RESEARCH PAPER

Mechanism of phytohormone involvement in feedback regulation of cotton leaf senescence induced by potassium deficiency

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Abstract

To elucidate the phytohormonal basis of the feedback regulation of leaf senescence induced by potassium (K) deficiency in cotton (*Gossypium hirsutum* L.), two cultivars contrasting in sensitivity to K deficiency were self- and reciprocally grafted hypocotyl-to-hypocotyl, using standard grafting (one scion grafted onto one rootstock), Y grafting (two scions grafted onto one rootstock), and inverted Y grafting (one scion grafted onto two rootstocks) at the seedling stage. K deficiency (0.03 mM for standard and Y grafting, and 0.01 mM for inverted Y grafting) increased the root abscisic acid (ABA) concentration by 1.6- to 3.1-fold and xylem ABA delivery rates by 1.8- to 4.6-fold. The K deficiency also decreased the delivery rates of xylem cytokinins [CKs; including the zeatin riboside (ZR) and isopentenyl adenosine (iPA) type] by 29–65% and leaf CK concentration by 16–57%. The leaf ABA concentration and xylem ABA deliveries were consistently greater in CCRI41 (more sensitive to K deficiency) than in SCRC22 (less sensitive to K deficiency) scions under K deficiency, and ZR- and iPA-type levels were consistently lower in the former than in the latter, irrespective of rootstock cultivar or grafting type, indicating that cotton shoot influences the levels of ABA and CKs in leaves and xylem sap. Because the scions had little influence on phytohormone levels in the roots (rootstocks) of all three types of grafts and rootstock xylem sap (collected below the graft union) of Y and inverted Y grafts, it appears that the site for basipetal feedback signal(s) involved in the regulation of xylem phytohormones is the hypocotyl of cotton seedlings. Also, the target of this feedback signal(s) is more likely to be the changes in xylem phytohormones within tissues of the hypocotyl rather than the export of phytohormones from the roots.

Key words: Absciscic acid, cotton, cytokinins, feedback regulation, potassium deficiency.

Introduction

The wide adoption of Bt cotton cultivars, noted for greater susceptibility to potassium (K) deficiency (Zhang *et al.*, 2007; Yang *et al.*, 2011), and inadequate input of K fertilizer has brought about an increasing occurrence of premature senescence in Chinese cotton-producing areas (Dong *et al.*, 2006; Tian *et al.*, 2008), characterized by early chlorophyll degradation and reduced

photosynthesis in mature leaves (Bednarz and Oosterhuis, 1995; Zhao *et al.*, 2001) during flowering and boll development. The widespread K deficiency caused by the use of higher yielding and faster fruiting cotton cultivars across the Cotton Belt of the USA was also reported about two decades ago (Oosterhuis *et al.*, 1995).

It is generally considered that cytokinins (CKs) and abscisic acid (ABA) are two major phytohormones involved in the initiation and progression of plant senescence. The results of measurement of endogenous CK levels before and during senescence (van Staden *et al.*, 1988; Singh *et al.*, 1992; Gan and Amasino, 1995), external application of CKs (van Staden *et al.*, 1988; Zavaleta-Mancera *et al.*, 1999), and manipulation of endogenous production of CKs in transgenic plants (Gan and Amasino, 1995; Rivero *et al.*, 2007; Ghanem, *et al.*, 2011a) showed that CKs can inhibit leaf senescence. The gain-of-function analyses also indicated that CK receptors AHK2 and AHK3 participate in regulating the onset of leaf senescence in *Arabidopsis* (Kim *et al.*, 2006; Riefler *et al.*, 2006). In contrast to CKs, the ABA level increases in senescing leaves (Gepstein and Thimann, 1980; Samet and Sinclair, 1980), and genes coding for proteins involved in ABA synthesis and signalling are up-regulated during leaf senescence (Buchanan-Wollaston *et al.*, 2005; van der Graaff *et al.*, 2006; Jukanti *et al.*, 2008). Furthermore, it has been known that exogenous application of ABA promotes leaf abscission and senescence (Zeevaart and Creelman, 1988), and exogenously applied ABA induces the expression of several *SAGs* (senescence-associated genes; Weaver *et al.*, 1998), which is consistent with its promotion of leaf senescence.

Nutrient deprivation (1% or 10% full-strength nutrient solution) not only decreased the levels of CKs in shoot and root (Kuiper *et al.*, 1989; Vysotskaya *et al.*, 2009), but also caused an accumulation of ABA in the shoot (Vysotskaya *et al.*, 2009). It is well known that CK levels in roots, shoots, and xylem sap are reduced in nitrogen-starved plants (Takei *et al.*, 2001; Dodd *et al.*, 2004). With respect to K, although increased ABA levels have been measured in grains and flag leaves of wheat (*Triticum aestivum*) (Haeder and Beringer, 1981), roots, xylem, and phloem sap of castor bean (*Ricinus communis* L.) (Jeschke *et al.*, 1997; Peuke *et al.*, 2002), and leaves of *Arabidopsis* (Kim *et al.*, 2009), the physiological consequences of this increase in ABA are less well known. One report (Salama and Wareing, 1979) showed that K deficiency also caused a marked decrease in CK levels in both roots and leaves.

A previous grafting study determined the roles of shoot and root in the regulation of premature leaf senescence induced by K deficiency in cotton (Li *et al.*, 2012). The results showed that the effect of rootstocks on leaf senescence was significant in some cases, but the scion cultivars explained a higher percentage of variation within grafting treatments. It was speculated that the shoot-to-root feedback signal(s) that mediates xylem phytohormone delivery was involved in the shoot regulation of premature senescence of cotton (Li *et al.*, 2012).

Several studies have shown that the root system dominates the root export of CKs via the xylem (Sitton *et al.*, 1967; McKenzie *et al.*, 1998; Dong *et al.*, 2008; Albacete *et al.*, 2009, 2010; Ghanem *et al.*, 2011b). However, results from studies on grafted pea branching mutants and *Arabidopsis* branching mutants suggested a feedback regulation of xylem sap CKs by some long-distance signals that move from shoot to root (Beveridge *et al.*, 1997; Foo *et al.*, 2007). Furthermore, the interaction of root and shoot in terms of xylem phytohormone delivery may exist when considering the recirculation of phytohormones between xylem and phloem (Dodd, 2005).

Although premature senescence is a typical symptom of K deficiency in cotton (Bednarz and Oosterhuis, 1995; Wright, 1999; Zhao *et al.*, 2001), there has not been much attention paid to the role of endogenous hormones in the leaf senescence induced by K deficiency. Therefore, the objectives of this study were to (i) examine the effects of K deficiency on the endogenous ABA, zeatin riboside (ZR) + zeatin (Z), and isopentenyl adenosine (iPA) + isopentenyladenine (iP) levels in roots, leaves, and xylem sap of cotton seedlings; (ii) determine whether there is a linkage between leaf senescence induced by K deficiency and changes in endogenous CK and ABA levels in leaves; and (iii) test the hypothesis that the leaf and xylem CK and ABA levels are dependent on feedback signal(s) derived from the shoot by using a grafting study as in previous work (Li *et al.*, 2012). The results should aid our understanding of mechanisms of phytohormone involvement in the feedback regulation of leaf senescence induced by K deficiency, and facilitate the development of approaches to managing this problem.

Materials and methods

Plant material

Two cotton cultivars contrasting in sensitivity to K deficiency were used in the present study: CCR141, developed by the Cotton Research Institute, Chinese Academy of Agricultural Sciences, which is more likely to senesce under K deficiency, and SCRC22, developed by the Cotton Research Center, Shandong Academy of Agricultural Sciences, which shows relatively late senescence under the same condition.

Growth conditions

The experiment was performed in a growth chamber with 12 h light/12 h dark at $30 \pm 2/22 \pm 2$ °C, 70–80% humidity, and $600 \mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetically active radiation. Seeds were surface-sterilized by soaking in 9% H_2O_2 for 30 min, rinsed with tap water, and then germinated in K-free sand medium for 4 d in the dark. After germination, uniform seedlings were cultured hydroponically by transferring them to 16 cm×13 cm×16 cm plastic pots filled with 2.2 litres of half-strength modified Hoagland's solution. The constituents of the solution were (mM) 2.5 Ca (NO_3)₂, 1 MgSO_4 , 0.5 (NH_4)₂ H_2PO_4 , 2×10^{-4} CuSO_4 , 1×10^{-3} ZnSO_4 , 0.1 Fe Na EDTA, 2×10^{-2} H_3BO_3 , 5×10^{-6} (NH_4)₆ Mo_7O_{24} , and 1×10^{-3} MnSO_4 . The concentration of K in the form of potassium sulphate (K_2SO_4) in the solution was 0.1 mM (mild K deficiency) before grafting and during graft recovery to ensure either a higher survival rate of grafts or faster occurrence of leaf senescence induced by severe K deficiency (0.03 mM or 0.01 mM) after graft establishment.

Grafting

Standard, Y, and inverted Y grafting of cotton seedlings were performed hypocotyl-to-hypocotyl at the cotyledonary or one-leaf stage as described previously (Li *et al.*, 2012). For each type of grafting, two cultivars were self- and reciprocally grafted; standard grafts are denoted as scion/rootstock, and 'Y' and 'inverted Y' grafts are denoted as (scion+scion)/rootstock and scion/(rootstock+rootstock), respectively. After establishment under high humidity and low light ($80 \mu\text{mol m}^{-2} \text{s}^{-1}$), surviving grafts were transferred to severe K-deficient (0.03 mM for standard and Y grafts, and 0.01 mM for inverted Y grafts because of its two rootstocks providing nutrients for only one scion) solutions to induce premature senescence, with a 2.5 mM (K-sufficient) medium as control. One week after establishment, the cotyledons and axillary bud from cotyledonary nodes of rootstock (standard and Y grafts) were removed.

Sampling of xylem sap

At the 8–9 leaf stage (~32 d after severe K deficiency treatment as above), xylem sap was collected basically according to Noodén *et al.* (1990). Ten grafts per treatment were decapitated 5–10 mm above the graft union to collect scion xylem sap, and another 10 grafts per treatment were cut below the graft union (~5 mm away) to collect rootstock xylem sap. The cut surface was wiped with distilled water to remove disrupted cells and residual cell elements, then a flexible silicon tube (length 15 mm, internal diameter 2 mm) was placed ~5–10 mm over the stump and tied tightly in place. The collection period started at 10:00 h and lasted for 24 h in the dark each time. After collection, the sap volume was quickly determined to calculate the sap flow rate over the collection period, and then the sap was freeze-dried (SIM FD5-6, LA, USA) and stored in the dark at -40 °C. The xylem ABA and CK delivery rates were calculated by multiplying phytohormone concentrations by the xylem sap flow rates.

Extraction and purification of phytohormone in leaves and roots

The fourth main-stem leaf from the apex and the whole roots were harvested before and after xylem sap collection to determine CKs and ABA. About 0.5 g of fresh samples was extracted and homogenized in 2 ml of 80% methanol (containing 40 mg l⁻¹ butylated hydroxytoluene as an antioxidant). The extract was incubated at 4 °C for 48 h, and then centrifuged at 4000 rpm for 15 min at 4 °C. The supernatant was passed through C18 Sep-Pak cartridges (Waters Corp., Millford, MA, USA), and the phytohormone fraction was eluted with 10 ml of 100% (v/v) methanol and then 10 ml of ether. The eluate was dried down by pure N₂ at 20 °C, and then stored at -40 °C.

Quantification of ABA and CKs by enzyme-linked immunosorbent assay (ELISA)

Freeze-dried xylem sap and N₂-dried extracts of leaf and root samples were dissolved in 2.0 ml of phosphate-buffered saline

(PBS) containing 0.1% (v/v) Tween-20 and 0.1% (w/v) gelatin (pH 7.5) to quantify free ABA, ZR, and iPA by ELISA following the protocol described in Zhao *et al.* (2006). The mouse monoclonal antigen and antibodies against free ABA, ZR, and iPA were produced at the Center of Crop Chemical Control, China Agricultural University, China, according to Weiler *et al.* (1981). As the anti-ZR antibody also detects Z, the CKs quantified by this antibody are described as ZR-type CKs. Similarly, the anti-iPA antibody detects iP and iPR, hence the CKs quantified by this antibody are described as iPA-type CKs. Calculations of the ELISA data were performed as described in Weiler *et al.* (1981). The recovery percentage obtained by using internal standards during extraction and analysis was all >90%.

Leaf photosynthesis measurement

In a separate experiment with four replicates and four plants for each replicate (Li *et al.*, 2012), the photosynthesis rate (*P_n*) of the fourth main-stem leaf from the apex (functional leaf) was measured by a Li-6400 portable photosynthesis system (LI-COR, Lincoln, NE, USA) with 1000 µmol m⁻² s⁻¹ quantum flux and 500 µmol mol⁻¹ CO₂ concentration.

Data analysis

Three replicates were used for each grafting treatment, and each replicate consisted of 10 plants for either rootstock or scion xylem sap samples and 20 plants for leaf and root samples. A similar trend of results was found in three independent repeat experiments; thus data were pooled across repeats. Analysis of variance (ANOVA) was performed using SAS statistical software (V8, SAS Institute Inc., Cary, NC, USA) to determine the significance of the effects of K supply, rootstock, and scion, and their interaction under K deficiency. Treatment means were compared using Duncan's multiple range test.

Fig. 1. Effect of K deficiency on ABA levels of cotton standard graft (scion/rootstock) at the 8–9 leaf stage. Grafting was performed hypocotyl-to-hypocotyl at the 1-leaf stage of the rootstock and the cotyledonary stage of the scion. Grafts were maintained in nutrient solution with 0.1 mM K during establishment, and transferred to solutions with either 2.5 mM (a) or 0.03 mM K (b) after establishment. The ABA concentrations (ng g⁻¹ FW) in roots (R) and the youngest fully expanded leaf (L), and xylem ABA delivery rates (ng plant⁻¹ 24 h⁻¹) below (B) and above the graft union (A) were determined. For each K level, means of the same sampling part (i.e. roots, leaf, and xylem sap collected both below and above the graft union) followed by the same letter are not significantly different according to Duncan's multiple range test, *P* < 0.05, *n*=4.

Results

Spatial pattern of ABA and CKs in cotton seedlings

As shown in Figs 1, 3, and 5, the ABA concentrations in cotton leaves were much higher than those in roots. The iPA-type concentrations in roots and leaves were higher than those of the ZR-type, whereas the xylem iPA-type delivery rate was much lower than that of the ZR-type (Figs 2, 4, 6), indicating that the ZR-type is the dominant type of CKs in cotton xylem sap, as in other plants (Singh *et al.*, 1992; Beveridge *et al.*, 1997; Foo *et al.*, 2007).

There were no significant differences in the xylem ABA and CK delivery rates (the product of phytohormone concentration in the xylem sap and sap flow rate) between rootstock (collected below the graft union) and scion (collected above the graft union) within a standard graft (Figs 1, 2), implying that the graft union itself had little influence on phytohormone delivery as previously demonstrated (Holbrook *et al.*, 2002; Dodd *et al.*, 2008). In addition, the sum of the xylem phytohormone levels of the two scions was more than that of the rootstock within a Y graft (Figs 3, 4), and the xylem phytohormone level in the scion was less than that of the sum of the two rootstocks within an inverted Y graft (Figs 5, 6), suggesting that the scion xylem phytohormone deliveries were independent of the xylem of the rootstocks.

ABA and CK levels in standard grafts

ABA

Under K sufficiency, there were no significant differences between SCRC22 and CCRI41 in either root (rootstocks) ABA concentrations or leaf (scion) ABA concentrations (Fig. 1a). Also, SCRC22 had similar xylem ABA delivery rates to CCRI41 both in rootstocks and in scions (Fig. 1a).

When submitted to K deficiency, the root ABA concentrations and xylem ABA delivery rates across grafts significantly ($P < 0.001$) increased by 1.6- and 4.6-fold, respectively, whereas the leaf ABA concentrations changed little (Fig. 1b; Table 1). Compared with SCRC22 self-grafts, CCRI41 self-grafts had 88, 90, and 28% greater ABA levels in roots, leaves, and xylem sap, respectively. In addition, it was observed that although the SCRC22 rootstock could reduce the ABA levels in leaves and xylem sap of CCRI41 scions compared with CCRI41 self-grafts, the corresponding values were greater than those of SCRC22 self-grafts. Similarly, the CCRI41 rootstock had a tendency to enhance the ABA levels in leaves and xylem sap of SCRC22 scions compared with SCRC22 self-grafts, but the corresponding values were lower than those of CCRI41 self-grafts (Fig. 1b). These results suggest a feedback regulation of leaf ABA concentrations and xylem ABA delivery rates by scion cultivars. Furthermore, the scion did not affect the rootstock in terms of root ABA concentrations under K deficiency (Fig. 1b; Table 1).

CKs

Under K sufficiency, the ZR- and iPA-type concentrations in roots and leaves of SCRC22 tended to be higher than those of CCRI41, but the differences were not significant in most cases. In addition, the xylem ZR-type delivery rates in SCRC22 scions

were significantly greater than those in CCRI41 scions regardless of rootstock cultivars. However, there were no significant variations in the xylem iPA-type delivery rates between scions of the two cultivars.

When exposed to K deficiency, the roots showed 73% ($P < 0.001$) more ZR-type concentrations across grafts compared with K sufficiency, and a slight but significant ($P = 0.009$) increase occurred in iPA-type concentrations (Fig. 2b; Table 1). Nonetheless, the ZR-type levels in leaves and xylem sap decreased by 32% and 29% ($P < 0.001$), and the iPA-type levels decreased 48% and 63% ($P < 0.001$), respectively. The ZR- and iPA-type levels in leaves and xylem sap of reciprocal grafts were altered insignificantly compared with self-grafts with the same scions as reciprocal grafts, suggesting a feedback regulation by scion cultivars (Fig. 2b). The same cultivar rootstock had similar root ZR- or iPA-type concentrations regardless of scion cultivar, indicating little influence of scion on rootstock. Considering genotypic variations, SCRC22 rootstocks across self- and reciprocal grafts showed 26% and 87% greater ZR- and iPA-type concentrations in roots (Fig. 2b). Also, SCRC22 scions showed 61% and 60% greater ZR- and iPA-type concentrations in leaves, and 26% and 42% greater ZR- and iPA-type delivery rates in xylem sap (averaged across above and below the graft union) than CCRI41 scions.

ABA and CK levels in Y grafts

ABA

Under K sufficiency, although there were no typical symptoms of premature senescence, genotypic variations in root and xylem ABA levels were observed between the two cultivars (Fig. 3a). Compared with SCRC22, CCRI41 rootstocks had greater ABA levels not only in roots but also in xylem sap (collected below the graft union) regardless of scion cultivars. Also, the xylem ABA delivery rates in CCRI41 scions were 93% significantly greater than those of SCRC22 scions regardless of rootstock cultivars. There were no significant differences in the leaf ABA concentrations between CCRI41 and SCRC22 scions.

Under K deficiency, the ABA levels significantly increased by 3.1-fold in roots, 1.9-fold in rootstock xylem sap, and 1.6-fold in scion xylem sap compared with K sufficiency (Fig. 3b; Table 1). However, there was no significant alteration in leaf ABA concentrations. CCRI41 rootstocks showed 86% and 32% greater ABA levels in roots and xylem sap than SCRC22 rootstocks; and its scions had consistently greater ABA levels in leaves and xylem sap than SCRC22 scions, even if they were grafted together onto the same rootstock, clearly suggesting a feedback regulation by scion cultivar. The scions did not affect root and xylem ABA levels in rootstocks (Fig. 3b).

CKs

Under K sufficiency, there were no significant differences in root ZR- and iPA-type concentrations between SCRC22 and CCRI41 rootstocks. However, the xylem ZR- and iPA-type delivery rates of SCRC22 rootstocks were much greater than those of CCRI41 rootstocks in most cases, regardless of scion cultivars. Similarly, SCRC22 scions showed greater xylem ZR- and iPA-type levels than CCRI41 scions, even if they were grafted together onto

Table 1. Summary of analysis of variance of ABA, ZR-, and iPA-type levels in the cotton grafting experiment*P*-values are presented for each main effect or interaction.

	Effect or interaction	Root	Xylem sap below the graft union	Xylem sap above the graft union	Leaf
Standard grafting					
ABA	K ^a	<0.001	<0.001	<0.001	0.061
	S-scion ^b	0.452	0.046	0.027	0.020
	S-rootstock ^c	0.096	0.129	0.881	0.690
	S-scion×rootstock ^d	0.541	0.276	0.591	0.308
	D-scion ^e	0.168	0.067	0.002	<0.001
	D-rootstock ^f	<0.001	0.033	0.057	0.010
	D-scion×rootstock ^g	0.315	0.756	0.825	0.060
ZR-type	K	<0.001	<0.001	<0.001	<0.001
	S-scion	0.097	0.003	<0.001	0.172
	S-rootstock	0.002	0.129	0.322	0.623
	S-scion×rootstock	0.295	0.085	0.651	0.470
	D-scion	0.024	0.031	<0.001	0.018
	D-rootstock	<0.001	0.051	0.040	0.392
	D-scion×rootstock	0.734	0.984	0.813	0.956
iPA-type	K	0.009	<0.001	<0.001	<0.001
	S-scion	0.166	0.613	0.005	0.037
	S-rootstock	0.262	0.574	0.864	0.505
	S-scion×rootstock	0.719	0.711	0.320	0.389
	D-scion	0.070	<0.001	<0.001	0.002
	D-rootstock	<0.001	0.094	0.043	0.030
	D-scion×rootstock	0.286	0.923	0.621	0.873
Y grafting					
ABA	K	<0.001	<0.001	<0.001	0.546
	S-rootstock	<0.001	<0.001	0.374	0.770
	D-rootstock	<0.001	<0.001	0.850	0.281
ZR-type	K	<0.001	<0.001	<0.001	<0.001
	S-rootstock	0.015	<0.001	0.662	0.305
	D-rootstock	<0.001	<0.001	0.657	0.097
iPA-type	K	<0.001	<0.001	<0.001	0.004
	S-rootstock	0.084	<0.001	0.251	0.248
	D-rootstock	<0.001	<0.001	0.159	0.641
Inverted Y grafting					
ABA	K	<0.001	<0.001	<0.001	0.308
	S-scion	0.118	0.089	0.021	<0.001
	D-scion	0.279	0.681	<0.001	<0.001
ZR-type	K	<0.001	<0.001	<0.001	<0.001
	S-scion	0.080	0.081	0.001	0.045
	D-scion	0.017	<0.001	<0.001	<0.001
iPA-type	K	<0.001	<0.001	<0.001	<0.001
	S-scion	0.100	0.002	<0.001	0.707
	D-scion	0.742	<0.001	<0.001	<0.001

^a K deficiency (0.03 mM for standard and Y grafts, and 0.01 mM for inverted Y grafts) relative to control (2.5 mM) was imposed on cotton grafts after establishment

^b Scion effect under K sufficiency (S-scion).

^c Rootstock effect under K sufficiency (S-rootstock).

^d Scion×rootstock under K sufficiency (S-scion×rootstock).

^e Scion effect under K deficiency (D-scion).

^f Rootstock effect under K deficiency (D-rootstock).

^g Scion×rootstock under K deficiency (D-scion×rootstock).

the same rootstock (Fig. 4a). No significant differences in leaf ZR- and iPA-type concentrations between the two cultivars were observed.

When subjected to K deficiency, the ZR-type levels in roots across grafts increased by 58% ($P < 0.001$) compared with K

sufficiency, and iPA-type levels changed slightly but significantly (Fig. 4b; Table 1). However, the ZR-type levels in xylem sap of rootstocks, xylem sap of scions, and leaves decreased by 39, 36, and 41% ($P < 0.001$); and the iPA-type levels decreased by 38, 43 ($P < 0.001$), and 16% ($P = 0.004$), respectively. Compared

Fig. 2. Effect of K deficiency on cytokinin (including the ZR- and iPA-type) levels of cotton standard graft (scion/rootstock) at the 8–9 leaf stage. Grafting was performed hypocotyl-to-hypocotyl at the 1-leaf stage of the rootstock and the cotyledonary stage of the scion. Grafts were maintained in nutrient solution with 0.1 mM K during establishment, and transferred to solutions with either 2.5 mM (a) or 0.03 mM K (b) after establishment. The ZR- and iPA-type concentrations (ng g^{-1} FW) in roots (R) and the youngest fully expanded leaf (L), and xylem ZR- and iPA-type delivery rates ($\text{ng plant}^{-1} 24 \text{ h}^{-1}$) below (B) and above the graft union (A) were determined. For each K level, means of the same sampling part (i.e. roots, leaf, and xylem sap collected both below and above the graft union) followed by the same letter are not significantly different according to Duncan's multiple range test, $P < 0.05$, $n=4$.

with CCRI41 self-graft, the rootstock of the SCRC22 self-graft had 37% and 55% greater ZR-type, and 37% and 43% greater iPA-type levels, in roots and xylem sap, respectively (Fig. 4b). When one of two scions of a self-graft was replaced by the other cultivar, the ZR- and iPA-type levels in both SCRC22 or CCRI41 rootstocks changed little. SCRC22 scions had more ZR- and iPA-type CKs in leaves and xylem sap than CCRI41 scions, even if they were grafted together onto the same rootstock (insignificant for the ZR type; Fig. 4b). The mean values of leaf ZR- and iPA-type concentrations and xylem ZR- and iPA-type delivery rates across SCRC22 scions were 72% and 32%, and 41% and 60% more, respectively, than those across CCRI41 scions.

ABA and CK levels in inverted Y grafts

ABA

Under K sufficiency, there were no significant differences in root and xylem ABA levels between CCRI41 and SCRC22 rootstocks, and in xylem ABA delivery rates between CCRI41 and SCRC22 scions. Nevertheless, CCRI41 scions showed greater leaf ABA concentrations than SCRC22 scions regardless of rootstock (Fig. 5a).

Under K deficiency, the ABA levels in roots and xylem sap across rootstocks strongly increased by 2.3- and 1.9-fold ($P < 0.001$) compared with K sufficiency; and those in xylem

Fig. 3. Effect of K deficiency on ABA levels of cotton Y graft (scion+scion/rootstock) at the 8–9 leaf stage. Grafting was performed hypocotyl-to-hypocotyl at the 1-leaf stage of the rootstock and the cotyledonary stage of the scion. Grafts were maintained in nutrient solution with 0.1 mM K during establishment, and transferred to solutions with either 2.5 mM (a) or 0.03 mM K (b) after establishment. The ABA concentrations (ng g⁻¹ FW) in roots (R) and the youngest fully expanded leaf (L), and xylem ABA delivery rates (ng plant⁻¹ 24 h⁻¹) below (B) and above the graft union (A) were determined. For each K level, means of the same sampling part (i.e. roots, leaf, and xylem sap collected both below and above the graft union) followed by the same letter are not significantly different according to Duncan's multiple range test, $P < 0.05$, $n=4$.

sap across scions increased by 78% ($P < 0.001$); no significant changes were observed in leaf ABA concentrations (Fig. 5b; Table 1). CCRI41 rootstocks showed significantly greater ABA levels in roots and xylem sap than SCRC22 rootstocks whether in self- or reciprocal grafts (except xylem ABA delivery rates in reciprocal grafts). In addition, the ABA levels in leaves and xylem sap of CCRI41 scions were more than those of SCRC22 scions, even if they were separately grafted onto the same combination of rootstocks, displaying the characteristic of feedback regulation. The mean values of ABA in leaves and xylem sap of CCRI41 scions were 53% and 31% more than those of SCRC22 scions.

CKs

Under K sufficiency, SCRC22 rootstocks had greater ZR- and iPA-type levels in roots and xylem sap than CCRI41 rootstocks, but not all differences were significant (Fig. 6a). Similarly, the xylem ZR- and iPA-type delivery rates in SCRC22 scions tended to be higher than those in CCRI41 scions. However, the leaf ZR- and iPA-type concentrations of SCRC22 scions were equivalent to those of CCRI41 scions.

Under K deficiency, the ZR- and iPA-type levels in roots across grafts increased by 90% and 47% ($P < 0.001$) but decreased by 33% and 63% ($P < 0.001$) in rootstock xylem sap, 42% and 73% ($P < 0.001$) in scion xylem sap, and 45% and 57% ($P < 0.001$) in leaves (Fig. 6b). SCRC22 rootstocks had more ZR- and iPA-type CKs in roots and xylem sap than CCRI41 rootstocks, even if they were grafted together with the same scion (Fig. 6b). In addition, SCRC22 scions showed more ZR- and iPA-type CKs in leaves and xylem sap than CCRI41 scions, even if they were separately grafted onto the same combination of rootstocks (Fig. 6b), suggesting a feedback

regulation by shoot cultivars. The mean ZR- and iPA-type levels in leaves and xylem sap of SCRC22 scions were 64% and 55% (leaves), and 1.2-fold and 61% (xylem sap) more than those of CCRI41 scions.

Discussion

Potassium deficiency increased ABA levels in roots and xylem sap but decreased CK levels in xylem sap and leaves of cotton seedlings

It was observed that the flow rates of xylem sap under K deficiency were lower than those under K sufficiency in the present study (data not shown) since K deficiency decreases root hydraulic conductance (Cabanero and Carvajal, 2007), which may result in the overestimation of the xylem phytohormone concentration (Dodd, 2005). Therefore, the phytohormone delivery rate (the product of xylem phytohormone concentration and sap flow rate) was used to compare the effects of different K supplies on xylem phytohormone levels.

In the present study, K deficiency (0.01 mM or 0.03 mM) increased root ABA concentrations by 1.6- to 3.1-fold, and xylem ABA flux by 1.8- to 4.6-fold across grafting types and cultivars, as compared with adequate K supply (2.5 mM). These results agreed with those of Peuke *et al.* (2002) showing that low K supply increased the deposition of ABA in the roots (1.9-fold), and root-to-shoot ABA signal in the xylem (4.6-fold). However, there was no significant change in leaf ABA concentrations across cultivars regardless of grafting type under K deficiency (Table 1), possibly due to the high degradation of xylem-sourced ABA in leaves (Zhang *et al.*, 1997; Holbrook *et al.*, 2002; Peuke *et al.*, 2002).

Fig. 4. Effect of K deficiency on cytokinin (including the ZR- and iPA-type) levels of cotton Y graft (scion+scion/rootstock) at the 8–9 leaf stage. Grafting was performed hypocotyl-to-hypocotyl at the 1-leaf stage of the rootstock and the cotyledonary stage of the scion. Grafts were maintained in nutrient solution with 0.1 mM K during establishment, and transferred to solutions with either 2.5 mM (a) or 0.03 mM K (b) after establishment. The ZR- and iPA-type concentrations (ng g⁻¹ FW) in roots (R) and the youngest fully expanded leaf (L), and xylem ZR- and iPA-type delivery rates (ng plant⁻¹ 24 h⁻¹) below (B) and above the graft union (A) were determined. For each K level, means of the same sampling part (i.e. roots, leaf, and xylem sap collected both below and above the graft union) followed by the same letter are not significantly different according to Duncan's multiple range test, $P < 0.05$, $n=4$.

The responses of ZR- and iPA-type CKs to K deficiency were different among parts of cotton seedlings. The levels of ZR- and iPA-type CKs decreased in leaves and xylem sap under K deficiency, but increased in the root tissue (albeit insignificantly for the iPA type in Y and inverted Y grafts). Salama and Wareing (1979) studied the effects of low supply of nitrogen (N), phosphorus (P), and K on endogenous CKs in sunflower (*Helianthus annuus*), and found that not only low N and low P but also low K decreased the levels of CKs in the roots. The difference between the present results and those of Salama and Wareing (1979) may be due to interspecific differences (cotton versus sunflower) in responses to K deficiency and the different intensities of K deficiency applied (~1/100 versus 1/10).

Recent studies showed potential co-regulation of the ABA and CK status in plants. Changes in ABA status can regulate shoot CK concentrations via altering cytokinin oxidase activity

(Vysotskaya *et al.*, 2009). Root overexpression of the *ipt* gene can decrease ABA accumulation in roots, xylem sap, and the mature leaf of salinized plants (Ghanem *et al.*, 2011a). In the present study, significant negative relationships of ABA with ZR- and iPA-type CKs were also found in the organs determined (data not shown). However, no causal relationship, or direct interaction, between them can be discerned.

ABA and CKs are responsible for premature senescence induced by K deficiency

Ghanem *et al.* (2008) and Albacete *et al.* (2010) have demonstrated that CKs in tomato was the hormonal parameter best related to photosystem II efficiency (F_v/F_m , indicative of leaf senescence) under salinity. In the present study, a close negative correlation of photosynthesis rate (P_n) in the fourth main-stem

Fig. 5. Effect of K deficiency on ABA levels of cotton inverted Y graft (scion/rootstock+rootstock) cotton at the 8–9 leaf stage. Grafting was performed hypocotyl-to-hypocotyl at the 1-leaf stage of both the rootstock and scion. Grafts were maintained in nutrient solution with 0.1 mM K during establishment, and transferred to solutions with either 2.5 mM (a) or 0.01 mM K (b) after establishment. The ABA concentrations (ng g⁻¹ FW) in roots (R) and the youngest fully expanded leaf (L), and xylem ABA delivery rates (ng plant⁻¹ 24 h⁻¹) below (B) and above the graft union (A) were determined. For each K level, means of the same sampling part (i.e. roots, leaf, and xylem sap collected both below and above the graft union) followed by the same letter are not significantly different according to Duncan's multiple range test, $P < 0.05$, $n=4$.

leaf from the apex (Li *et al.*, 2012), indicative of leaf senescence, with leaf ABA concentrations, scion xylem ABA delivery rates, and ratios of ABA/(ZR+iPA) type in leaves and xylem sap under K deficiency were found; a close positive correlations was also noted between Pn and CK levels in leaves and xylem sap (Figs 7, 8).

In previous work (Li *et al.*, 2012), a close positive relationship between leaf K content and senescence induced by K deficiency was observed. In order to investigate whether leaf K content influences leaf senescence via phytohormones, K content in the fourth main-stem leaf from the apex (Li *et al.*, 2012) was correlated with ABA and CK concentrations in the same leaf under K deficiency. There was a significant negative relationship between K content and ABA concentration and a significant positive relationship between K content and ZR-type concentration (except standard grafting; Fig. 9). In addition, significant relationships were found between K deliveries and phytohormone levels in rootstock xylem sap of standard (except iPA-type CKs) and inverted Y grafts and in scion xylem sap of Y grafts (data not shown). However, there were no significant relationships between root K content and ABA and CK concentrations in root tissues.

Feedback regulation of ABA and CK levels in leaves and scion xylem sap (collected above the graft union) is guaranteed by the different grafting types

Grafting is a useful tool to test whether there was a feedback regulation of some physiological responses, including leaf senescence. For the seedlings exposed to K deficiency, the rootstocks

have a tendency to alter phytohormone levels in leaves and scion xylem sap in some cases (Table 1), even significantly for the leaf ABA concentration of a CCRI41 scion grafted onto a SCRC22 rootstock (compared with CCRI41 self-graft; Fig. 1b), but the scion cultivars explained a higher percentage of variation within grafting treatments under K deficiency irrespective of grafting types. No interactions were found between rootstock and scion in the standard grafting experiment regardless of plant organs and phytohormone types (Table 1), indicating that rootstock and scion are autonomous to a great extent in terms of ABA and CK levels.

The levels of ZR- and iPA-type CKs were consistently lower in leaves and xylem of CCRI41 scions than those of SCRC22 scions, and the leaf ABA concentrations and scion xylem ABA flux were consistently greater in grafts with CCRI41 as scions than those with SCRC22 as scions. These results revealed that the cotton shoot can modify (i.e. feedback regulate) the import of phytohormones from the root, as reported in Beveridge *et al.* (1997) and Foo *et al.* (2007) for pea and *Arabidopsis*, and the leaf phytohormone concentrations. Furthermore, it was noticed that it is the xylem phytohormone concentration rather than sap volume that was regulated by shoot cultivars. With respect to seedlings grown under K sufficiency, although significant genotypic differences in scion xylem CK flux were observed in some cases, the leaf CK concentrations were similar between CCRI41 and SCRC22 scions (Figs 2a, 4a, 6a; Table 1).

Consistent with the present results, Gan and Amasino (1995) and Faiss *et al.* (1997) found that the CK-overproducer rootstock failed to delay leaf senescence via a grafting study. Due to the absence of data for xylem CKs in grafts which they performed, it

Fig. 6. Effect of K deficiency on cytokinin (including the ZR- and iPA-type) levels of cotton inverted Y graft (scion+scion/rootstock) cotton at the 8–9 leaf stage. Grafting was performed hypocotyl-to-hypocotyl at the 1-leaf stage of both the rootstock and scion. Grafts were maintained in nutrient solution with 0.1 mM K during establishment, and transferred to solutions with either 2.5 mM (a) or 0.01 mM K (b) after establishment. The ZR- and iPA-type concentrations (ng g⁻¹ FW) in roots (R) and the youngest fully expanded leaf (L), and xylem ZR- and iPA-type delivery rates (ng plant⁻¹ 24 h⁻¹) below (B) and above the graft union (A) were determined. For each K level, means of the same sampling part (i.e. roots, leaf, and xylem sap collected both below and above the graft union) followed by the same letter are not significantly different according to Duncan's multiple range test, $P < 0.05$, $n=4$.

remained unclear whether or not there was a feedback regulation of CKs exported from roots by the shoots. However, the present results are different from the classic notion that the roots play an important role in regulation of leaf senescence by delivering hormones to shoots (van Staden *et al.*, 1988; McKenzie *et al.*, 1998; Dong *et al.*, 2008; Albacete *et al.*, 2009, 2010; Ghanem *et al.*, 2011b), reflecting the diversity and complexity of the long-distance signalling system in higher plants. As for ABA, studies with reciprocal grafts of wild-type plants and ABA-deficient mutants indicated that the mutant rootstocks had no/little effect on ABA concentrations in xylem sap (Dodd *et al.*, 2009) and in the leaves (Holbrook *et al.*, 2002) of wild-type scions, which are similar to those in the present study.

The site of feedback regulation of xylem ABA and CK levels is the hypocotyl

Xylem sap was collected below and above the graft union, and the phytohormone levels were measured separately. This permits the site of feedback regulation to be deduced more precisely. The three types of grafting were all performed hypocotyl-to-hypocotyl. Because scions did not influence the root (rootstock) ABA and CK concentrations (Figs 1b, 2b, 3b, 4b, 5b, 6b), it is postulated that the site of feedback regulation of xylem phytohormones by scion lies beyond the root tissue. For standard grafts, no significant differences in xylem ABA and CK delivery between rootstock (collected below the graft union) and scion

Fig. 7. Relationships between leaf ABA, ZR-, and iPA-type concentrations, and ABA/(ZR+iPA) ratios (x) with photosynthetic rate (Pn) of the youngest fully expanded leaf (the fourth leaf from the top of the plant) grown under K deficiency (0.03 mM for standard and Y grafts, and 0.01 mM for inverted Y grafts). Open and filled circles denote CCR141 and SCRC22 scions of standard grafts, respectively; open and filled triangles denote CCR141 and SCRC22 scions of Y grafts; and open and filled inverted triangles denote CCR141 and SCRC22 scions of inverted Y grafts. Each point represents the mean of a grafting treatment averaged across three or four replicates, and each replicate consisted of 4–20 plants. The linear regressions were fitted with Sigmaplot 11.0.

(collected above the graft union) within a graft were found (Figs 1b, 2b), suggesting that the site of feedback regulation of xylem phytohormones in standard grafts is most probably the rootstock hypocotyl (from the root–shoot junction to the graft union). In terms of Y and inverted Y grafts, the scion(s) did not significantly affect the xylem ABA and CK flux in rootstock(s) (collected below the graft union) (Figs 3b, 4b, 5b, 6b), thus implying that the main site of feedback regulation of xylem phytohormones by scion(s) is the scion hypocotyl (above the graft union), and the target of feedback signal(s) is more likely to be the changes in xylem phytohormone levels within hypocotyl tissues rather than the export of phytohormones from the roots.

It is considered that when/after xylem phytohormones passed through the graft union from rootstock(s) to scion(s) in Y and inverted Y grafts, they experienced great changes in metabolism, and/or exchanges with xylem parenchyma cells via the action of feedback signal(s). This presumption can at least partly

explain the imbalance between rootstock(s) and scion(s) within either a Y graft or an inverted Y graft in terms of xylem phytohormone delivery rates (Figs 3–6), and is supported by the literature. Sauter and Hartung (2002) demonstrated that the lateral transport of ABA in the stem (from the stem parenchyma to the xylem, and vice versa) may contribute to modifying the xylem ABA levels, and xylem sap can be enriched with ABA sourced from xylem parenchyma cells by the higher xylem pH (Li *et al.*, 2011). Moreover, it is known that CKs may be modulated along their transport pathway, for example by movement from xylem to parenchyma cells in the stem or petioles (Singh *et al.*, 1992). Future studies need to confirm the site of feedback regulation of xylem phytohormones further by using interstock grafting and top grafting (on the main stem above the cotyledons, as opposed to the hypocotyl in the present study), and the physiological and molecular mechanisms underlying the changes in xylem phytohormones levels within hypocotyl tissues.

Fig. 8. Relationships between ABA, ZR-, and iPA-type delivery rates, and ABA/(ZR+iPA) ratios (x) in scion xylem sap (collected above the graft union) and photosynthetic rate (P_n) of the youngest fully expanded leaf (the fourth leaf from the top of the plant) grown under K deficiency (0.03 mM for standard and Y grafts, and 0.01 mM for inverted Y grafts). Open and filled circles denote CCR141 and SCRC22 scions of standard grafts, respectively; open and filled triangles denote CCR141 and SCRC22 scions of Y grafts; and open and filled inverted triangles denote CCR141 and SCRC22 scions of inverted Y grafts. Each point represents the mean of a grafting treatment averaged across three or four replicates, and each replicate consisted of 4–20 plants. The linear regressions were fitted with Sigmaplot 11.0.

The mechanism of feedback regulation of leaf ABA and CK concentrations remains unclear

Theoretically, the level of phytohormones in leaf is regulated at diverse steps, including *de novo* synthesis, activation, conjugation, and degradation, as well as local and long-distance transport. Since the differences in xylem CKs levels between CCR141 and SCRC22 scions were similar to those differences in leaf CKs concentrations under K deficiency, we cannot exclude the xylem contribution to leaf phytohormone status by long-distance transport in the present study.

The capacity of the leaf to biosynthesize ABA and CKs has been well demonstrated (Miyawaki *et al.*, 2004; Endo *et al.*, 2008). Furthermore, both ABA inactivation (the conjugation with glucose by ABA glucosyltransferase to form ABA glucose ester; Xu *et al.*, 2002) and CK degradation by cytokinin oxidase (Vysotskaya *et al.*, 2009) can occur in the leaf. Therefore, further metabolic studies such as on *de novo* synthesis, activation,

conjugation, and degradation, and local and long-distance transport need to be undertaken to find the exact mechanism underlying the feedback regulation of leaf phytohormone concentrations.

Conclusion

K deficiency strongly increased ABA levels in roots and xylem sap of cotton seedlings and decreased those of ZR- and iPA-type CKs in xylem sap and leaves. Correlation analysis indicated that ABA, ZR-, and iPA-type levels in leaves and scion xylem sap (collected above the graft union) under K deficiency were closely associated with leaf senescence. The results of both standard (one scion grafted onto one rootstock) and Y (two scions grafted onto one rootstock) or inverted Y (one scion grafted onto two rootstocks) grafting showed that the ABA and CK levels in leaves and scion xylem sap were feedback regulated by scion cultivars under K deficiency. The main action site of basipetal feedback signal(s) involved in xylem phytohormones is the

Fig. 9. Relationships between K content (x) and ABA, ZR-, and iPA-type concentrations in the youngest fully expanded leaf (the fourth leaf from the top of plant) grown under K deficiency (0.03 mM for standard and Y grafts, and 0.01 mM for inverted Y grafts). Open and filled circles denote CCR141 and SCRC22 scions of standard grafts, respectively; open and filled triangles denote CCR141 and SCRC22 scions of Y grafts; and open and filled inverted triangles denote CCR141 and SCRC22 scions of inverted Y grafts. Each point represents the mean of a grafting treatment averaged across three or four replicates, and each replicate consisted of 4–20 plants. The linear regressions were fitted with Sigmaplot 11.0.

rootstock hypocotyl (below the graft union) in standard grafting and the scion hypocotyl (above the graft union) in Y and inverted Y grafting. The target of this feedback signal(s) is more likely to be the changes in xylem phytohormones within hypocotyl tissues rather than the export of phytohormones from the roots. The mechanism of feedback regulation of leaf ABA and CK concentrations remains unclear. The results of this study have provided an improved understanding of communication between shoot and root, and are beneficial for the development of approaches to managing this problem.

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References

- Albacete A, Ghanem ME, Dodd IC, Pérez-Alfocea F.** 2010. Principal component analysis of hormone profiling data suggests an important role for cytokinins in regulating leaf growth and senescence of salinized tomato. *Plant Signaling and Behavior* **5**, 45–48.
- Albacete A, Martinez-Andujar C, Ghanem ME, Acosta M, Sanchez-Bravo J, Asins MJ, Cuartero J, Lutts S, Dodd IC, Pérez-Alfocea F.** 2009. Rootstock-mediated changes in xylem ionic and hormonal status are correlated with delayed leaf senescence, and increased leaf area and crop productivity in salinized tomato. *Plant, Cell and Environment* **32**, 928–938.
- Bednarz CW, Oosterhuis DM.** 1995. Plant potassium partitioning during progression of deficiency symptoms in cotton (*Gossypium hirsutum*). *Better Crops. Potash and Phosphate Institute* **79**, 12–14.
- Beveridge CA, Murfet IC, Kerhoas L, Sotta B, Miginiac E, Rameau C.** 1997. The shoot controls zeatin riboside export from pea roots. Evidence from the branching mutant rms4. *The Plant Journal* **11**, 339–345.
- Buchanan-Wollaston V, Page T, Harrison E, Breeze E, Lim PO, Nam HG, Lin JF, Wu SH, Swidzinski J, Ishizaki K, and Leaver CJ** 2005. Comparative transcriptome analysis reveals significant differences in gene expression and signalling pathways between developmental and dark/starvation-induced senescence in *Arabidopsis*. *The Plant Journal* **42**, 567–585.
- Cabanero FJ, Carvajal M.** 2007. Different cation stresses affect specifically osmotic root hydraulic conductance, involving aquaporins, ATPase and xylem loading of ions in *Capsicum annuum*, L. plants. *Journal of Plant Physiology* **164**, 1300–1310.
- Dodd IC.** 2005. Root-to-shoot signalling: assessing the roles of 'up' in the up and down world of long-distance signalling in planta. *Plant and Soil* **274**, 251–270.
- Dodd IC, Egea G, Davies WJ.** 2008. Accounting for sap flow from different parts of the root system improves the prediction of xylem

ABA concentration in plants grown with heterogeneous soil moisture. *Journal of Experimental Botany* **59**, 4083–4093.

Dodd IC, Ngo C, Turnbull CGN, Beveridge CA. 2004. Effects of nitrogen supply on xylem cytokinin delivery, transpiration and leaf expansion of pea genotypes differing in xylem-cytokinin concentration. *Functional Plant Biology* **31**, 903–911.

Dodd IC, Theobald JC, Richer SK, Davies WJ. 2009. Partial phenotypic reversion of ABA-deficient flacca tomato (*Solanum lycopersicum*) scions by a wild-type rootstock: normalizing shoot ethylene relations promotes leaf area but does not diminish whole plant transpiration rate. *Journal of Experimental Botany* **60**, 4029–4039.

Dong HZ, Li WJ, Tang W, Li ZH, Zhang DM. 2006. Effects of genotypes and plant density on yield, yield components and photosynthesis in *Bt* transgenic cotton. *Journal of Agronomy and Crop Science* **192**, 132–139.

Dong HZ, Niu YH, Li WJ, Zhang DM. 2008. Effects of cotton rootstock on endogenous cytokinins and abscisic acid in xylem sap and leaves in relation to leaf senescence. *Journal of Experimental Botany* **59**, 1295–1304.

Endo A, Sawada Y, Takahashi H, Okamoto M, Ikegami K, Koiwai H, Seo M, Toyomasu T, Mitsuhashi W, Shinozaki K, Nakazono M, Kamiya Y, Koshiha T, Nambara E. 2008. Drought induction of *Arabidopsis* 9-cis-epoxycarotenoid dioxygenase occurs in vascular parenchyma cells. *Plant Physiology* **147**, 1984–1993.

Faiss M, Zalubilová J, Strnad M, Schmülling T. 1997. Conditional transgenic expression of the *ipt* gene indicates a function for cytokinins in paracrine signaling in whole tobacco plants. *The Plant Journal* **12**, 401–415.

Foo E, Morris SE, Parmenter K, Young N, Wang H, Jones A, Rameau C, Turnbull CGN, Beveridge CA. 2007. Feedback regulation of xylem cytokinin content is conserved in pea and *Arabidopsis*. *Plant Physiology* **143**, 1418–1428.

Gan SS, Amasino RM. 1995. Inhibition of leaf senescence by autoregulated production of cytokinin. *Science* **270**, 1986–1988.

Gepstein S, Thimann KV. 1980. Changes in the abscisic acid content of oat leaves during senescence. *Proceedings of the National Academy of Sciences, USA* **77**, 2050–2053.

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

Ghanem ME, Albacete A, Smigocki AC, Frébort I, Pospíšilová H, Martínez-Andújar C, Acosta M, Sánchez-Bravo J, Lutts S, Dodd IC, Pérez-Alfocea F. 2011a. Root-synthesized cytokinins improve shoot growth and fruit yield in salinized tomato (*Solanum lycopersicum* L.) plants. *Journal of Experimental Botany* **62**, 125–140.

Ghanem ME, Hichri I, Smigocki AC, Albacete A, Fauconnier ML, Diatloff E, Martinez-Andujar C, Lutts S, Dodd IC, Pérez-Alfocea F. 2011b. Root-targeted biotechnology to mediate hormonal signalling and improve crop stress tolerance. *Plant Cell Reports* **30**, 807–823.

Haeder HE, Beringer H. 1981. Influence of potassium nutrition and water stress on the content of abscisic acid in grains and flag leaves

of wheat during grain development. *Journal of the Science of Food and Agriculture* **32**, 552–556.

Holbrook NM, Shashidhar VR, James RA, Munns R. 2002. Stomatal control in tomato with ABA-deficient roots: response of grafted plants to soil drying. *Journal of Experimental Botany* **53**, 1503–1514.

Jeschke WD, Peuke AD, Pate JS, Hartung W. 1997. Transport, synthesis and catabolism of abscisic acid (ABA) in intact plants of castor bean (*Ricinus communis* L.) under phosphate deficiency and moderate salinity. *Journal of Experimental Botany* **48**, 1737–1747.

Jukanti AK, Heidlebaugh NM, Parrott DL, Fischer IA, McInerney K, Fischer AM. 2008. Comparative transcriptome profiling of near-isogenic barley (*Hordeum vulgare*) lines differing in the allelic state of a major grain protein content locus identifies genes with possible roles in leaf senescence and nitrogen reallocation. *New Phytologist* **177**, 333–349.

Kim HJ, Ryu H, Woo HR, Lim PO, Lee IC, Sheen J, Nam HG, Hwang I. 2006. Cytokinin-mediated control of leaf longevity by AHK3 through phosphorylation of ARR2 in *Arabidopsis*. *Proceedings of the National Academy of Sciences, USA* **106**, 814–819.

Kim MJ, Shin R, Schachtman DP. 2009. A nuclear factor regulates abscisic acid responses in *Arabidopsis*. *Plant Physiology* **151**, 1433–1445.

Kuiper D, Kuiper PJC, Lambers H, Schuit J, Staal M. 1989. Cytokinin concentration in relation to mineral nutrition and benzyladenine treatment in *Plantago major* ssp. *pleiosperma*. *Physiologia Plantarum* **75**, 511–517.

Li B, Wang Y, Zhang ZY, Wang BM, Eneji AE, Duan LS, Li ZH, Tian XL. 2012. Cotton shoot plays a major role in mediating senescence induced by potassium deficiency. *Journal of Plant Physiology* **169**, 327–335.

Li BB, Feng ZG, Xie M, Sun MZ, Zhao YX, Liang LY, Liu GJ, Zhang JH, Jia WS. 2011. Modulation of the root-sourced ABA signal along its way to the shoot in *Vitis riparia* × *Vitis labrusca* under water deficit. *Journal of Experimental Botany* **62**, 1731–1741.

McKenzie MJ, Mett V, Reynolds PHS, Jameson PE. 1998. Controlled cytokinin production in transgenic tobacco using a copper-inducible promoter. *Plant Physiology* **116**, 969–977.

Miyawaki K, Matsumoto-Kitano M, Kakimoto T. 2004. Expression of cytokinin biosynthetic isopentenyltransferase genes in *Arabidopsis*: tissue specificity and regulation by auxin, cytokinin, and nitrate. *The Plant Journal* **37**, 128–138.

Noodèn LD, Singh S, Letham DS. 1990. Correlation of xylem sap cytokinin levels with monocarpic senescence in soybean. *Plant Physiology* **93**, 33–39.

Oosterhuis DM. 1995. Potassium nutrition of cotton with particular reference to foliar fertilization. In: Constable GA, Forrester NW, eds. *World Cotton Research Conference*. Brisbane, Australia: CSIRO, 133–146.

Peuke AD, Jeschke WD, Hartung W. 2002. Flows of elements, ions and abscisic acid in *Ricinus communis* and site of nitrate reduction under potassium limitation. *Journal of Experimental Botany* **53**, 241–250.

- Riefler M.** 2006. *Arabidopsis* cytokinin receptor mutants reveal functions in shoot growth, leaf senescence, seed size, germination, root development, and cytokinin metabolism. *The Plant Cell* **18**, 40–54.
- 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. *Proceedings of the National Academy of Sciences, USA* **104**, 19631–19636.
- Salama AMSEA, Wareing PF.** 1979. Effects of mineral nutrition on endogenous cytokinins in plants of sunflower (*Helianthus annuus* L.). *Journal of Experimental Botany* **30**, 971–981.
- Samet JS, Sinclair TR.** 1980. Leaf senescence and abscisic acid in leaves of field-grown soybean. *Plant Physiology* **66**, 1164–1168.
- Sauter A, Hartung W.** 2002. The contribution of internode and mesocotyl tissues to root-to-shoot signalling of abscisic acid. *Journal of Experimental Botany* **53**, 297–302.
- Singh S, Letham DS, Palni LMS.** 1992. Cytokinin biochemistry in relation to leaf senescence. VIII. Translocation, metabolism and biosynthesis of cytokinins in relation to sequential leaf senescence of tobacco. *Physiologia Plantarum* **86**, 398–406.
- Sitton D, Itai C, Kende H.** 1967. Decreased cytokinin production in the roots as a factor in shoot senescence. *Planta* **73**, 296–300.
- Takei K, Sakakibara H, Taniguchi M, Sugiyama T.** 2001. Nitrogen-dependent accumulation of cytokinins in root and the translocation to leaf: implication of cytokinin species that induces gene expression of maize response regulator. *Plant and Cell Physiology* **42**, 85–93.
- Tian XL, Wang GW, Yang FQ, Yang PZ, Duan LS, Li ZH.** 2008. Differences in tolerance to low-potassium supply among different types of cultivars in cotton (*Gossypium hirsutum*). *Acta Agronomica Sinica* **34**, 1770–1780.
- van der Graaff E, Schwacke R, Schneider A, Desimone M, Flügge UI, Kunze R.** 2006. Transcription analysis of *Arabidopsis* membrane transporters and hormone pathways during developmental and induced leaf senescence. *Plant Physiology* **141**, 776–792.
- van Staden J, Cook E, Noodén LD.** 1988. Cytokinins and senescence. In: Noodén LD, Leopold A, eds. *Senescence and aging in plants*. London: Academic Press, 281–328.
- Vysotskaya LB, Korobova AV, Veselov SY, Dodd IC, Kudoyarova GR.** 2009. ABA mediation of shoot cytokinin oxidase activity: assessing its impacts on cytokinin status and biomass allocation of nutrient-deprived durum wheat. *Functional Plant Biology* **36**, 66–72.
- Weaver LM, Gan SS, Quirino B, Amasino RM.** 1998. A comparison of the expression patterns of several senescence-associated genes in response to stress and hormone treatment. *Plant Molecular Biology* **37**, 455–469.
- Weiler EW, Jourdan PS, Conrad W.** 1981. Levels of indole-3-acetic acid in intact and decapitated coleoptiles as determined by a specific and highly sensitive solid-phase enzyme immunoassay. *Planta* **153**, 561–571.
- Wright PR.** 1999. Premature senescence of cotton (*Gossypium hirsutum* L.): predominantly a postassium disorder caused by an imbalance of source and sink. *Plant and Soil* **211**, 231–239.
- Xu ZJ, Nakajima M, Suzuki Y, Yamaguchi I.** 2002. Cloning and characterization of the abscisic acid-specific glucosyltransferase gene from adzuki bean seedlings. *Plant Physiology* **129**, 1285–1295.
- Yang FQ, Wang GW, Zhang ZY, Eneji AE, Duan LS, Li ZH, Tian XL.** 2011. Genotypic variations in potassium uptake and utilization in cotton. *Journal of Plant Nutrition* **34**, 83–91.
- Zavaleta-Mancera HA, Franklin KA, Ougham HJ, Thomas H, Scott IM.** 1999. Regreening of senescent *Nicotiana* leaves. I. Reappearance of NADPH-protochlorophyllide oxidoreductase and light-harvesting chlorophyll a/b binding protein. *Journal of Experimental Botany* **50**, 1677–1682.
- Zeevaart JAD, Creelman RA.** 1988. Metabolism and physiology of abscisic acid. *Annual Review of Plant Physiology and Plant Molecular Biology* **39**, 39–473.
- Zhang J, Jia W, Zhang DP.** 1997. Effect of leaf water status and xylem pH on metabolism of xylem-transported abscisic acid. *Plant Growth Regulation* **21**, 51–58.
- Zhang ZY, Tian XL, Duan LS, Wang BM, He ZP, Li ZH.** 2007. Differential responses of conventional and Bt-transgenic cotton (*Gossypium hirsutum* L.) to potassium deficiency. *Journal of Plant Nutrition* **30**, 659–671.
- Zhao D, Oosterhuis DM, Bednarz CW.** 2001. Influence of potassium deficiency on photosynthesis, chlorophyll content, and chloroplast ultrastructure of cotton plants. *Photosynthetica* **39**, 103–109.
- Zhao J, Li G, Yi GX, Wang BM, Deng AX, Nan TG, Li ZH, Li QX.** 2006. Comparison between conventional indirect competitive enzyme-linked immunosorbent assay (icELISA) and simplified icELISA for small molecules. *Analytica Chimica Acta* **571**, 79–85.