Effects of cotton rootstock on endogenous cytokinins and abscisic acid in xylem sap and leaves in relation to leaf senescence

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Abstract

Leaf senescence varies greatly among cotton cultivars, possibly due to their root characteristics, particularly the root-sourced cytokinins and abscisic acid (ABA). Early-senescence (K1) and late-senescence (K2) lines, were reciprocally or self-grafted to examine the effects of rootstock on leaf senescence and endogenous hormones in both leaves and xylem sap. The results indicate that the graft of K1 scion onto K2 rootstock (K1/K2) alleviated leaf senescence with enhanced photosynthetic (Pn) rate, increased levels of chlorophyll (Chl) and total soluble protein (TSP), concurrently with reduced malondialdehyde (MDA) contents in the fourth leaf on the main-stem. The graft of K2 scion onto K1 rootstock enhanced leaf senescence with reduced Pn, Chl, and TSP, and increased MDA, compared with their respective self-grafted control plants (K1/K1 and K2/K2). Reciprocally grafted plants differed significantly from their self-grafted control plants in levels of zeatin and its riboside (Z+ZR), isopentenyl and its adenine (iP+iPA), and ABA, but not in those of dihydrozeatin and its riboside (DHZ+DHZR) in leaves in late season, which was consistent with variations in leaf senescence between reciprocally and self-grafted plants. The results suggest that leaf senescence is closely associated with reduced accumulation of Z+ZR, and iP+iPA rather than DHZ+DHZR, or enhanced ABA in leaves of cotton. Genotypic variation in leaf senescence may result from the difference in root characteristics, particularly in Z+ZR, iP+iPA, and ABA which are regulated by the root system directly or indirectly.

Key words: Abscisic acid, cotton, cytokinins, grafting, leaf senescence.

Introduction

As an integral part of plant growth and development, leaf senescence is an important step in the life cycle of a plant (Samet and Sinclair, 1980; He et al., 2005). Visual symptoms of leaf senescence include loss of chlorophyll pigments (yellowing), desiccation, and eventual abscission. Physiological and molecular events such as chloroplast disintegration, decline in photosynthesis, breakdown of protein, and loss of nucleic acids also occur during leaf senescence (Buchanan-Wollaston, 1997; Buchanan-Wollaston et al., 2003; Chandlee, 2001). As the senescence ultimately leads to the death of a leaf, it could be regarded as a form of programmed cell death (Thomas et al., 2003; van Doorn and Woltering, 2004). Although leaf senescence is a degenerative process, it plays a vital role in nutrient recycling (Himelblau and Amasino, 2001). Thus leaf senescence is a key to ensure survival of plant species in the following season, and is also a major determinant of yield in many crops (Thomas, 1992).

Leaf senescence usually occurs in an age-dependent manner in many plant species including cotton. However, it may occur too early or too late in the season due to environmental stresses or internal factors (Guin, 1985). Senescence too late in the season would interfere with nutrient remobilization, thereby compromising photosynthetic activity in young leaves and reproductive success. By contrast, too early senescence would reduce the plant’s overall capacity to assimilate CO₂ (Winger et al., 2006). Too early senescence for 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 (Bt) cotton (*Gossypium hirsutum* L.) cultivars were introduced for commercial production (Dong et al., 2006). Wright (1999) indicated that premature senescence frequently developed during the period of rapid boll filling. It results in reduced lint yield and poor fibre properties, thus constituting an important constraint to cotton yield and quality (Wright, 1998). An understanding of leaf senescence may help to improve yield and quality through appropriate management to avoid too-early or too-late senescence.

Compared with other important traits, however, the mechanisms controlling leaf senescence are still not well understood (Wingler et al., 2006). The initiation and progression of leaf senescence in cotton can be affected by a number of environmental factors such as nutrient deficiency (Wright, 1998), drought (Radin, 1981; Marani et al., 1985), elevated CO$_2$ (Kakani et al., 2004), and salinity (Ganieva et al., 1998), or internal factors such as phytohormones (Yong et al., 2000; Dong et al., 2005). Large boll load and potassium deficiency are likely to constitute the most important factors causing premature senescence (Wright, 1999; Pettigrew, 2003). Plant hormones, especially cytokinins and ABA, are generally thought to be the main internal factors involved in the initiation and progression of leaf senescence (Yang et al., 2004). Cytokinins are a structurally diverse group of N$_6$-substituted purine derivatives capable of inducing plant cell division. Zeatin riboside (ZR), dihydrozeatin riboside (DHZR), and isopentenyl adenine (iPA) are the most commonly detected physiologically active cytokinins in plants (Mok et al., 2000). Cytokinins can delay leaf senescence (Garrison et al., 1984; Van Staden et al., 1988; Yong et al., 2000), and the progression of leaf senescence is usually correlated with a decrease in cytokinin in the leaves (Buchanan-Wollaston, 1997). By contrast, abscisic acid (ABA) is considered to be a senescence enhancer. Leaf senescence is usually followed by a increase in ABA (Guin, 1985; Samet and Sinclair, 1980).

The sources of cytokinins and ABA that control leaf senescence are still not clear. Many researchers considered that cytokinins are predominantly root-sourced plant hormones, and the cytokinins translocated from the roots through the xylem to the aerial plant parts will control shoot development (Letham and Palni, 1983; Letham, 1994). The assumption that roots are the main site of cytokinin synthesis in plants was activated by the discovery of IPT-genes that control cytokinin synthesis in plants (McCabe et al., 2001; Chang et al., 2003; Khodakovskaya et al., 2005). The root is also a major site for producing ABA, and at least part of the ABA in leaves is translocated from the root (Zhao et al., 1991; Yang et al., 2004; Thompson et al., 2007). However, many of these findings are based on an analysis of plant hormones in the xylem sap and root. The presence of phytohormones in either the xylem sap or root does not prove that they are only synthesized in the roots. Either cytokinins or ABA in the xylem and root could also be translocated from the recirculation of leaf-sourced phytohormone in the phloem (Wolf et al., 1990; Hoad, 1995). There is evidence that shoot meristematic tissues and leaves are capable of synthesizing cytokinin and ABA (Chen et al., 1985; Yong et al., 2000; Miyawaki et al., 2004). Phytohormones in leaves that regulate leaf senescence may be multi-sourced, and varied with plant species or genotypes in the same plant species (He et al., 2005). Further studies on temporal changes in cytokinins and ABA in both xylem sap and leaves with different senescent types of cultivars may better explain their role in the regulation of leaf senescence in cotton.

A grafting experiment was thus conducted to study the effects of rootstocks and cytokinins and ABA on leaf senescence in cotton. Self- and reciprocal-grafting was performed on two transgenic Bt cotton lines (K1 and K2) with different leaf senescence. Leaf senescence and levels of cytokinins and ABA in both leaves and xylem sap of grafted plants were determined.

### Materials and methods

The experiment was conducted from 2005 to 2006 in Jinan, China. Two transgenic Bt cotton lines, K1 and K2, developed by the Cotton Research Center, Shandong Academy of Agricultural Sciences, Jinan, were used in the experiment. Our previous field trial showed that leaves of K1 started to senesce earlier than those of K2 after flowering. K1 and K2 were therefore considered as early and late senescence lines, respectively.

In 2005, a preliminary study was conducted to examine plant growth and development, as well as leaf senescence determined from chlorophyll (Chl) content and net photosynthetic rate in the fourth leaf from the main-stem terminal. Analysis of hormones and physiological parameters associated with leaf senescence was conducted on self- and reciprocally-grafted plants in 2006.

### Plant culture and grafting experiment

Acid-delinted seeds from each transgenic cotton line were sown in plastic pots (50 cm in height and 40 cm in diameter) filled with fertile soil (1.2% organic matter, 500 mg kg$^{-1}$ total N, 15 mg kg$^{-1}$ available P, and 120 mg kg$^{-1}$ available K), and allowed to germinate and grow in a greenhouse. Temperature in the greenhouse averaged 30 °C in the day and 20 °C at night. When the first fully expanded true leaf occurred, seedlings were thinned to four per pot.

When most seedlings reached the 3–4 leaf stage, self- and reciprocal- graftings were performed according to a modified method of Luo and Gould (1999). Briefly, the native shoot above the cotyledons of a stock seedling was removed and cotyledons were retained. After removing the shoot, the main-stem of a stock was split vertically to a depth of 2 cm. The base of each shoot scion with a true leaf and growth point was cut to form a deep ‘V’. The scion was inserted into the base of the vertically split rootstock and closely wrapped with parafilm. Grafted seedlings were sprayed with...
water and immediately covered with large plastic bags to prevent wilting. Two weeks later, after a leaf bud emerged from the scion, the plastic bag and parafilm were removed. Grafted seedlings were allowed to grow in the greenhouse for another week, thinned to one vigorous plant per pot and moved out to continue growth under natural environment conditions. Potted plants were watered to 75% of the field water capacity daily to minimize water stress. Seedlings were fertilized with 5 g compound fertilizer (25% N, 25% P, and 20% K) per pot at flowering.

For convenience, the reciprocally grafted plants of the two lines were designated as K1/K2 and K2/K1, and self-grafted plants as K1/K1 and K2/K2, respectively. The experiment was a completely random design with four replications. Each treatment had 10 pots per replication. The grafting experiment was repeated twice by planting cotton in early April and early May in 2006, respectively.

**Physiological measurement**

Chlorophyll (Chl) contents were determined as described by He et al., (2002). Briefly 0.25 g of fresh leaves was placed in a 100 ml test tube. The tissues were homogenized with a polytron after 10–15 ml pure methanol was added. 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 nm and 665 nm for Chl a and Chl b, respectively. Four leaves per treatment were measured separately and the measurement was repeated twice.

Net photosynthesis (Pn) rate of the fourth leaf was measured using a LI-6400 portable photosynthesis system (LI-COR, Lincoln, NE, USA). The measurements were taken between 09.00 h and 11.00 h on cloudless days when ambient photosynthetic photon flux density exceeded 1500 μmol m⁻² s⁻¹ at a 15-d interval from flowering to boll-opening. Three plants were examined and the means were calculated across replicates.

The peroxidation of leaf lipids during leaf senescence was estimated by the formation of malondialdehyde (MDA), an end-product of lipid peroxidation, as described by Iturbe-Ormaetxe et al., (1998). Lipid peroxides were extracted from 0.5 g fresh leaf samples with 5 ml of 5% (w/v) metaphosphoric acid and 100 μl of 2% (w/v) butyl hydroxytoluene (in ethanol). After centrifugation at 15 000 × g for 20 min, the chromatogram was formed by mixing 0.5 ml of supernatant, 50 μl of 2% (w/v) butyl hydroxytoluene, 0.25 ml of 1% (w/v) TBA (in 50 mM NaOH), and 0.25 ml of 25% (v/v) HCl. The reaction mixtures were incubated at 100 °C for 30 min and cooled to room temperature. The formed chromatogram was extracted by adding 1 ml of n-butanol to the mixture followed by vigorous shaking. Butanol and aqueous phases were separated by centrifugation. The absorbance of the thiobarbituric acid reactive substance (TBARS) was determined as TBA-MDA complex at 532 nm using a spectrophotometer. The absorbance readings were converted using BSA as standard curve.

**Phytohormone extraction, purification, and quantification**

The fourth leaf on the main-stem from the terminal bud was also used for phytohormone quantification, because it is the most functional leaf of a cotton plant and is particularly appropriate when monitoring plants during their development. The extraction and purification of Z+ZR, DHZ+DHZR, iP+iPA, and ABA in leaves were done according to the modified procedure described by He et al. (2005).

About 0.2 g fresh leaves were extracted and homogenized in 2 ml of 80% methanol (containing 40 mg l⁻¹ butylated hydroxytoluene) and stored at −20 °C for 48 h. After centrifugation at 20 000 g for 15 min, sediments were resuspended in 1 ml 80% methanol at −20 °C for 16 h. The combined extracts were purified by passing them through C₁₈ Sep-Pak cartridges (Waters, Milford, USA). Afterwards, samples were evaporated under vacuum to remove the organic solvent, and dissolved in 2.0 ml of TBS buffer (TRIS-buffered saline; 50 mM TRIS, pH 7.8, 1 mM MgCl₂, 10 mM NaCl, 0.1% Tween, 0.1% gelatin) to determine Z+ZR, DHZ+DHZR, iP+iPA, and ABA.

The DHZ, ZR, and iP 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 et al. (1982). As the antibodies of cytokinins also recognize free bases, nucleotides, and 9N-glucosides, what was measured was the sum of free bases, ribosides, nucleotides, and 9N-glucosides of the corresponding cytokinins. For simplicity, the cytokinins would be identified as Z+ZR, DHZ+DHZR, and iP+iPA, respectively.

Analysis of ABA was conducted also 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 which were provided by Professor BM Wang from China Agricultural University, Beijing. The anti-ABA antibody shows complete reactivity with (+/–) ABA and very low cross-reactivity with compounds structurally similar to ABA. In order 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, 0.5 mM MgCl₂ (pH 9.8). Absorbance of the developed colour was measured at 405 nm, and the ABA concentration was calculated.

Xylem sap was collected between 19.00 h and 07.00 h from four plants per treatment according to Noodén et al. (1990). The plants were cut off about 1 cm below the grafting point and the cut surface was wiped with methanol/formic acid/H₂O (14.1.2, by vol.) to inhibit phosphatase action and thereby prevent loss of the nucleotides. The cut stem was connected to low-volume plastic tubing which ran 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 darkness at −40 °C. Z+ZR, DHZ+DHZR, iP+iPA, and ABA in samples were analysed as described above.

**Plant biomass and root:shoot ratio**

For both years, the weight of the above-ground portion (leaves, stem, and branches) and below-ground portion (root system), as well as the total number of bolls per plant, boll weight, and seed cotton yield were determined at harvest. Roots of plants in a pot were collected with a sieve and dried to a constant weight. Seed cotton was manually harvested and weighted after air-drying for 5 d. Average boll weight and root:shoot ratio were also calculated.

**Statistical analysis**

Data were statistically analysed with DPS Data Processing System (Tang and Feng, 1997). Means were separated using Duncan’s Multiple Range Test at P=0.05 or P < 0.01.
Results

Plant biomass, yield, yield components, and leaf senescence

Data for 2005 were mainly on plant growth, development, and leaf senescence performance of the two cotton lines. Seedling emergence, squaring, flowering, peak flowering, peak boll-setting, and boll-opening for both K1 and K2 occurred at about 7, 45, 65, 80, 95, and 110 d after planting (DAP). Both lines grew and developed in a similar time-course and performed similarly in shoot and root weights, shoot:root ratio, and plant biomass (Table 1). However, seed cotton yield and leaf senescence based on Chl content and Pn in the late season differed significantly between the two cotton lines (Fig. 1).

Shoot weight and total plant biomass for the grafted K1 with K2 as the rootstock (K1/K2) were significantly greater than those for self-grafted K1 (K1/K1), but those for grafted K2 with K1 rootstock (K2/K1) were significantly lower than those for self-grafted K2. Seed cotton yield of K1/K2 increased 21.6% relative to that of K1/K1, while that of K2/K1 decreased 9.1%. The increased yield for K1/K2 was attributed to enhanced boll weight, while the decreased yield for K2/K1 was attributed to reduced boll weight (Table 1).

There were no statistical differences in photosynthetic rate, chlorophyll, MDA and soluble protein contents either between K1/K2 and its control (K1/K1), or between K2/K1 and its control (K2/K2) at 65 DAP and 80 DAP. However, these senescence-related physiological parameters in reciprocally grafted plants significantly differed from those in self-grafted controls at 95 (P <0.05) and 110 (P <0.05 or P <0.01) DAP (Figs 2, 3). The photosynthetic rate and the chlorophyll and soluble protein contents of K1/K2 were much greater than those of K1/K1, but the MDA content of K1/K2 was much lower than that of K1/K1, at either 95 DAP or 110 DAP. By contrast, K2/K1 contained more Z+ZR than its control (K2/K2), but K2/K1 contained less Z+ZR than its control (K2/K2) in main-stem leaves in late season (95–110 DAP). A similar difference between self-grafted and reciprocally grafted plants was also detected in iP+IPA contents in the main-stem leaves in the late season, whereas no significant difference in leaf DHZ+DHZR was observed between self-grafted and reciprocally grafted plants in the late season. The results indicated that the K2 rootstock increased cytokinin (Z+ZR and iP+iPA) levels in K1 leaves, while of the K1 rootstock reduced them in K1 leaves in the late season.

Temporal differences between self-grafted and reciprocally grafted plants in cytokinins in rootstock sap were similar to those in leaves (Fig. 5). The graft of K1/K2 contained more Z+ZR and iP+iPA than K1/K1. The K2/K1 graft contained less Z+ZR than K2/K2 in the late season. No significant difference in DHZ+DHZR was found between K1/K2 and K1/K1, and between K2/K1 and K2/K2 at 95 DAP and 110 DAP. Levels of Z+ZR (n=16, R²=0.556, P <0.05) and iP+iPA (n=16, R²=0.604, P <0.05) in the main-stem leaves were significantly correlated with those in the bleeding sap.

ABA in leaves and bleeding sap

In both self- and reciprocally grafted plants, ABA contents in the fourth leaf decreased constantly from 65 DAP to 110 DAP (Fig. 6). K1/K2 contained more ABA at 105 DAP than K1/K1, but K2/K1 contained less ABA than its control (K2/K2) at 95 DAP and 110 DAP. These results suggested that the rootstock of K2 alleviated leaf senescence in the K1 scion, but that of K1 enhanced it in the K2 scion in the late season, compared with their respective self-grafted controls.

Cytokinin levels in leaves and xylem sap

In both self- and reciprocally grafted plants, Z+ZR contents in the fourth leaf decreased constantly from 65 DAP to 110 DAP (Fig. 4). K1/K2 contained more Z+ZR than its control (K1/K1), but K2/K1 contained less Z+ZR than its control (K2/K2) in main-stem leaves in late season (95–110 DAP). A similar difference between self-grafted and reciprocally grafted plants was also detected in iP+iPA contents in the main-stem leaves in the late season, whereas no significant difference in leaf DHZ+DHZR was observed between self-grafted and reciprocally grafted plants in the late season. The results indicated that the K2 rootstock increased cytokinin (Z+ZR and iP+iPA) levels in K1 leaves, while of the K1 rootstock reduced them in K1 leaves in the late season.

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ABA in leaves and bleeding sap

In both self-grafted and reciprocally grafted plants, ABA contents in the fourth main-stem leaves increased consistently from 65 DAP to 110 DAP (Fig. 6), but K1/K2 accumulated significantly less ABA than its control

<table>
<thead>
<tr>
<th>Treatment</th>
<th>DW of shoot (g⁻¹ plant)</th>
<th>DW of root (g⁻¹ plant)</th>
<th>Root:shoot ratio</th>
<th>Total DW (g⁻¹ plant)</th>
<th>Seed cotton (g⁻¹ plant)</th>
<th>No. of bolls plant⁻¹</th>
<th>Boll weight (g)</th>
</tr>
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<tbody>
<tr>
<td>2005</td>
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<tr>
<td>K1</td>
<td>64.7 a</td>
<td>20.8 a</td>
<td>0.321 a</td>
<td>85.5 a</td>
<td>51.4 a</td>
<td>10.1 a</td>
<td>5.09 a</td>
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<tr>
<td>K2</td>
<td>65.3 a</td>
<td>21.6 a</td>
<td>0.331 a</td>
<td>86.9 a</td>
<td>58.5 b</td>
<td>10.4 a</td>
<td>5.65 b</td>
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<tr>
<td>2006</td>
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<tr>
<td>K1/K1</td>
<td>67.9 b</td>
<td>22.5 a</td>
<td>0.331 a</td>
<td>90.4 a</td>
<td>53.2 a</td>
<td>10.9 a</td>
<td>4.88 a</td>
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<tr>
<td>K1/K2</td>
<td>75.8 a</td>
<td>23.9 a</td>
<td>0.315 a</td>
<td>99.7 b</td>
<td>64.7 b</td>
<td>11.8 b</td>
<td>5.49 b</td>
</tr>
<tr>
<td>K2/K1</td>
<td>68.2 b</td>
<td>23.1 a</td>
<td>0.339 a</td>
<td>91.3 a</td>
<td>57.4 a</td>
<td>10.5 a</td>
<td>5.46 b</td>
</tr>
<tr>
<td>K2/K2</td>
<td>62.6 c</td>
<td>22.3 a</td>
<td>0.356 b</td>
<td>84.9 c</td>
<td>52.2 a</td>
<td>10.6 a</td>
<td>4.92 a</td>
</tr>
</tbody>
</table>

* The shoot includes main stem, branches, bolls and the remaining leaves.
* For each year, means within a column followed by same letters are not significantly different at P=0.05.
practice to enhance production in some plant species like vegetables and trees (Estan et al., 2005). This strategy was also used to study the interaction of scion and stock on leaf senescence in soybean (Ookawa et al., 2005) and improve recovery of seedlings from *in vitro* culture in cotton (Luo and Gould, 1999). Grafting is also a useful technique to examine long-distance signalling between scions and rootstocks. Faiss et al. (1997) reported no influence over apical dominance or senescence in wild-type tissue grafted onto *ipt*-transgenic rootstock, and claimed that an elevated level of cytokinins in the root led to only a slight increase in cytokinin levels in the xylem and had no phenotypic consequences in the scion. Thus, they concluded that cytokinin may act as paracrine signal, at least with respect to apical dominance and leaf senescence (Haberer and Keiber, 2002; Hirose et al., 2008). However, this conclusion has been debated by other studies. Catterou et al. (2002) showed that the axillary buds appeared when wild-type scions were grafted onto roots of mutant plants overproducing cytokinins and their grafting experiments clearly pointed to the roots as the major source of cytokinins in scions. McKenzie et al. (1998) studied transgenic plants in which the expression of the *ipt*-gene was limited to the roots and showed a delayed senescence of the plants. In the present study, self-grafting and reciprocal-grafting experiments were conducted with an early senescing line (K1) and a late senescing line (K2). As expected, the leaf senescence of K1 grafted onto the K2 rootstock was significantly delayed, but that of K2 grafted onto the K1 rootstock was significantly accelerated. This suggests that leaf senescence is considerably affected by the root characteristics, and genotypic variation in leaf senescence is at least in part attributed to the root features. The results also suggest that leaf senescence could be affected by substances transferred from roots. Since the two different senescent lines were comparable in growth and development, root and shoot weights, and their ratios, there is a possibility that plant hormones regulating leaf senescence are transported from rootstocks to leaves as previously reported for other plant species (He et al., 2005; Hirose et al., 2008).

Leaf senescence might follow changes in endogenous hormone levels that are known to cause lipid peroxidation and subsequent degradation of Chl and protein (He and Jin, 1999). The importance of phytohormones, especially of cytokinins in the control of leaf senescence has been reported in many previous works (Tao et al., 1983; Garrison et al., 1984; Noodén and Letham, 1984, 1986). During senescence, endogenous signals up-regulate certain genes that show high homology to enzymes known to degrade protein, RNA, lipids, and chlorophyll (Buchanan-Wollaston, 1997). Although major plant hormones are involved in the senescence process, cytokinins and ABA have been shown conclusively to regulate senescence (Smart, 1994). Therefore, the relationship between leaf

(K1/K1). The K2/K1 graft contained more ABA than its control K2/K2 in main-stem leaves in the late season. Temporal changes in ABA contents in rootstock sap were different from those in leaves (Fig. 6). ABA levels in bleeding sap peaked at 80 DAP and decreased to its lowest level at 95 DAP. There was a significant difference in sap ABA levels between self-grafted and reciprocally grafted plants in the late season. Grafted plants with the K1 rootstock accumulated more ABA, while those with the K2 rootstock accumulated less.

**Discussion**

To investigate the intrinsic factors resulting in different patterns of leaf senescence in cotton, an early senescence line (K1) and a late senescence line (K2) were reciprocally and self-grafted in this study. The effects of rootstock, as well as the endogenous hormones in leaves and xylem sap, on leaf senescence were examined. Results in the first year showed similar temporal changes in the two cotton lines for growth and development, plant biomass, and root:shoot ratio, but a considerable difference in seedcotton yield and leaf senescence. Therefore, the difference between the two lines in senescence as a physiological parameter should contribute more to variation in yield than morphological or growth parameters.

Grafting plant shoots onto rootstocks to reduce the effect of external stress on the shoot is a widely-used practice to enhance production in some plant species like

![Fig. 1](https://example.com/fig1.png) Chlorophyll (Chl) content and net photosynthetic (Pn) rate of the fourth leaf from the apex on the main-stem at 65–110 d after planting. Values are means ±SD, n = 4. * and ** show significant differences at P < 0.05 and P < 0.01 from the self-grafted control. Initial flowering, peak flowering, peak boll-setting, and initial boll-opening occurred at 65, 80, 95, and 110 d after planting, respectively.

![Fig. 2](https://example.com/fig2.png) Chlorophyll (Chl) content and net photosynthetic (Pn) rate of the fourth leaf from the apex on the main-stem at 65–110 d after planting. Values are means ±SD, n = 4. * and ** show significant differences at P < 0.05 and P < 0.01 from the self-grafted control. Initial flowering, peak flowering, peak boll-setting, and initial boll-opening occurred at 65, 80, 95, and 110 d after planting, respectively.
senescence and hormone contents in the leaf and the xylem sap is very important for determining both sources and roles of hormones. Reciprocally grafted plants significantly differed from their corresponding self-grafted control plants in \( Z+ZR \), \( iP+iPA \), and ABA in leaves, but not in \( DHZ+DHZR \). Since the phytohormone difference between reciprocally and self-grafted plants was consistent with variations in senescence, it is suggested that leaf senescence is closely associated with contents of \( Z+ZR \), \( iP+iPA \), and ABA in leaves. The results are in agreement with previous reports that increased contents of cytokinins delayed leaf senescence, and elevated ABA accumulation enhanced leaf senescence (Zeevaart and Creelman, 1988; Noode`n et al., 1990; He et al., 2005). Therefore, it is

Fig. 2. Effects of rootstock on leaf chlorophyll (Chl) content and net photosynthetic (Pn) rate in self-grafted (controls) and reciprocally grafted plants of two different senescent cotton lines. K1 (an early senescence line) and K2 (a late senescence line) scions grafted onto K2 and K1 rootstocks are expressed as K1/K2 and K2/K1, respectively. Values are means ±SD, \( n=4 \); * and ** show significant differences at \( P<0.05 \) and \( P<0.01 \) from the self-grafted control, respectively. Initial flowering, peak flowering, peak boll-setting, and initial boll-opening occurred at 65, 80, 95, and 110 d after planting.

Fig. 3. Effects of rootstock on malondialdehyde (MDA) and soluble protein contents in the fourth leaf of self-grafted (controls) and reciprocally grafted plants of two different senescent cotton lines. K1 (an early senescence line) and K2 (a late senescence line) scions grafted onto K2 and K1 rootstocks are expressed as K1/K2 and K2/K1, respectively. Values are means ±SD, \( n=4 \); * and ** show significant differences at \( P<0.05 \) and \( P<0.01 \) from the self-grafted control, respectively. Initial flowering, peak flowering, peak boll-setting, and initial boll-opening occurred at 65, 80, 95, and 110 d after planting.
suggested that root-sourced cytokinins and ABA play very important roles in regulating leaf senescence in cotton. Samet and Sinclair (1980) indicated that increased free ABA was not the cause of the leaf senescence process in field-grown soybean under normal growing conditions, but this has not been confirmed in this study.

Cytokinins have been thought to act as a long-distance signal because it was found in the xylem sap of several different plants (Yong et al., 2000; Morris et al., 2001; Takei et al., 2001; Kuroha et al., 2002; Kudoyarova et al., 2007). The present self-grafting and reciprocal grafting experiments indicate that K1/K2 contained more Z+ZR and iP+iPA than K1/K1, but K2/K1 contained less Z+ZR and iP+iPA than K2/K2 in leaves in late season. Such pattern was well matched by the temporal changes in Z+ZR and iP+iPA in xylem sap in reciprocally and self-grafted plants. The levels of Z+ZR and iP+iPA in xylem sap were significantly correlated with those in the leaf. The K2 rootstock enhanced the accumulation of Z+ZR and iP+iPA in leaves and consequently delayed leaf senescence in K1/K2. By contrast, the K1 rootstock reduced the accumulation of Z+ZR and iP+iPA in leaves, resulting in rapid leaf senescence in K2/K1. We consider that genotypic variation in leaf senescence may at least in part result from the difference in levels of root-sourced Z+ZR and iP+iPA between the two lines. The production and translocation of root-sourced Z+ZR and iP+iPA in the root of late senescence line K2 is much higher than that in the root of the early senescence line K1. The results support roots as a major site of cytokinins production, and cytokinins plays a role as a root-to-shoot acropetal signal (Hirose et al., 2008).

In contrast to cytokinins, the ABA content in leaves was different from that in the xylem sap at 65–110 DAP. The most distinctive difference occurred at 80 DAP when the ABA in xylem sap reached the maximum level, although that in leaves was still very low. It is not clear if the enhanced accumulation of ABA in xylem sap at 80 DAP was a precursor for leaf ABA accumulation. Corresponding differences in ABA in leaves and the xylem sap between the reciprocally grafted and self-grafted control plants also suggest that ABA in the xylem sap might be involved in leaf ABA accumulation and in regulation of leaf senescence.

Since there was no significant difference in DHZ+DHZR in leaves and xylem sap as well as between K1/K1 and K1/K2, or K2/K2 and K2/K1, our present data suggest that DHZ+DHZR might not be involved as much

![Fig. 4. Effects of rootstock on endogenous cytokinin (Z+ZR, DHZ+DHZR, iP+iPA) contents in the fourth leaf of self-grafted (controls) and reciprocally grafted plants of two different senescent cotton lines. K1 (an early senescence line) and K2 (a late senescence line) scions grafted onto K2 and K1 rootstocks are expressed as K1/K2 and K2/K1, respectively. Values are means ±SD; n=4, * and ** show significant differences at P<0.05 and P<0.01 from the self-grafted control. Initial flowering, peak flowering, peak boll-setting, and initial boll-opening occurred at 65, 80, 95, and 110 days after planting, respectively.](image-url)
Fig. 5. Effects of rootstock on endogenous cytokinin (Z+ZR, DHZ+DHZR, iP+iPA) contents in rootstock sap of self-grafted (controls) and reciprocally grafted plants of two different senescent cotton lines. K1 (an early senescence line) and K2 (a late senescence line) scions grafted onto K2 and K1 rootstocks are expressed as K1/K2 and K2/K1, respectively. Values are means ± SD; * and ** show significant difference at P < 0.05 and P < 0.01 from the self-grafted control, respectively. Initial flowering, peak flowering, peak boll-setting, and initial boll-opening occurred at 65, 80, 95, and 110 d after planting, respectively.

Fig. 6. Effects of rootstock on endogenous abscisic acid (ABA) contents in the fourth leaf on main-stem from terminal and the rootstock sap of self-grafted (controls) and reciprocally grafted plants of two different senescent cotton lines. K1 (an early senescence line) and K2 (a late senescence line) scions grafted onto K2 and K1 rootstocks are expressed as K1/K2 and K2/K1, respectively. Values are means ± SD; * and ** show significant difference at P < 0.05 and P < 0.01 from the self-grafted control. Initial flowering, peak flowering, peak boll-setting, and initial boll-opening occurred at 65, 80, 95, and 110 d after planting, respectively.
in leaf senescence. The result is not in agreement with Garrison et al. (1984) who reported that DHZ and DHZR were more effective than zeatin in controlling leaf senescence. However, it should be noted that DHZ+DHZR declined as the plant aged like Z+ZR and ip+iPA. This phenomenon also suggests that the involvement of DHZ+DHZR in the control of senescence should not simply be excluded. The exact role of DHZ+DHZR in the control of cotton leaf senescence can be evaluated further in future studies.

In addition to plant hormones, differences in nutrient uptake by the rootstock may be another factor affecting leaf senescence (Wright, 1999). Further research on the relationship between leaf senescence and nutrient uptake, and the genotypic variation in nutrient allocation is necessary.

Taken together, the results in this study clearly show that Z+ZR, ip+iPA and ABA are involved in the regulation of leaf senescence, and leaf senescence is largely affected by the roots through changing levels of Z+ZR, ip+iPA, and ABA in leaves directly or indirectly.

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