. These results suggested that main-stem girdling accelerated leaf senescence due to reduced levels of cytokinin and/or increased ABA. Cytokinin and ABA are involved in leaf senescence following main-stem girdling.

Keywords Abscisic acid · Cotton · Cytokinins · Stem girdling · Leaf senescence

Abbreviations

ABA	Abscisic acid
Chl	Chlorophyll
Pn	Photosynthetic rate
DHZ + DHZR	Dihydrozeatin and its riboside
iP + iPA	Isopentenyladenine and its riboside
t-Z + t-ZR	*Trans*-zeatin and its riboside
MDA	Malondialdehyde
DAG	Days after girdling
JA	Jasmonic acid
MeJA	Methyl jasmonate
DAS	Days after squaring
PBS	Phosphate-buffered saline
PGRs	Plant growth regulators

Introduction

Stem or shoot girdling obstructs the downward phloem transport, resulting in the accumulation of photosynthates in the canopy and improving the availability of metabolites for developing organs above the cutting (Wallerstein et al. 1978). Therefore, girdling has been widely used to improve flowering, fruit set and fruit size in citrus, grape, peach and other fruit tree crops (Trueman and Turnbull 1994; Agusti et al. 1998; Gusti et al. 2005). Since girdling also disrupted

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root respiration (Johnsen et al. 2007), and increased expression of certain genes, especially those involved in carbohydrate metabolism (Pourtau et al. 2006; Li et al. 2003), it has been used experimentally to test the relationship between foliar parts and root systems in many plant species. Some precise physiological, biochemical and molecular changes resulting from girdling have been reported in tree crops (Berüter and Feusi 1997; Goren et al. 2003; Li et al. 2003). It has been shown that fruit from scored trees at the onset of cell enlargement stage initiate ethylene production before fruit from unscored trees (Juan et al. 2009). Moreover, girdling increases the levels of leaf carbohydrates such as fructose, glucose, sucrose, fructan and starch in spinach, barley, citrus and sugar maple (Krapp and Stitt 1995; Parrott et al. 2007; Rivas et al. 2008; Murakami et al. 2008), and high carbohydrate contents are associated with the onset of the senescence process (Pourtau et al. 2004, 2006; Wingler et al. 2006; Parrott et al. 2005, 2007). However, the effect of stem girdling on leaf senescence and the underlying mechanisms in field crops, especially in cotton (*Gossypium hirsutum* L.), is poorly understood.

Leaf senescence is a form of programmed cell death and is characterized by decline in both chlorophyll and photosynthesis and by an increase in MDA content (Kakani et al. 2004). Degradation of chloroplast components is one of the earliest and most visible symptoms of leaf senescence, which starts at the leaf margins and progresses to the interior of the leaf (Thomas and Stoddart 1980). The onset and progression of leaf senescence were accompanied by a number of physiological and molecular events such as chloroplast disintegration, decline in photosynthesis and breakdown of protein and nucleic acids (Buchanan-Wollaston 1997; Buchanan-Wollaston et al. 2003; Chandlee 2001). The loss of assimilatory capacity following leaf senescence contributed to low fiber yield, and delayed leaf senescence might increase cotton productivity (Wright 1999).

Leaf senescence is considered the result of complex interaction between external factors such as drought, high salinity and low temperatures (Marani et al. 1985; Lim and Nam 2007) and internal factors such as plant hormones (Yong et al. 2000; Dong et al. 2008). Cytokinin and ABA are commonly identified phytohormones during research on leaf senescence. Cytokinins are a structurally diverse group of N^6-substituted purine derivatives capable of inducing plant cell division. *Trans*-zeatin riboside (t-ZR), dihydrozeatin riboside (DHZR) and isopentenyladenine (iPA) are the most commonly detected physiologically active cytokinins in plants (Mok et al. 2000). Plants overexpressing the isopentenyl transferase (IPT) gene showed massive cytokinin overproduction and delayed leaf senescence (Smart et al. 1991). In contrast, abscisic acid (ABA) is considered a senescence enhancer. Water-deficit stress accelerated leaf senescence through more accumulation of ABA in plant leaves (Pic et al. 2002). Although previous studies have reported possible correlation of leaf senescence with cytokinins and ABA in cotton (Dong et al. 2008, 2009), their contribution to leaf senescence and plant integrity after stem girdling has not been studied.

In the present study, stem girdling at the early squaring stage was used to determine the physiological parameters of cotton plants and the correlation between foliar and root development, particularly the correlation of leaf senescence to phytohormones in cotton.

Materials and methods

Field experiments were conducted in 2007 and 2008 at the Cotton Experimental Station of Shandong Cotton Research Center (SCRC), Linqing (36°61′N, 115°42′E), Shandong, China. SCRC21, a *Bacillus thuringiensis* Berliner (Bt) transgenic cotton (*Gossypium hirsutum* L.) cultivar, developed by SCRC, Shandong Academy of Agricultural Sciences, was used in both years.

Experiment design and field management

SCRC 21 was sown on 22 April 2007 and on 26 April 2008. After complete emergence each year, seedlings were thinned to the targeted plant density (4 plants m^{-2}) by retaining one vigorous plant per hill. There were two treatments, girdling and non-girdling each year. Girdling treatments were conducted 5 days after squaring (DAS) for both years (21 June 2007 and 24 June 2008). Girdling was done by carefully removing the outer bark and phloem tissues from the main stem (above the cotyledon node) with a standard razor blade to form two separate semicircular wounds on each plant. These two wounds were on opposite sides with 1.5 cm vertical distance on the main stem. The width of each wound was about 2 mm. After being sterilized with 70% ethanol, the wounds were tightly wrapped around with parafilm to protect the girdled area from pathogen invasion. Non-girdled plants served as the corresponding controls.

The experiment was arranged into a randomized complete block design with three replications. Each plot (32 m^2) contained four rows, with row length of 10 m and in-row spacing of 80 cm. Vegetative branches and growth terminals on the main stems in each plot were removed manually at peak squaring and peak boll-setting stages following local management requirements. Each year before sowing, the cotton field was fertilized with 15 tons of chicken manure and 600 kg of commercial compound fertilizer containing (by weight) 20% N, 13% P_2O_5 and

33% K_2O per hectare. Side dressing with 90 kg ha^{-1} N was done 90 days after sowing. Water stress was minimized with timely irrigation. Other management practices were conducted according to local agronomic recommendations unless otherwise indicated.

Data collection

For both years, the weight of the above-ground (leaves, stem, fruiting forms and branches) and below-ground portions (root system) were determined at 5, 20, 50 and 70 days after girdling (DAG). Roots were collected with a sieve and weighed after drying at 80°C for 48 h to a constant weight. Seed cotton was manually harvested and weighed after air drying for 5 days. The plant biomass and root/shoot ratio (*w*/*w*) were also recorded.

During the growth season, the fourth true leaf on the main stem from the top was used for physiological analysis. Leaf chlorophyll (Chl) contents were determined as described in He et al. (2002). Briefly, 0.20 g of fresh leaves was placed in a 100-ml test tube. The tissues were homogenized with polytron after adding 10–15 ml pure methanol. The homogenate was then filtered and made up to 100 ml with pure methanol. The Chl concentration in the supernatant was spectrophotometrically determined by measuring the absorbances at 652 and 665 nm for Chla and Chlb, respectively. Leaf photosynthetic (Pn) rate was measured using LI-6400 portable photosynthesis system (Li-Cor, Lincoln, NE, USA). The measurements were taken between 09:00 and 11:00 h on cloudless days when ambient photosynthetic photon flux density exceeded 1,500 μmol m^{-2} s^{-1}. Three plants per replicate were examined and the mean values were calculated.

Leaf malondialdehyde (MDA) content was estimated following the procedure of Heath and Packer (1968). Samples of 200 mg were ground in 5 ml distilled water. In the same solution, 5 ml TBA–TCA reagent (0.5% thiobarbituric acid and 20% TCA dissolved in 100 ml of distilled water) was added. The slurry was kept in water bath at 95°C for 30 min. Cooled solution was obtained by placing in ice bath and centrifuged at 10,000*g* for 10 min. Optical density of supernatant was recorded at 600 and 535 nm. The difference in optical density gave the actual intensity of color developed by MDA.

Fresh samples of main-stem leaves and the xylem sap were used for phytohormone quantification. The extraction and purification of t-Z + t-ZR, DHZ + DHZR, iP + iPA and ABA in leaves were conducted according to Fernández et al. (1995). Tissues (0.5 g fresh leaves) were extracted by mixing through repeated inversion with 25 ml of 80% (v/v) cold methanol containing 10 mg l^{-1} butylated hydroxytoluene during 14 h at 4°C in the darkness. The resulting extract was filtered through GF/A glass-fiber filters (Whatman Sci. Ltd.) and the solid residue was re-extracted with 15 ml of the same solvent (7 h). The combined methanolic extracts were evaporated under reduced pressure at 35°C and the resulting aqueous solutions were made to a final volume of 5 ml with distilled water. Samples were cleared by centrifugation (10,000×*g* for 25 min at 4°C) and the supernatants adjusted to pH 3 with acetic acid. The extracts were percolated through equilibrated Sep-Pak C18 cartridges (Waters, Milford, USA). After loading, the cartridges were washed twice with 5 mL of distilled water and the PGRs retained were eluted by passing 5 ml of 80% methanol (v/v) twice. Methanolic fractions were reduced to dryness, redissolved in 2 ml of 10 mM phosphate-buffered saline (PBS) (pH 7.4) (0.01 M Na_2HPO_4 and 0.15 M NaCl) and applied to an immunoaffinity column against zeatin-type (ZR and DHZR) and isopentenyl-type (iPA) cytokinins. The column was washed with 30 ml of PBS, and then with 30 ml of distilled water that was discarded. The retained cytokinins were finally washed from the column with 30 ml pure methanol. The separation of the different cytokinins was accomplished following the method reported by Horgan and Scott (1987).

The DHZR, t-ZR (raised against *trans*-Zs potential cross reaction with cis-Zs), and iPA were determined by ELISA using monoclonal antibodies (Phytodetek, Agdia, Elkhart, IN, USA) following the protocol provided by the manufacturer. The absorbance was recorded at 405 nm. Calculation of the ELISA data was performed as described in Weiler (1982). As antibodies of cytokinins also recognize free bases, nucleotides and 9*N*-glucosides, what was measured might be the sum of free bases, ribosides, nucleotides and 9*N*-glucosides of the corresponding cytokinins. For simplicity, the cytokinins would be identified as t-Z + t-ZR, DHZ + DHZR and iP + iPA, respectively.

Concentration of ABA was also determined by the ELISA method as described in Gawronska et al. (2003), using anti-IgG polyclonal antibody, anti-ABA monoclonal antibody and ABA-conjugated alkaline phosphatase as tracer, all of which were provided by the China Agricultural University, Beijing. The anti-ABA antibody shows complete reactivity with (±) ABA and very low cross-reactivity with compounds structurally similar to ABA. To check if the samples contained any immunoreactive compounds, several dilutions of the standard curve were spiked with three dilutions of the samples with the highest and lowest concentrations of ABA. The spiked standard curves were parallel to the original one in its linear range (data not shown). The alkaline phosphatase activity was visualized with *p*-nitrophenylphosphate as a substrate in 1 M diethanolamine and 0.5 mM $MgCl_2$ (pH 9.8). Absorbance of the resulting color was measured at 405 nm and the ABA concentration was calculated.

Xylem sap was collected between 19:00 and 07:00 hours from three plants per treatment according to

Noodén et al. (1990). The plants were cut off about 1 cm below the position of cotyledons and the cut surface was wiped with methanol/formic acid/H_2O (14/1/2, by volume) to inhibit phosphatase action and thereby prevent the loss of nucleotides. The cut stem was connected to low-volume plastic tubing, which extended into a glass tube with 0.1 ml concentrated formic acid in an ice bath. The volumes of the sap samples were then determined, and samples were frozen, freeze dried and stored in the dark at −40°C. Samples were analyzed for t-Z + t-ZR, DHZ + DHZR, iP + iPA and ABA as described above.

Statistical analysis

Data were statistically analyzed with DPS Data Processing System (Tang and Feng 1997). The initial combined data for yield and physiological parameters showed interactions with year. Thus, the data are presented separately for each year (Steel and Torrie 1980). Means were separated using a *t* test.

Results

Root/shoot ratio and cotton yield

In 2007, the dry root weight and root/shoot ratio of girdled plants were significantly lower than those of non-girdled control (Table 1). Compared with the control, stem girdling decreased the dry root weight by 32.1% at 20 DAG, 38.4% at 50 DAG and 42.6% at 70 DAG. It also decreased the root/shoot ratio by 25.8% at 5 DAG, 19.2% at 20 DAG, 34.1% at 50 DAG and 28.9% at 70 DAG. Similar results were also obtained in 2008; stem girdling significantly reduced dry root weight and root/shoot ratio from 5 to 70 DAG. It significantly reduced seed cotton yield by 18.7% in 2007 and 20.9% in 2008 (Table 1).

Photosynthetic rate, chlorophyll content and MDA content

For both years, stem girdling significantly inhibited leaf photosynthesis from 5 to 70 DAG (Fig. 1). In 2007, girdling progressively reduced the net Pn by 10.5% at 5 DAG, 25.9% at 20 DAG, 27.7% at 50 DAG and 29.7% at 70 DAG; in 2008, the reductions were 6.7, 21.1, 25.9 and 27.5%, respectively. Stem girdling decreased the Chl (Chla + Chlb) content, but increased accumulation of MDA in the main-stem leaves in both years (Table 2). Averaged across the 2 years, it reduced Chl by 4.3% at 5 DAG, 4.8% at 20 DAG and 9.7% at 50 DAG, and increased MDA by 14.6, 70.2 and 24.7%, respectively.

Table 1 Dry weight of root, root/shoot ratio and seed cotton yield after girdling

Treatments	Dry root weight (g plant^{-1})				Root/shoot				Seed cotton yield (kg ha^{-1})
	5 DAG	20 DAG	50 DAG	70 DAG	5 DAG	20 DAG	50 DAG	70 DAG	
2007									
Control	1.65 ± 0.05a	7.23 ± 0.19a	7.76 ± 0.07a	8.52 ± 0.19a	0.155 ± 0.007a	0.125 ± 0.005a	0.085 ± 0.014a	0.069 ± 0.014a	4,421 ± 32a
Girdling	1.57 ± 0.07a	4.91 ± 0.31b	4.78 ± 0.14b	4.89 ± 0.11b	0.115 ± 0.008b	0.101 ± 0.007b	0.056 ± 0.007b	0.049 ± 0.011b	3,596 ± 36b
2008									
Control	1.93 ± 0.08a	6.71 ± 0.19a	8.90 ± 0.8a	10.2 ± 1.2a	0.142 ± 0.010a	0.117 ± 0.012a	0.093 ± 0.006a	0.075 ± 0.007a	4,428 ± 49a
Girdling	1.71 ± 0.05b	5.20 ± 0.17b	5.99 ± 0.11b	6.22 ± 0.19b	0.103 ± 0.011b	0.095 ± 0.003b	0.065 ± 0.008b	0.042 ± 0.009b	3,504 ± 26b

The shoot includes main stem, branches, fruiting forms and leaves. For each year, values represent mean ± SD of three replications with three plants per replication. Different letters within a column for each year indicate significant differences at $p = 0.05$

DAG days after stem girdling

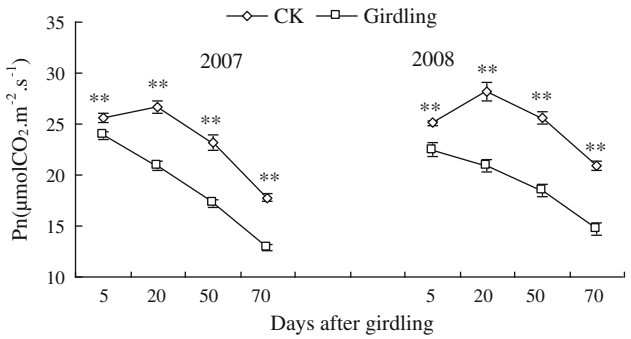

Fig. 1 Changes in photosynthetic rate (*Pn*) in the main-stem leaf at different days after girdling (*DAG*) in 2007 and 2008. Cotton was sown on 22 April 2007 and 26 April 2008; girdling was done on 21 June 2007 and 24 June 2008 (5 days after squaring). The Pn of the main-stem leaf was measured 22 and 51 DAG instead of 20 and 50 DAG due to cloudy weather in 2007. In 2008, the Pn was measured 23 and 69 DAG instead of 20 and 70 DAG due to cloudy weather. Values are mean ± SD of three replications per experiment, and each replication includes three plants. *,**Significant differences from control at $p = 0.05$ and $p = 0.01$, respectively

Cytokinin and ABA levels in xylem sap

Plants attained peak levels of t-Z + t-ZR, DHZ + DHZR and iP + iPA in the xylem sap at 20 DAG, while their ABA levels steadily increased from 5 to 70 DAG, regardless of treatment. Girdling significantly decreased the levels of t-Z + t-ZR, DHZ + DHZR and iP + iPA in xylem sap starting at 5 DAG (Table 3). In contrast to cytokinin, girdling significantly increased the ABA concentration (Table 4). Averaged across 2 years, decreases of 40.6, 57.3 and 67.2% in t-Z + t-ZR level, 50, 66 and 87.8% in DHZ + DHZR level, and 47.1, 38.6 and 38.5% in iP + iPA level were observed at 5, 20 and 70 DAG for girdled plants. Girdling also increased the ABA concentration in xylem sap by 12.2, 37.9 and 31.2%, respectively.

Cytokinin and ABA levels in main-stem leaves

As expected, stem girdling also affected levels of cytokinin and ABA in the main-stem leaves. Levels of t-Z + t-ZR,

Table 2 Effects of girdling on leaf chlorophyll (Chl) and malondialdehyde (MDA) content at 5, 20 and 50 days after girdling (DAG)

Treatments	Chl (mg g^{-1} FW)			MDA (μmol g^{-1} FW)		
	5 DAG	20 DAG	50 DAG	5 DAG	20 DAG	50 DAG
2007						
Control	2.16 ± 0.05a	2.23 ± 0.03a	1.51 ± 0.04a	4.64 ± 0.06b	6.00 ± 0.34b	16.95 ± 0.18b
Girdling	2.02 ± 0.07b	2.12 ± 0.04b	1.36 ± 0.05b	5.02 ± 0.12a	10.43 ± 0.22a	20.72 ± 0.18a
2008						
Control	2.04 ± 0.10a	2.36 ± 0.04a	2.09 ± 0.05a	4.76 ± 0.09b	4.59 ± 0.08b	14.46 ± 0.70b
Girdling	2.00 ± 0.04a	2.25 ± 0.04b	1.89 ± 0.07b	5.75 ± 0.12a	7.59 ± 0.07a	18.44 ± 0.16a

For each year, values represent mean ± SD of three replications with three plants per replication. Different letters within a column for each year indicate significant differences at $p = 0.05$

Table 3 Effects of girdling on cytokinin (t-Z + t-ZR, DHZ + DHZR and iP + iPA) and ABA levels in xylem sap at 5, 20 and 70 days after girdling (DAG)

Treatments	t-Z + t-ZR (ng g^{-1})			DHZ + DHZR (ng g^{-1})			iP + iPA (ng g^{-1})		
	5 DAG	20 DAG	70 DAG	5 DAG	20 DAG	70 DAG	5 DAG	20 DAG	70 DAG
2007									
Control	60 ± 3.1a	119 ± 7.3a	78 ± 5.4a	148 ± 5.2a	1,181 ± 45a	928 ± 18.6a	73.7 ± 2.4a	90 ± 3.1a	39 ± 2.3a
Girdling	45 ± 4.0b	54 ± 3.6b	29 ± 2.8b	77 ± 5.1b	390 ± 18b	123 ± 5.7b	40 ± 1.3b	53 ± 1.4b	25 ± 1.2b
2008									
Control	65 ± 5.3a	139 ± 6.5a	99 ± 6.4a	168 ± 8.2a	1,254 ± 52.1a	1,151 ± 16.4a	81 ± 1.6a	112 ± 2.2a	44 ± 2.6a
Girdling	47 ± 3.4b	56 ± 4.3b	29 ± 3.0b	81 ± 3.9b	438 ± 18.0b	131 ± 12.1b	42 ± 1.7b	71 ± 1.1b	26 ± 2.7b

t-Z + t-ZR, DHZ + DHZR and iP + iPA represent trans-zeatin and its riboside, dihydrozeatin and its riboside and isopentenyladenine and its riboside, respectively. For each year, values represent mean ± SD of three replications with three plants per replication. Different letters within a column each year indicate significant differences at $p = 0.05$

Table 4 Effects of girdling on abscisic acid (ABA) levels in xylem sap at 5, 20 and 70 days after girdling (DAG)

Treatments	ABA (ng g^{-1})		
	5 DAG	20 DAG	70 DAG
2007			
Control	221 ± 5.2b	617 ± 6.3b	1,136 ± 8.4b
Girdling	259 ± 7.3a	817 ± 7.1a	1,497 ± 11.5a
2008			
Control	205 ± 6.2b	528 ± 3.1b	995 ± 7.3b
Girdling	219 ± 4.2a	762 ± 9.1a	1,300 ± 13.6a

For each year, values represent mean ± SD of three replications with three plants per replication. Different letters within a column for each year indicate significant differences at $p = 0.05$

DHZ + DHZR and iP + iPA in the main-stem leaves were markedly reduced, but those of ABA were raised from 5 to 70 DAG (Fig. 2). Girdling decreased concentrations of total cytokinins (t-Z + t-ZR, DHZ + DHZR and iP + iPA) in 2007 by 48.3% at 5 DAG, 35.2% at 20 DAG, 78.4% at 50 DAG and 84.8% at 70 DAG; in 2008, the decreases were 48.1, 74.3, 60.3 and 83.9%, respectively (Fig. 2a, b, c). In contrast, stem girdling increased ABA levels in 2007 by 53.6% at 5 DAG, 21.0% at 20 DAG, 66.5% at 50 DAG and 32.5% at 70 DAG; in 2008, the increases were 32.9, 36.8, 59.2 and 31.2%, respectively (Fig. 2d).

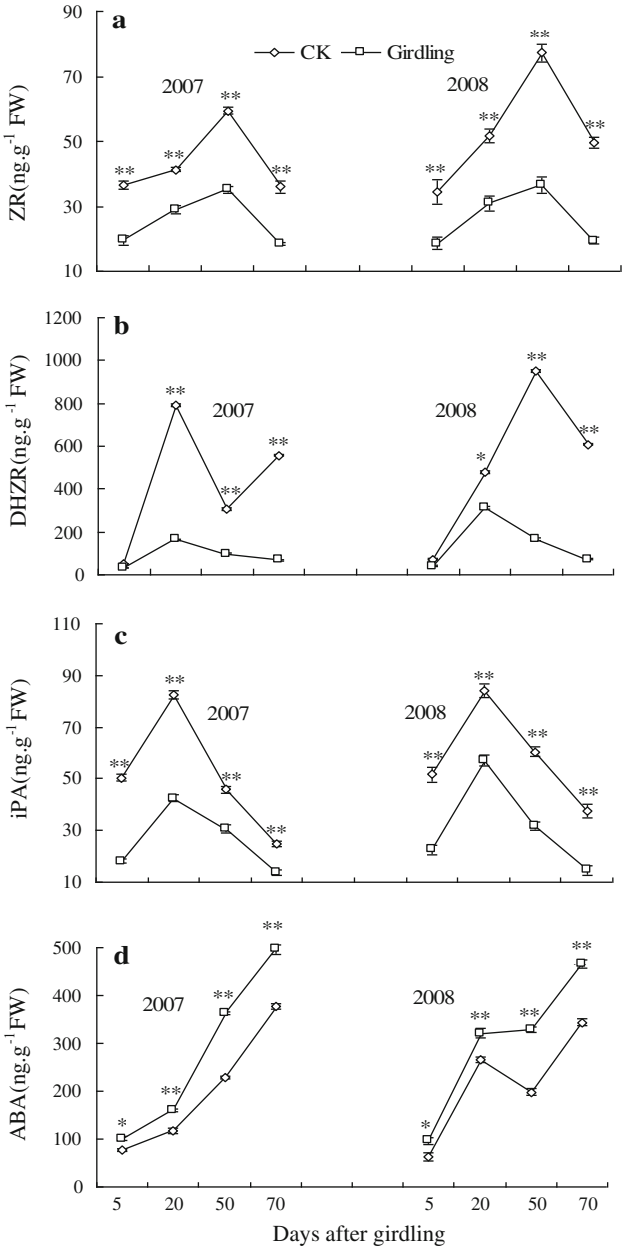

Fig. 2 Changes in zeatin and its riboside (t-Z + t-ZR), dihydrozeatin and its riboside (DHZ + DHZR), isopentenyl and its adenine (iP + iPA), and abscisic acid (ABA) in cotton main-stem leaves in 2007 and 2008. Cotton was planted on 22 April 2007 and 26 April 2008 and girdled on 21 June 2007 and 24 June 2008 (5 days after squaring). Values are mean ± SD of three replications per experiment, and each replication includes three plants. *,**Significant differences from control at $p = 0.05$ and $p = 0.01$, respectively

Discussion

Girdling refers to the destruction of phloem, such as the removal of a strip of bark from the stem or major limbs and heat treatment in leaf petioles. It blocks the translocation of photosynthates and metabolites in phloem (Cohen 1981; Li et al. 2003). In the present study, we not only found significant effects of girdling on root growth and development, root/shoot ratio and economic yield, but also marked effects on leaf senescence and some phytohormones.

Leaf senescence is a degenerative process that involves orderly and sequential change in physiology and biochemistry (Thomas 1992). Senescence too early or too late would reduce the yield and quality (Wright 1999; Wingler et al. 2006). Too early senescence of a whole plant in cotton is referred to as premature senescence, which has been occurring on an increasing scale since modern transgenic *Bacillus thuringiensis* Berliner cotton cultivars were introduced for commercial production (Dong et al. 2006). The loss in chlorophyll and decline in photosynthesis are early symptoms of leaf senescence (Smart 1994; Bleecker and Patterson 1997). In the absence of other acceptors in mitochondria and chloroplasts, electrons from the electron transport chains are leaked and react with O_2 to form activated oxygen species such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (.OH) and singlet oxygen ($^1O_2$) (Halliwell and Gutteridge 1985). If the activated oxygen species were not eliminated immediately, the membranes of most plant cell organelles would

be destroyed, and then more malondialdehyde (MDA) would accumulate. Thus, MDA together with Chl content and Pn has been used as valid indication of leaf senescence (Dong et al. 2008). In our study, the Pn and Chl contents were lower, and MDA content higher in girdled plants than in non-girdled control plants, suggesting that girdling significantly accelerated leaf senescence through promotion of chlorophyll degradation and membrane lipid peroxidation. The decreased seed cotton yield as a result of stem girdling was at least partially attributed to the accelerated leaf senescence following girdling.

Plant hormones play an important role in the process of leaf senescence. Different classes of plant hormones are involved in the process, such as IAA, gibberellins, ethylene, cytokinins and ABA (Gan and Amasino 1995; Ghanem et al. 2008; He et al. 2002; Zacarias and Reid 1990; Tao et al. 1983; Garrison et al. 1984; Smart 1994). Our data showed that girdling significantly decreased cytokinin levels in main-stem leaf and substantially increased the ABA level. Cytokinins (t-Z + t-ZR, DHZ + DHZR and iP + iPA) are anti-senescence agents (McGaw and Burch 1995; Perrin et al. 1997; Badenoch-Jones et al. 1996; Robson et al. 2004), while ABA is an endogenous promoter of leaf senescence (Ray et al. 1983; He and Jin 1999). Much evidence suggests that exogenous application of cytokinins or the overproduction of CKs in transgenic plants transformed with the isopentenyl transferase (*IPT*) and zeatin *O*-glucosyltransferase (*ZOGl*) can delay leaf senescence (Van Staden et al. 1994; Rivero et al. 2007; Cowan et al. 2005; Havlova et al. 2008). ABA could provoke carbohydrate accumulation in mature leaves (Estruch et al. 1989) and influence the metabolic fluxes (Schippers et al. 2007). It is also indicated that ABA increase in leaves could be partially responsible for the decreased CK levels, thereby indirectly enhancing senescence (Brugière et al. 2003). Therefore, we suggest that cytokinin and ABA might be involved in the regulation of leaf senescence following girdling in cotton.

Previous grafting studies showed that plant hormones such as cytokinin and ABA can be transported from root to leaves to regulate leaf senescence (McKenziel et al. 1998; Catterou et al. 2002; Dong et al. 2008). Our data also showed that girdling significantly reduced the levels of t-Z + t-ZR, DHZ + DHZR and iP + iPA, but increased the ABA level in the xylem sap, which were in accordance with phytohormone changes in main-stem leaves. These findings further suggest that the cytokinins and ABA involved in leaf senescence following girdling might be at least partially root-sourced as reported in Van Staden et al. (1994).

In conclusion, girdling accelerated the main-stem leaf senescence as indicated by declines in Chl content and Pn, and an increase in MDA content. Correspondingly, it also decreased levels of cytokinins and increased those of ABA in both leaves and xylem sap. Cytokinins and ABA are involved in leaf senescence following girdling. The results of the study add new evidences to the role of cytokinins and ABA in leaf senescence in relation to plant girdling.

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References

Agusti M, Andreu I, Juan M, Almela V, Zacarias L (1998) Effect of ringing branches on fruit size and maturity of peach and nectarine cultivars. J Hortic Sci Biotechnol 73:537–540

Badenoch-Jones J, Parker CW, Letham DS, Singh S (1996) Effect of cytokinins supplied via the xylem at multiples of endogenous concentrations on transpiration and senescence in derooted seedlings of oat and wheat. Plant Cell Environ 19:504–516

Berüter J, Feusi MES (1997) The effect of girdling on carbohydrate partitioning in the growing apple fruit. J Plant Physiol 151:277–285

Bleecker AB, Patterson SE (1997) Last exit: senescence abscission and meristem arrest in *Arabidopsis*. Plant Cell 9:1169–1179

Brugière N, Jiao S, Hantke S, Zinselmeier C, Roessler JA, Niu X, Jones RJ, Habben JE (2003) Cytokinin oxidase gene expression in maize is localized to the vasculature, and is induced by cytokinins, abscisic acid and abiotic stress. Plant Physiol 132:1228–1240

Buchanan-Wollaston V (1997) The molecular biology of leaf senescence. J Exp Bot 48:181–199

Buchanan-Wollaston V, Earl S, Harrison E, Mathas E, Navabpour S, Page T, Pink D (2003) The molecular analysis of leaf senescence: a genomics approach. Plant Biotechnol J 1:3–22

Catterou M, Dubois F, Smets R, Vaniet S, Kichey T, Van Onckelen H, Sangwan-Norreel BS, Sangwan RS (2002) hoc: an *Arabidopsis* mutant overproducing cytokinins and expressing high in vitro organogenic capacity. Plant J 30:273–287

Chandlee JM (2001) Current molecular understanding of the genetically programmed process of leaf senescence. Physiol Plantarum 113:1–8

Cohen A (1981) Recent developments in girdling citrus trees. Proc Int Soc Citric 1:196–199

Cowan AK, Freeman M, Bjŏrkman PO, Nicander B, Sitbon F, Tillberg E (2005) Effects of senescence-induced alteration in cytokinin metabolism on source–sink relationships and ontogenic and stress-induced transitions in tobacco. Planta 221:801–814

Dong HZ, Li WJ, Tang W, Zhang DM, Li ZH (2006) Yield, quality and leaf senescence of cotton grown at varying planting dates and plant densities in the Yellow River Valley of China. Field Crops Res 98:106–115

Dong HZ, Niu YH, Li WJ, Zhang DM (2008) Effects of cotton root stock on endogenous cytokinins and abscisic acid in xylem sap and leaves in relation to leaf senescence. J Exp Bot 59:1295–1304

Dong HZ, Niu YH, Kong XQ, Luo Z (2009) Effect of early-fruit removal on endogenous cytokinins and abscisic acid in relation to leaf senescence in cotton. J Plant Growth Regul 59:93–101

Estruch JJ, Pereto JG, Vercher Y, Beltran JP (1989) Sucrose loading in isolated veins of *Pisum sativum*: regulation by abscisic acid, gibberellic acid, and cell turgor. Plant Physiol 91:259–265

Fernández B, Centeno ML, Feito I, Sánchez-Tamés R, Rodríguez A (1995) Simultaneous analysis of cytokinins, auxins and abscisic acid by combined immunoaffinity chromatography, high performance liquid chromatography and immunoassay. Phytochem Anal 6:49–54

Gan S, Amasino RM (1995) Inhibition of leaf senescence by autoregulated production of cytokinin. Science 270:1986–1988

Garrison FR, Brinker AM, Noodén LD (1984) Relative activities of xylem-supplied cytokinins in retarding soybean leaf senescence and sustaining pod development. Plant Cell Physiol 25:213–224

Gawronska H, Deji A, Sakakibara H, Sugiyama T (2003) Hormone-mediated nitrogen signaling in plants: implication of participation of abscisic acid in negative regulation of cytokinin-inducible expression of maize response regulator. Plant Physiol Biochem 41:605–610

Ghanem ME, Albacete A, Martínez-Andújar C, Acosta M, Romero-Aranda R, Lutts S, Pérez-Alfocea F (2008) Hormonal changes during salinity-induced leaf senescence in tomato (*Solanum lycopersicum* L.). J Exp Bot 59:3039–3050

Goren R, Huberman M, Goldschmidt EE (2003) Girdling: physiological and horticultural aspects. Hortic Rev 30:1–36

Gusti MA, Gariglio N, Juan M, Almela V, Mesejo C (2005) Effect of branch scoring on fruit development in loquat. J Hortic Sci Biotechnol 80:370–374

Halliwell B, Gutteridge JMC (1985) Free radicals in biology and medicine. Clarendon Press, Oxford

Havlova M, Dobrev PI, Motyka V, Storchova H, Libus J, Dobra J (2008) The role of cytokinins in responses to water deficit in tobacco plants over-expressing *trans*-zeatin *O*-glucosyltransferase gene under *35S* or *SAG12* promoters. Plant Cell Environ 31:341–353

He P, Jin JY (1999) Relationships among hormone changes, transmembrane flux of Ca^{2+} and lipid peroxidation during leaf senescing in spring maize. Acta Bot Sin 41:1221–1225

He YH, Fukushige H, Hildebrand DF, Gan SS (2002) Evidence supporting a role of jasmonic acid in *Arabidopsis* leaf senescence. Plant Physiol 128:876–884

Heath RL, Packer L (1968) Photoperoxidation in isolated chloroplast. I. Kinetics and stoichiometry of fatty acids peroxidation. Arch Biochem Biophys 125:189–198

Horgan R, Scott IM (1987) Cytokinins. In: Rivier L, Crozier A (eds) The principles and practice of plant hormone analysis. Academic Press, London, pp 303–365

Johnsen K, Maier C, Sanchez F, Anderson P, Butnor J, Waring R, Linder S (2007) Physiological girdling of pine trees via phloem chilling: proof of concept. Plant Cell Environ 30:128–134

Juan M, Mesejo C, Martínez-Fuentes A, Reig M, Agustí M (2009) Branch scoring encourages fruit development and climacteric in persimmon. Sci Hortic 122:497–500

Kakani VG, Reddy KR, Zhao D, Gao W (2004) Senescence and hyperspectral reflectance of cotton leaves exposed to ultraviolet-B radiation and carbon dioxide. Physiol Plantarum 121:250–257

Krapp A, Stitt M (1995) An evaluation of direct and indirect mechanisms for the 'sink regulation' of photosynthesis in spinach: changes in gas exchange, carbohydrates, metabolites, enzyme activities and steady-state transcript events after cold-girdling source leaves. Planta 195:313–323

Li CY, Weiss D, Eliezer E (2003) Girdling affect carbohydrate-related gene expression in leaves, bark and roots of alternate-bearing citrus trees. Ann Bot 92:137–143

Lim PO, Nam HG (2007) Aging and senescence of the leaf organ. J Plant Biol 50:291–300

Marani A, Baker DN, Reddy VR, McKinion JM (1985) Effect of water stress on canopy senescence and carbon exchange rates in cotton. Crop Sci 25:798–802

McGaw BA, Burch LR (1995) Cytokinin biosynthesis and metabolism. In: Davies PJ (ed) Plant hormones. Kluwer Academic Publishers, Dordrecht, pp 98–117

McKenziel MJ, Mett V, Reynolds PHS, Jameson PE (1998) Controlled cytokinin production in transgenic tobacco using a copper-inducible promoter. Plant Physiol 116:969–977

Mok MC, Martin RC, Mok DWS (2000) Cytokinins: biosynthesis, metabolism and perception. In Vitro Cell Dev Biol 36:102–107

Murakami PF, Schaberg PG, Shane JB (2008) Stem girdling manipulates leaf sugar concentrations and anthocyanin expression in sugar maple trees during autumn. Tree Physiol 28:1467–1473

Noodén LD, Singh S, Letham DS (1990) Correlation of xylem sap cytokinin levels with monocarpic senescence in soybean. Plant Physiol 93:33–39

Parrott DL, Yang L, Shama L, Fischer AM (2005) Senescence is accelerated, and several proteases are induced by carbon 'feast' conditions in barley (*Hordeum vulgare* L.) leaves. Planta 222:989–1000

Parrott DL, Mclnnerney K, Feller U, Fischer AM (2007) Steam-girdling of barley (*Hordeum vulgare*) leaves leads to carbohydrate accumulation and accelerated leaf senescence, facilitating transcriptomic analysis of senescence-associated genes. New Phytol 176:56–69

Perrin Y, Doumas P, Lardets L, Carron MP (1997) Endogenous cytokinins as biochemical markers of rubber-tree (*Hevea brasiliensis*) clone rejuvenation. Plant Cell Tiss Org Cult 47:239–245

Pic E, Serve BT, Tardieu F, Turc O (2002) Leaf senescence induced by mild water deficit follows the same sequence of macroscopic, biochemical, and molecular events as monocarpic senescence in pea. Plant Physiol 128:236–246

Pourtau N, Marès M, Purdy S, Quentin N, Wingler A (2004) Interactions of abscisic acid and sugar signalling in the regulation of leaf senescence. Planta 219:765–772

Pourtau N, Jennings R, Pelzer E, Pallas J, Wingler A (2006) Effect of sugar-induced senescence on gene expression and implications for the regulation of senescence in *Arabidopsis*. Planta 224:556–568

Ray S, Mondal WA, Choudhuri MA (1983) Regulation of leaf senescence, grain filling and yield of rice by kinetin and abscisic acid. Physiol Plantarum 59:343–346

Rivas F, Fornes F, Agustí M (2008) Girdling induces oxidative damage and triggers enzymatic and non-enzymatic antioxidative defences in *citrus* leaves. Environ Exp Bot 64:256–263

Rivero RM, Kojima M, Gepstein A, Sakakibara H, Mittler R, Gepstein S, Blumwald E (2007) Delayed leaf senescence induces extreme drought tolerance in a flowering plant. Proc Natl Acad Sci USA 104:19631–19636

Robson PRH, Donnison IS, Wang K, Frame B, Pegg SE, Thomas A, Thomas H (2004) Leaf senescence is delayed in maize expressing the *Agrobacterium IPT* gene under the control of a novel maize senescence-enhanced promoter. Plant Biotechnol J 2:101–112

Schippers JHM, Jing HC, Hille J, Dijkwel PP (2007) Developmental and hormonal control of leaf senescence. In: Gan S (ed) Senescence processes in plants. Blackwell Publishing, Oxford, pp 145–170

Smart CM (1994) Gene expression during leaf senescence. New Phytol 126:419–448

Smart CM, Scofield SR, Bevan MW, Dyer TA (1991) Delayed leaf senescence in tobacco plants transformed with *tmr*, a gene for cytokinin production in *Agrobacterium*. Plant Cell 3:647–656

Steel RGD, Torrie JH (1980) Analysis of covariance. In: Principles and procedures of statistics: a biometrical approach, 2nd edn. McGraw-Hill, New York, pp 401–437

Tang QY, Feng MG (1997) Practical statistics and DPS data processing system. China Agri Press, Beijing, pp 1–407

Tao GQ, Letham DS, Plani LMS, Summons RE (1983) Cytokinin biochemistry in relation to leaf senescence. I. The metabolism of

6-benzylaminopurine and zeatin in oat leaf segments. J Plant Growth Regul 2:89–102

Thomas H (1992) Canopy survival. In: Thomas H, Baker N (eds) Crop photosynthesis: spatial and temporal determinants. Elsevier, Amsterdam, pp 11–41

Thomas H, Stoddart JL (1980) Leaf senescence. Plant Physiol 31:83–111

Trueman SJ, Turnbull CGN (1994) Fruit set, abscission and dry matter accumulation on girdled branches of macadamia. Ann Bot 74:667–674

Van Staden J, Upfold SJ, Altman A (1994) Metabolism of [^{14}C] *trans*-zeatin and [^{14}C] benzyladenine by detached yellow, green and variegated leaves of *Schefflera*. Physiol Plant 90:73–78

Wallerstein I, Goren R, Monselise SP (1978) Rapid and slow translocation of ^{14}C sucrose and ^{14}C-assimilates in *Citrus* and *Phaseollus* with special reference to ringing effect. J Hortic Sci 53:203–208

Weiler EW (1982) Plant hormone immunoassay. Physiol Plantarum 54:230–234

Wingler A, Purdy S, MacLean JA, Pourtau N (2006) The role of sugars in integrating environmental signals during the regulation of leaf senescence. J Exp Bot 57:391–399

Wright PR (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

Yong JWH, Wong SC, Letham DS, Hocart CH, Farquhar GD (2000) Effects of elevated [CO_2] and nitrogen nutrition on cytokinins in the xylem sap and leaves of cotton. Plant Physiol 124:767–779

Zacarias L, Reid M (1990) Role of growth regulators in the senescence of *Arabidopsis thaliana* leaves. Physiol Plant 80:549–554