Journal of Plant Physiology 169 (2012) 327–335

Contents lists available at SciVerse ScienceDirect

Journal of Plant Physiology

journal homepage: www.elsevier.de/jplph

Cotton shoot plays a major role in mediating senescence induced by potassium deficiency

Bo Li[a,b,1], Ye Wang[a,1], Zhiyong Zhang[a,c,1], Baomin Wang[a], A. Egrinya Eneji[d], Liusheng Duan[a], Zhaohu Li[a], Xiaoli Tian[a,*]

[a] State Key Laboratory of Plant Physiology and Biochemistry, Key Laboratory of Crop Cultivation and Farming System, Center of Crop Chemical Control, China Agricultural University, Beijing 100193, China
[b] Shandong Kingenta Ecological Engineering Co., Ltd., China
[c] School of Life Science and Technology, Henan Institute of Science and Technology, Xinxiang, Henan, 453003, China
[d] Department of Soil Science, Faculty of Agriculture, Forestry and Wildlife Resources Management, University of Calabar, Nigeria

ARTICLE INFO

Article history:
Received 13 April 2011
Received in revised form 18 October 2011
Accepted 19 October 2011

Keywords:
Cotton (*Gossypium hirsutum* L.)
Grafting
Leaf senescence
Potassium deficiency

ABSTRACT

The objective of this study was to determine the roles of shoot and root in the regulation of premature leaf senescence induced by potassium (K) deficiency in cotton (*Gossypium hirsutum* L.). Two contrasting cultivars (CCRI41, more sensitive to K deficiency; and SCRC22, a less sensitive cultivar) were selected for self- and reciprocal-grafting, using standard grafting (one scion/one rootstock), Y grafting (two scions/one rootstock) and inverted Y grafting (one scion/two rootstocks) at the seedling stage. Standard grafting was studied in the field in 2007 and 2008. There were no obvious differences in senescence between CCRI41 and SCRC22 scions while supplied with sufficient K. However, SCRC22 scions showed significantly greater K content, SPAD values (chlorophyll content), soluble protein content and net photosynthetic rates than CCRI41 scions while grown in K deficient solution or soil, regardless of rootstock cultivars, grafting types, growth stage and growth conditions. Also, SCRC22 scions had greater yield and less variation in boll weight either between upper- and lower sympodials, or between proximal and distal fruit positions from the main stem in the field under K deficiency, probably owing to reduced leaf senescence. Although the effect of rootstocks on leaf senescence under K deficiency was significant in some cases, the scion cultivars explained the highest percentage of variations within grafting treatments. The shoot-to-root feedback signal(s), rather than high shoot demand for K nutrition, was involved in the shoot regulation of premature senescence in cotton plants, achieved possibly by altering root K uptake.

Introduction

Transgenic *Bacillus thuringiensis* Berliner (Bt) cotton (*Gossypium hirsutum* L.) has been adopted in most major cotton countries including China, where its adoption reached 70% in recent years (James, 2009). However, Bt cotton cultivars are more susceptible to potassium (K) deficiency (Zhang et al., 2007; Yang et al., 2011), although the mechanisms underlying this response are not well understood. In parallel with the inadequate input of K fertilizer, premature senescence, characterized by early chlorophyll degradation and reduced photosynthesis in mature leaves (Bednarz and Oosterhuis, 1995; Zhao et al., 2001) during flowers and boll development (Wright, 1999), has been occurring on an increasing scale (Dong et al., 2006; Tian et al., 2008) and this has limited cotton productivity (Zhao et al., 2001).

Premature senescence of cotton induced by K deficiency was believed to result from faster fruit set, greater boll load and/or reduced root growth (Brouder and Cassman, 1990; Wright, 1999; Pettigrew, 2003), since the former would enhance remobilization of K from leaves and the latter would impair K supply to leaves. Dong et al. (2008) selected two Bt cotton lines with the same yield potential but contrasting senescence properties to study the factors controlling leaf senescence, and found that leaf senescence may result from root characteristics. Also, we observed that premature senescence/K-deficiency symptoms can occur in some cotton cultivars before first bloom when boll load is negligible in fields with low available K (unpublished data). Therefore, whether shoot demand or root supply or both dominate the premature senescence caused by K deficiency in cotton remains unclear and this has impeded the development of management solutions to the problem.

* Corresponding author at: Department of Agronomy, College of Agronomy and Biotechnology, China Agricultural University, No. 2 Yuanmingyuan West Road, Haidian, Beijing 100193, China. Tel.: +86 10 62732567; fax: +86 10 62731569.
E-mail addresses: tianxl@cau.edu.cn, tian_xiaoli@163.com (X. Tian).
[1] These authors contributed to this paper equally.

0176-1617/$ – see front matter © 2011 Elsevier GmbH. All rights reserved.
doi:10.1016/j.jplph.2011.10.009

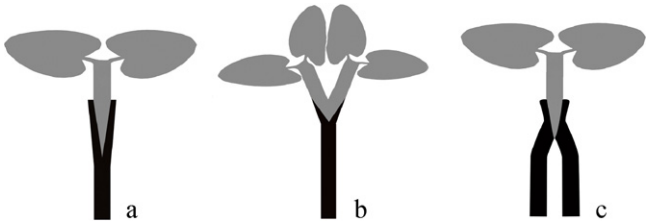

Fig. 1. Sketch of the three types of grafting studied: (a) standard graft with one scion and one rootstock; (b) "Y" graft with two scions grafted onto one rootstock; (c) "inverted Y" graft with one scion grafted onto two rootstocks.

Grafting is commonly used for horticultural crops, and also has been used as a tool to explore root–shoot interactions governing a range of physiological responses, including abiotic stress tolerance (Ghanem et al., 2011b). Data for the relative importance of root (rootstock) and shoot (scion) in regulating growth and some physiological processes are variable. There is considerable evidence that elite rootstocks can mediate resistance to biotic (Anwar et al., 2002) and abiotic stress in plants, such as thermal stress (Rivero et al., 2003) and salt stress (Ruiz et al., 2006; Albacete et al., 2009, 2010; Ghanem et al., 2011a), thereby influencing scion growth and yield (Van Norman et al., 2004; Dodd et al., 2009; Jones et al., 2009) as well as delaying leaf senescence (Dong et al., 2008; Ghanem et al., 2011a). The importance of the shoot, in the regulation of growth (Chen et al., 2003; Tandonnet et al., 2010), leaf senescence (Faiss et al., 1997), branching (Beveridge et al., 1997; Foo et al., 2007), and drought tolerance (Holbrook et al., 2002) was also documented. Grafting studies indicate that both shoot and root are effective in the regulation of cambium development (Matsumoto-Kitano et al., 2008), growth (Werner et al., 2010), and the response of the plants to salt stress (Etehadnia et al., 2008). Other studies have shown that the relative role of root and shoot in grafted plants depended on the environment (Cornish and Zeevaart, 1988), species (Rivero et al., 2004), genotype (Beveridge, 2000; Holbrook et al., 2002; Dodd et al., 2009), growth stage (Ookawa et al., 2001), and growth processes (Holbrook et al., 2002).

To investigate rootstock and scion effects on premature senescence in cotton, we have developed three types of grafting between two contrasting cultivars (Li et al., 2009). The first type is standard grafting involving one scion and one rootstock (Fig. 1a); this can evaluate the relative role of root and shoot in regulating senescence. The second type is "Y" grafting with two scions grafted onto one rootstock (Fig. 1b) to more precisely evaluate the role of shoot physiology at the same level of mineral and phytohormone supply. The third type is "inverted Y" grafting with one scion grafted onto two rootstocks (Fig. 1c) to evaluate interactions between two contrasting roots in regulation of leaf senescence. In the present study, the three types of grafts were studied at high (2.5 mM, as control) and low (0.03 or 0.01 mM, to induce premature senescence) levels of K in the growth chamber at the seedling stage. The standard grafts were also studied in the field at different levels of K fertilizer. A better understanding of rootstock and scion regulation of premature senescence in cotton would facilitate the development of approaches to manage this problem.

Materials and methods

Plant material

Two transgenic insect-resistant cotton (*Gossypium hirsutum* L.) cultivars, CCRI41 [containing *Bacillus thuringiensis* (Bt) and cowpea trypsin inhibitor (CpTI), developed by the Cotton Research Institute, Chinese Academy of Agricultural Sciences] and SCRC22 (containing Bt, developed by the Cotton Research Center, Shandong Academy of Agricultural Sciences), were used in the present study. Although both genotypes contain the *Bt* gene, they exhibit different degrees of leaf senescence. CCRI41 is susceptible to senescence induced by K deficiency, whereas SCRC22 has relatively late senescence under K deficiency.

Growth chamber experiment

Growth conditions

The experiment was performed in a growth chamber with 12 h light/12 h dark at $30 \pm 2/22 \pm 2\,^{\circ}C$, 70–80% humidity and 600 $\mu mol\,m^{-2}\,s^{-1}$ photosynthetically active radiation. Seeds were surface-sterilized with 9% H_2O_2 for 30 min, and then germinated in a K-free sand medium. After emergence (4 d after germination), uniform seedlings were cultured hydroponically by transferring to 16 cm × 13 cm × 16 cm plastic pots filled with 2.2 L of 1/2-strength modified Hoagland's solution. The constituents of the solution were (mM) 2.5 Ca $(NO_3)_2$, 1 $MgSO_4$, 0.5 $(NH_4)H_2PO_4$, 2×10^{-4} $CuSO_4$, 1×10^{-3} $ZnSO_4$, 0.1 Fe Na EDTA, 2×10^{-2} H_3BO_3, 5×10^{-6} $(NH_4)_6Mo_7O_{24}$ and 1×10^{-3} $MnSO_4$. The concentration of K in the form of potassium sulphate (K_2SO_4) in solutions varied before and after grafting (see below).

Four uniform seedlings were raised per pot. All solutions were changed twice per week. De-ionized water was added daily to replace the water lost by evapo-transpiration. Solution pH was maintained at 6.5 by adding concentrated solution of NaOH and air was bubbled into the solution to provide O_2 and achieve nutrient homogeneity.

Grafting

Preliminary studies indicated that grafting did not affect the growth and development of cotton plants, irrespective of the type of grafting and growth environment (growth chamber or field). For example, there were no significant differences in SPAD values of the youngest fully expanded leaves (the 4th leaf from the top of plant) between ungrafted control and self-grafts in the growth chamber experiment, as well as lint yield in the field (Table 1). Therefore, only self-grafts were used as controls in the present study.

To properly evaluate the contribution of root and shoot to K deficiency-induced senescence, grafting at the root–shoot junction is necessary. However, the survival rate was too low for this kind of grafting to yield meaningful results. Consequently, the graft union in the present study was located at the cotyledonary node of the rootstock or just below it.

Standard grafting: When the rootstocks (4 d after germination, i.e. 4 DAG) were transferred to solutions containing 0.1 mM K, the scions started to germinate in sand. After full expansion of scion cotyledons (5 DAG), the 1st true leaf of the rootstock appeared, and grafting was carried out. Scions and rootstocks were joined at the cotyledonary node by the wedge-grafting technique (Fig. 1a), and grafted joints were wrapped with Parafilm (American National Can Inc., Chicago, USA) to prevent dehydration.

"*Y*" *grafting*: The sizes of scions and rootstock for Y grafting were the same as for the standard grafting. The scions from two separate seedlings were cut with a razor blade 2–3 cm below the apex. The rootstock was prepared by removing the shoot and cutting vertically downward approximately 2 cm at the cotyledonary node without damaging the cotyledons. Then two wedge-cut scions were placed into the gap of the rootstock, with the cut surfaces of scions and rootstock in complete contact (Fig. 1b). Parafilm was used to reinforce the union.

"*Inverted Y*" *grafting*: The scion plant and two rootstock plants were germinated at the same time. When their first true leaf appeared, two separate rootstocks were severed at the hypocotyls and a diagonal slice was made in each. An elastic band was placed over the two rootstocks, creating a V-shape slit at the junction of the

Table 1
Comparison of SPAD values (indicating chlorophyll content) in the youngest fully expanded leaf (the 4th leaf from the top of plant) at the 7–8 leaf stage in the growth chamber and lint yield (kg ha^{-1}) in the field between ungrafted control and self-grafts under K deficiency. Standard grafting was performed with one scion and one rootstock. "Y" grafting indicates two scions grafted onto one rootstock, and "inverted Y" grafting is constructed with one scion grafted onto two rootstocks. In the growth chamber, the ungrafted and self-grafted plants were grown hydroponically in low K solutions (0.03 mM K for standard and Y grafting, 0.01 mM K for inverted Y grafting); in the field, available K in soil was 58.7 mg kg^{-1} and no K fertilizer was applied. For each parameter, means within a row followed by the same letter are not significantly different according to Duncan's multiple range test, $p < 5\%$, $n = 4$.

SPAD values											
Standard grafting scion/rootstock				Y grafting scion + scion/rootstock				Inverted Y grafting scion/rootstock + rootstock			
41[a] ungrafts	41/41	22[b] ungrafts	22/22	41 ungrafts	(41 + 41)/41	22 ungrafts	(22 + 22)/22	41 ungrafts	41/(41 + 41)	22 ungrafts	22/(22 + 22)
20.8b	20.0bc	29.3a	30.5a	13.8d	14.4d	27.6a	23.7b	22.8c	25.3c	29.4b	33.5a

Lint yield (kg ha^{-1})							
2007 (standard grafting)				2008 (standard grafting)			
41 ungrafts	41/41	22 ungrafts	22/22	41 ungrafts	41/41	22 ungrafts	22/22
545b	444b	1090a	938a	43b	48b	114a	139a

[a] CCRI41.
[b] SCRC22.

two cut surfaces. The individual scion was cut into a wedge shape and inserted into the incision of both rootstocks (Fig. 1c), and the union was reinforced by parafilm. The diameter of scion and rootstocks cuttings were carefully selected in order that all cut surfaces fit well at the graft unions.

For each of the grafting procedures above, two cultivars were reciprocal- or self-grafted; standard grafts were denoted as scion/rootstock, "Y" and "inverted Y" grafts were denoted as (scion + scion)/rootstock and scion/(rootstock + rootstock), respectively.

Grafted seedlings, grown in above modified Hoagland's solution with 0.1 mM K (mild K deficiency) to ensure either higher survival rate of grafts or faster occurrence of leaf senescence induced by severe K deficiency (0.03 mM) after recovery, were immediately covered with perforated plastic bags, and transferred to 80 μmol m^{-2} s^{-1} photosynthetically active radiation, to maintain high humidity and minimize transpiration. Five days after grafting, the plastic bags were removed, but plants were still retained in a low light environment. Two days later, when the graft union was well established, plants were transferred to the normal growth condition indicated above, and exposed to either severe K deficiency (0.03 mM K) or normal K nutrition (2.5 mM K). Because inverted Y grafts had two rootstocks providing nutrients for one scion, they were grown in solution containing only 0.01 mM K after establishment so that leaf senescence was induced faster. One week after establishment, the cotyledons and auxiliary bud from cotyledonary nodes of the rootstock (standard and Y grafts) were removed.

Leaf analysis

At the 7–8 leaf stage, SPAD value, which had a linear relationship with chlorophyll content in the preliminary study ($Y = 0.0411X + 0.2023$; Y and X being chlorophyll content and SPAD value, respectively, $r^2 = 0.8080$, $p < 0.01$, and $n = 15$), and photosynthesis rate (Pn) of the youngest fully expanded leaf were measured by SPAD-502 Chlorophyll Meter (Minolta Inc., Tokyo, JP) and Li-6400 (LI-COR, Lincoln, USA) with 1000 μmol m^{-2} s^{-1} quantum flux and 500 μmol mol^{-1} CO_2 concentration. The same leaves were sampled and separated into two halves. One half was stored at −80 °C for determination of soluble protein with Coomassie Blue dye-binding assay (Bradford, 1976), and the other was oven-dried at 80 °C for analysis of K with atomic absorption spectrophotometry (SpectAA-50/55, Varian) (Zhang et al., 2009).

A completely randomized design was used with four replications (pots). Each replicate consisted of four plants. Similar trends of results were found in five independent repeat experiments, and thus data are given for one of them.

Field experiment

Field trials were conducted in 2007–2008 in Beijing at the Shangzhuang Experiment Station (40°08′N; 116°10′E) of China Agricultural University.

Grafting

Standard grafting was performed in the greenhouse in mid-April of both years. Two cultivars were germinated simultaneously, and the seedlings were raised in paper cups (250 mL) filled with sandy loam from the field. When most seedlings reached the two-leaf stage, self- and reciprocal-grafting were carried out just below the cotyledonary node of the rootstock, retaining the cotyledons and true leaves of the scion.

Grafts were immediately covered with perforated plastic bags, and then exposed to reduced light intensity (60 μmol m^{-2} s^{-1}) created by a shadecloth. A large quantity of water was poured into this area per day to maintain higher humidity. Seven days after grafting, the plastic bags and shadecloth were gradually removed over a period of three days. The established grafts were selected to acclimate in the greenhouse for three days, and were transplanted to the field on May 9, 2007 and May 4, 2008.

Growth conditions

The soil was a sandy loam, with a pH of 8.0 (water:soil = 2.5:1.0), organic matter content of 0.65% (digested with potassium dichromate under strong acid), available nitrogen of 24.9 mg kg^{-1} (extracted with 1 M KCl), available P of 14.2 mg kg^{-1} (extracted with 0.5 M $NaHCO_3$), and available K of 58.7 mg kg^{-1} (extracted with 1 M NH_4OAC). Based on the deficiency criteria for available K for cotton (Qin and Zhang, 1983), the soil used in this experiment was K-deficient (<70 mg kg^{-1}).

A split plot design with four replications was used for the field experiment. The main plots were assigned the K fertilizer rates (0 and 195 kg K ha^{-1} in the form of potassium sulphate), while the grafting treatments (CCRI41/CCRI41, CCRI41/SCRC22, SCRC22/CCRI41, and SCRC22/SCRC22) constituted the sub-plots.

Each plot contained three rows 6 m long with an inter-row spacing of 1.2 m and an intra-row spacing of 0.4 m, for a plant density of 20,800 plant ha^{-1}. The field management followed conventional practices. One hundred and fifty two kg N ha^{-1} as urea and di-ammonium phosphate and 138 kg P ha^{-1} as di-ammonium phosphate, as well as 97.5 kg K ha^{-1} were applied before sowing.

At the early flowering stage, urea-N (for both low and high K treatment) and K (for only high K treatment) were top-dressed at 69 kg N ha^{-1} and 97.5 kg K ha^{-1}. Plots were specially maintained insect-free by applying chemical pesticides such as omethoate and imidacloprid.

Sampling

Cotton plants were de-topped (by removing the tip of the main stem to enhance the growth of reproductive organs via changing direction of assimilates allocation, which is a conventional measure for cotton production in China) on July 26 and July 28, 2007 and 2008, respectively. Prior to that, the first expanded leaves (denoted as 0 d old) from the apex were labeled. At least five 23 d old leaves were used for SPAD value and Pn determination for each plot in 2008, with the same methods as those in the growth chamber experiment (except Li-6400 with 1200 μmol m^{-2} s^{-1} quantum flux and 400 μmol mol^{-1} CO_2 concentration).

In both years, five representative plants from the central row of each plot were selected to determine boll numbers per plant, boll weight at each fruit position and lint percentage (%). The 1st–4th, 5th–8th and 9th–12th sympodials from the bottom were denoted as lower, medium and upper sympodials, respectively. Yield was determined by harvesting all plants including the five representatives in a plot.

Data analysis

Analysis of variance was performed using SAS statistical software (V8, SAS Institute Inc., Cary, USA), and means of the grafts were compared using Duncan's multiple range test. Because excessive precipitation in July and August 2008 (353.8 mm vs. 226.3 mm in the same period in 2007) reduced yield dramatically, the data for yield and its components were presented separately for each year.

Results

Role of shoots in K deficiency-induced senescence at the seedling stage

Standard grafts

Grafts grew well in K-sufficient solution (2.5 mM), but K deficiency (0.03 mM) resulted in pronounced leaf senescence. Under K sufficiency, the K content in the 4th leaf from the top was above 3.0% and no difference was found between the two genotypes. Also, SPAD values, soluble protein content, and photosynthesis rates (Pn) in the same leaf of SCRC22 scions were similar to those of CCRI41 scions (Table 2). Under K deficiency, the K content in leaf was reduced to 0.15–0.22%, accompanied with lower SPAD values, protein content, and Pn compared with K sufficiency. Moreover, K deficiency caused significant differences in leaf K content and senescence between genotypes, and SCRC22 scions had greater K content and those variables were related to senescence rather than CCRI41 regardless of rootstock genotypes (Table 2).

When grown in K sufficient solutions, scion effect was only significant for K content ($p < 0.001$) and SPAD values ($p = 0.002$), and no significant rootstock effect and interaction between scion and rootstock were found (Table 2). Scion effect was significant for all four traits ($p < 0.001$) determined under K deficiency, and rootstock effect was only significant for Pn ($p = 0.039$) (Table 2). Coinciding with this, the mean K content, SPAD value, soluble protein content and Pn across SCRC22 scions grown in K-deficient solution were 37, 40, 70 and 68% greater than those across CCRI41 scions, whereas these four traits across SCRC22 rootstocks were only −3, 1, −7 and 6% higher than those across CCRI41 rootstocks (Table 2). These results suggest that the scion genotype explained the greatest percentage of variations in leaf senescence induced by K deficiency among grafts.

In addition, the interaction between scion and rootstock was significant for SPAD value ($p = 0.014$) and Pn ($p = 0.046$) under K deficiency (Table 2), indicating that leaf senescence in terms of chlorophyll content (SPAD value) and Pn was influenced by interaction of scion and rootstock, besides the scion effect (SPAD value and Pn) and rootstock effect (Pn).

Y grafts

Similar to standard grafts, we found no significant differences in K content, SPAD value, soluble protein content and Pn among scions in Y grafts receiving sufficient K. However, SCRC22 and CCRI41 scions growing on the same rootstock (either SCRC22 or CCRI41) exhibited different K contents and senescence under K deficiency (0.03 mM), the former having significantly greater values for the above-mentioned traits than CCRI41 scions (Table 3).

The effects of the rootstock on the SPAD values and soluble protein content in scions of Y grafts were significant, but did not exceed the effects of the scion. For example, the SPAD value of the CCRI41 scion in graft combination of (CCRI41 + SCRC22)/SCRC22 was increased 13% by the SCRC22 rootstock compared with the average value of the two CCRI41 scions in (CCRI41 + CCRI41)/CCRI41, whereas the SPAD value of the SCRC22 scion in (CCRI41 + SCRC22)/CCRI41 was 60% greater than the average value of the two CCRI41 scions in (CCRI41 + CCRI41)/CCRI41. In addition, the soluble protein content of the SCRC22 scion was reduced 14% by the CCRI41 rootstock in (CCRI41 + SCRC22)/CCRI41 relative to the average value of the two SCRC22 scions in (SCRC22 + SCRC22)/SCRC22, whereas this trait for the CCRI41 scion in (CCRI41 + SCRC22)/SCRC22 was 43% lower than the average value of the two SCRC22 scions in (SCRC22 + SCRC22)/SCRC22.

The mean K content, SPAD value, soluble protein content and Pn across SCRC22 scions grown in K-deficient solution were 23, 58, 37 and 71% greater than those across CCRI41 scions, whereas these four traits across SCRC22 rootstocks were only 11, 31, 14 and 25% greater than those across CCRI41 rootstocks (Table 3).

Inverted Y grafts

Potassium-sufficient solution (2.5 mM) enabled inverted Y grafts to grow well, and K content, SPAD values, soluble protein content and Pn did not differ significantly between scions (Table 4). When K concentration in solution was reduced to 0.01 mM, low leaf K content and senescence as well as their variations between SCRC22 and CCRI41 scions were induced as in standard and Y grafts (Table 4). The mean K content, SPAD value, soluble protein content and Pn across SCRC22 scions were 32, 30, 21 and 39% greater than those across CCRI41 scions.

When compared with self-grafts, reciprocal-grafts had the same variables related to leaf senescence (except Pn), suggesting that the integration of two contrasting rootstocks does not change the shoot dominance in regulation of leaf senescence. With respect to Pn, when the SCRC22 scion was grafted onto one SCRC22 and one CCRI41 rootstock [SCRC22/(SCRC22 + CCRI41)], the Pn decreased by 14% relative to SCRC22/(SCRC22 + SCRC22), but was 27% greater than that of the CCRI41 scion grafted onto the same rootstocks [CCRI41/(SCRC22 + CCRI41)], indicating that the CCRI41 shoot had more influence on Pn than on CCRI41 root.

Influence of shoots on K deficiency-induced senescence in the field

Physiological variables involved in senescence at the late boll filling stage

The SPAD values, soluble protein content and Pn of 23 d old main stem leaves located at the first node from the top of

Table 2
Effect of K deficiency on the leaf senescence of standard graft (scion/rootstock) cotton at the 7–8 leaf stage. Grafting was performed at the 1-leaf stage of the rootstock and cotyledonary stage of the scion. Grafts were maintained in nutrient solution with 0.1 M K during establishment, and transferred to solutions with either 0.03 or 2.5 mM K after establishment. The K content (%), SPAD values (indicating chlorophyll (chl) content), soluble protein and photosynthetic rate (Pn) in the youngest fully expanded leaf (4th leaf from the top of plant) were determined. For each K level, means within a column followed by the same letter are not significantly different according to Duncan's multiple range test, $p < 5\%$, $n = 4$. *P* values are presented for each main effect or interaction.

K level (mM)	Scion/rootstock	K content (%)	Chl (SPAD)	Protein (mg g^{-1})	Pn (μmol CO_2 m^{-2} s^{-1})
2.5	CCRI41/CCRI41	3.4a	34.2a	0.9a	13.9a
	CCRI41/SCRC22	3.2a	33.4a	0.9a	13.1a
	SCRC22/SCRC22	3.5a	37.6a	0.9a	14.7a
	SCRC22/CCRI41	3.2a	36.1a	1.0a	14.5a
	Scion	<0.001	0.002	0.103	0.066
	Rootstock	0.073	0.243	0.455	0.420
	Scion × rootstock	0.940	0.400	0.421	0.940
0.03	CCRI41/CCRI41	0.15b	25.5b	0.5b	7.2b
	CCRI41/SCRC22	0.15b	27.1b	0.5b	8.6b
	SCRC22/SCRC22,	0.20a	35.8a	0.8a	13.6a
	SCRC22/CCRI41	0.21a	37.8a	0.9a	12.9a
	Scion	<0.001	<0.001	<0.001	<0.001
	Rootstock	0.755	0.824	0.117	0.039
	Scion × rootstock	0.073	0.014	0.575	0.046

Table 3
Effect of K deficiency on the leaf senescence of "Y" graft cotton (scion + scion/rootstock) at the 7–8 leaf stage. Grafting was performed at the 1-leaf stage of the rootstock and cotyledonary stage of the scion. Grafts were maintained in nutrient solution with 0.1 M K during establishment, and transferred to solutions with either 0.03 or 2.5 mM K after establishment. The K content (%), SPAD values (indicating chlorophyll (chl) content), soluble protein and photosynthetic rate (Pn) in the youngest fully expanded leaf (4th leaf from the top of plant) of each scion were determined. For each K level, means within a column followed by the same letter are not significantly different according to Duncan's multiple range test, $p < 5\%$, $n = 4$.

K level (mM)	Scion + scion/rootstock	K content (%)	Chl (SPAD)	Protein (mg g^{-1})	Pn (μmol CO_2 m^{-2} s^{-1})
2.5	(**CCRI41**[a] + CCRI41)/CCRI41	2.4a	38.0a	1.0a	11.2a
	(CCRI41 + **CCRI41**)/CCRI41	2.4a	38.9a	1.2a	10.7a
	(**CCRI41** + SCRC22)/CCRI41	2.5a	36.1a	1.0a	10.2a
	(CCRI41 + **SCRC22**)/CCRI41	2.7a	37.7a	1.2a	11.9a
	(**SCRC22** + SCRC22)/SCRC22	2.8a	38.6a	1.2a	12.6a
	(SCRC22 + **SCRC22**)/SCRC22	2.7a	39.1a	1.3a	13.1a
	(CCRI41 + **SCRC22**)/SCRC22	2.9a	38.7a	1.2a	12.5a
	(**CCRI41** + SCRC22)/SCRC22	2.7a	36.6a	1.2a	11.3a
0.03	(**CCRI41** + CCRI41)/CCRI41	0.37b	14.4c	0.5c	6.9b
	(CCRI41 + **CCRI41**)/CCRI41	0.33b	15.6bc	0.5c	5.7b
	(**CCRI41** + SCRC22)/CCRI41	0.37b	13.4c	0.5c	5.1b
	(CCRI41 + **SCRC22**)/CCRI41	0.42a	24.0a	0.6b	10.5a
	(SCRC22 + **SCRC22**)/SCRC22	0.48a	23.7a	0.7a	10.8a
	(**SCRC22** + SCRC22)/SCRC22	0.42a	22.9a	0.7a	9.6a
	(CCRI41 + **SCRC22**)/SCRC22	0.40a	24.9a	0.6b	9.0a
	(**CCRI41** + SCRC22)/SCRC22	0.36b	16.9b	0.4c	5.7b

[a] The bold scion was measured.

de-topped cotton plants were determined on August 20, 2008. Owing to excessive precipitation in July and August of 2008 (353.8 mm vs. 226.3 mm in the same period in 2007) and consequent poor root growth, the application of 195 kg ha^{-1} K fertilizer did not prevent premature senescence completely, as indicated by lower K content (about 0.50%) compared with reference (Reddy et al., 2000) and K deficiency symptoms were observed during the boll filling period, although to a lesser extent compared with 0 kg ha^{-1} of K fertilizer.

In terms of the differences among grafting treatments, SCRC22 scions still had greater SPAD values and Pn than CCRI41 scions, irrespective of rootstock genotypes and amount of K fertilizer (Table 5), as observed in seedlings grown in nutrient solution (Table 2). However, the range of variations between grafts supplied with more K

Table 4
Effect of K deficiency on the leaf senescence of "inverted Y" graft cotton (scion/rootstock + rootstock) at the 7–8 leaf stage. Grafting was performed at the 1-leaf stage of both rootstock and scion. Grafts were maintained in nutrient solution with 0.1 M K during establishment, and transferred to solutions with either 0.01 or 2.5 mM K after establishment. The K content (%), SPAD values (indicating chlorophyll (chl) content), soluble protein and photosynthetic rate (Pn) in the youngest fully expanded leaf (4th leaf from the top of plant) were determined. For each K level, means within a column followed by the same letter are not significantly different according to Duncan's multiple range test, $p < 5\%$, $n = 4$.

K level (mM)	Scion/rootstocks + rootstocks	K content (%)	Chl (SPAD)	Protein (mg g^{-1})	Pn rate (μmol CO_2 m^{-2} s^{-1})
2.5	CCRI41/(CCRI41 + CCRI41)	3.8a	34.5a	1.4a	13.9a
	CCRI41/(CCRI41 + SCRC22)	3.7a	34.1a	1.4a	13.8a
	SCRC22/(SCRC22 + SCRC22)	3.9a	37.3a	1.4a	15.7a
	SCRC22/(CCRI41 + SCRC22)	3.8a	36.5a	1.5a	15.5a
0.01	CCRI41/(CCRI41 + CCRI41)	0.25b	25.3b	0.5b	4.7c
	CCRI41/(CCRI41 + SCRC22)	0.24b	24.3b	0.5b	4.8c
	SCRC22/(SCRC22 + SCRC22)	0.34a	33.5a	0.6a	7.1a
	SCRC22/(CCRI41 + SCRC22)	0.31a	31.1a	0.6a	6.1b

Table 5
Effect of K deficiency on leaf senescence of standard graft (scion/rootstock) cotton in the field. Grafting was performed at the 2-leaf stage of both rootstock and scion grown in 250 mL paper cup filled with sandy loam in the greenhouse. Grafts were transferred to the field with available K of 58.7 mg kg^{-1} after establishment. Zero and 195 kg K ha^{-1} in the form of potassium sulphate were split-applied, half before sowing and half at early flowering. SPAD values (indicating chlorophyll (chl) content), soluble protein and photosynthetic rate (Pn) of 23 d old leaves from the first node of de-topped plants were determined in 2008. For each K rate, means within a column followed by the same letter are not significantly different according to Duncan's multiple range test, $p < 5\%$, $n = 4$.

K rate (kg K_2SO_4 ha^{-1})	Scion/rootstock	Chl (SPAD)	Protein (mg g^{-1})	Pn (μmol CO_2 m^{-2} s^{-1})
195	CCRI41/CCRI41	47.3b	3.6a	19.8c
	CCRI41/SCRC22	47.1b	3.6a	22.1b
	SCRC22/SCRC22	52.1a	4.1a	22.6ab
	SCRC22/CCRI41	52.9a	4.0a	23.6a
0	CCRI41/CCRI41	34.1b	2.6b	9.8b
	CCRI41/SCRC22	33.3b	2.8b	10.7b
	SCRC22/SCRC22	47.2a	3.4a	19.1a
	SCRC22/CCRI41	45.7a	3.3a	17.5a

Table 6
Effect of K deficiency on yield and yield components of standard grafts (scion/rootstock) in the field (2007–2008). Grafting was performed at the 2-leaf stage of both rootstock and scion grown in 250 mL paper cup filled with sandy loam in greenhouse. Grafts were transferred to the field with available K of 58.7 mg kg^{-1} after establishment. Zero and 195 kg K ha^{-1} in the form of potassium sulphate were split-applied, half before sowing and half at early flowering. For each K rate, means within a column followed by the same letter are not significantly different according to Duncan's multiple range test, $p < 5\%$, $n = 4$.

Year	K rate (kg K_2SO_4 ha^{-1})	Scion/rootstock	Boll no.	Boll wt[a] (g)	Lint pt[b] (%)	Lint yield (kg ha^{-1})
2007	195	CCRI41/CCRI41	50.3a	4.3b	41.3b	1013b
		CCRI41/SCRC22	52.6a	4.7b	41.5b	980b
		SCRC22/SCRC22	49.5a	6.0a	43.4a	1085ab
		SCRC22/CCRI41	44.3a	6.2a	42.9a	1291a
	0	CCRI41/CCRI41	36.3a	3.0c	40.0b	444b
		CCRI41/SCRC22	41.2a	3.0c	40.4b	542b
		SCRC22/SCRC22	43.4a	5.1a	43.1a	938a
		SCRC22/CCRI41	41.8a	4.4b	42.8a	931a
2008	195	CCRI41/CCRI41	34.1a	2.7b	39.0b	343b
		CCRI41/SCRC22	32.4a	3.0b	38.8b	287b
		SCRC22/SCRC22	30.2a	3.7a	41.0a	532a
		SCRC22/CCRI41	30.5a	3.5ab	40.9a	454a
	0	CCRI41/CCRI41	9.9b	1.4b	37.3b	48b
		CCRI41/SCRC22	13.1b	1.7ab	36.8b	61b
		SCRC22/SCRC22,	23.5a	2.1a	40.7a	139a
		SCRC22/CCRI41	19.8a	2.0ab	40.4a	125a

[a] wt, weight.
[b] pt, percentage.

fertilizers was narrower than that without K fertilizer (Table 5). Furthermore, for those grafts without K fertilizer application, soluble protein content in the leaf of SCRC22 scions was also greater than that of CCRI41 scions as well.

Yield and its components

Lint yield in 2008 was significantly less than that in 2007, being independent of K and grafting treatments (Table 6), which could be explained by less boll numbers, less boll weight and lint percentage due to excessive wet weather during the period of boll forming and filling. In addition, the application of K fertilizer (195 kg ha^{-1}) increased lint yield by elevating boll numbers and boll weight in both years (Table 6), which may be partly attributed to the alleviation of leaf senescence.

The lint yield of SCRC22 scions was significantly greater than CCRI41 scions except with high K fertilizer in 2007, regardless of rootstock genotypes (Table 6). Both greater boll weight and greater lint percentage, but not boll number (except without K fertilizer in 2008), of SCRC22 scions contributed to their greater yield (Table 6). The mean lint yields across SCRC22 scions were 89, 56 and 141% greater than those across CCRI41 scions for 0 kg ha^{-1} K fertilizer in 2007, and 195 and 0 kg ha^{-1} K fertilizer in 2008, respectively. Rootstocks had no significant effect on lint yield (Table 6).

The spatial distribution of boll weight can reflect the extent of leaf senescence, because leaf senescence can decrease the biosynthesis and supply of assimilates to bolls, especially those produced at the upper sympodials and lateral distal fruit positions from the main stem. Considering less variation in lint yield among grafts receiving high K fertilizer in 2007, and low yields in 2008, we focused on the spatial distribution of boll weight in the low K treatment in 2007.

The boll weight of SCRC22 scions at all sympodial and fruit positions were significantly greater than those of CCRI41, irrespective of rootstock genotypes (Tables 7 and 8). In terms of boll weight variations within one grafting treatment, we found that those within SCRC22 scions were much smaller than those within CCRI41 scions (Tables 7 and 8), indicating later and reduced leaf senescence of the former. For example, the mean boll weight at the second and third lateral fruit positions of fruiting branches across SCRC22 scions

Table 7
Comparison of boll weight (g) at different lateral fruit positions of sympodials among grafts (scion/rootstock) without K fertilizer (2007). Means within each column followed by the same letter are not significantly different according to Duncan's multiple range test, $p < 5\%$.

Scion/rootstock	BP1[a]	BP2	BP3	$LSD_{0.05}$
CCRI41/CCRI41	4.1d	3.3d	2.8d	0.45
CCRI41/SCRC22	4.2c	3.5c	2.9c	0.76
SCRC22/SCRC22	6.1a	5.6a	5.1a	0.76
SCRC22/CCRI41	5.9b	5.5b	4.7b	0.67

[a] BP1, BP2, BP3: lateral position 1, 2, and 3 in the fruiting branches from main stem.

Table 8
Comparison of boll weight (g) at different sympodials among grafts (scion/rootstock) without K fertilizer (2007). Means within each column followed by the same letter are not significantly different according to Duncan's multiple range test, $p < 5\%$.

Scion/rootstock	1st–4th[a]	5th–8th	9th–12th	$LSD_{0.05}$
CCRI41/CCRI41	4.3c	3.5d	2.3d	0.48
CCRI41/SCRC22	3.9d	3.9c	2.7c	1.01
SCRC22/SCRC22	5.7b	5.9a	5.2a	0.68
SCRC22/CCRI41	5.8a	5.5b	4.7b	0.61

[a] 1st–4th, 5th–8th, 9th–12th: 1st–4th, 5th–8th, and 9th–12th of the sympodials from the bottom of main stem.

were 7 and 18% lower than that at the first position whereas these differences for CCRI41 scions were 19 and 31%, respectively. In addition, the mean boll weight at middle (5th–8th), and upper (9th–12th) sympodials across SCRC22 scions were 0 and 12% lower than that at lower (1st–4th) sympodials whereas these differences for CCRI41 scions were 10%, and 37%, respectively.

Discussion

Grafting is useful for exploring how changing the genetic make-up of the root or shoot affects the corresponding phenotype of shoot or root. We developed grafts with cotton seedlings under mild K deficiency to determine the roles of the shoot and root on K deficiency-induced leaf senescence at the seedling stage and hence the effect of interaction between shoot and root within the same genotype on the roles of the shoot and root could be eliminated as early as possible.

The results of our grafting study (Tables 2–8) revealed that leaf senescence induced by K deficiency in the two cotton cultivars appeared largely shoot dependent. Moreover, we found a close positive relationship between leaf K content and senescence indicated by SPAD values (Fig. 2a), protein content (Fig. 2b) and Pn (Fig. 2c) under K deficiency for those grafts grown in a growth chamber at the seedling stage. Thus, we consider that cotton leaf senescence due to K deficiency can be attributed to K uptake and mobilization in root rather than shoot sensitivity to K, and the shoot can regulate the uptake of K by the root as reported in Marschner (1995).

Historically, the root system has been regarded as a major factor of leaf senescence by producing and/or delivering cytokinins to aerial parts through the xylem (Sitton et al., 1967; Garrison et al., 1984). Our unpublished data indicate that the late senescence of SCRC22 scions is closely associated with increased cytokinins (CKs) in leaves and rootstock xylem sap, even when grafted onto CCRI41 (early senescence genotype) rootstock and vice versa for the early senescence of CCRI41 scions. When a single tomato cultivar was grafted onto rootstocks from a recombinant inbred line population and grown with moderate (75 mM) salinity, xylem K and zeatin concentrations were strongly correlated with maintenance of the photosynthetic apparatus, indicating a rootstock effect (Albacete et al., 2009). Therefore, we assume that K and CKs from roots are critical for leaf senescence under either K deficiency or salt stress, whether they are regulated by shoot or root.

Faiss et al. (1997) obtained similar results to ours when demonstrating that grafting non-transformed (wild-type) tobacco scions onto the root system of cytokinin-overproducing transgenic plants did not prevent normal leaf senescence of wild-type scions. However, Dong et al. (2008) reported conflicting results for cotton. Using two lines contrasting in leaf senescence (one of which was the same late senescence SCRC22 as in our experiment) to perform reciprocal- and self-grafts, Dong et al. (2008) found that the graft of early senescence scions onto late senescence rootstocks alleviated leaf senescence, whereas that of late senescence scions onto early senescence rootstocks enhanced leaf senescence. Thus, they inferred that leaf senescence is considerably affected by the root

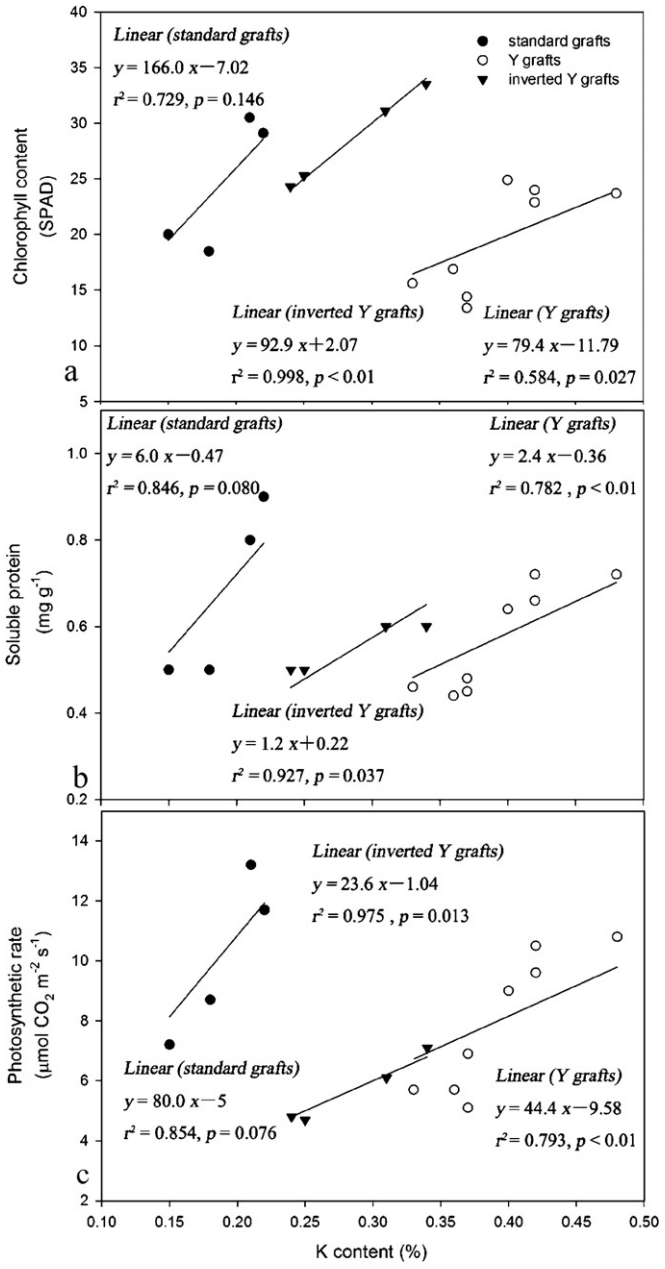

Fig. 2. Relationships between leaf K content (%, x) and SPAD values (indicating chlorophyll (Chl) content) (a), soluble protein (b), and photosynthetic rate (Pn) (c) of the youngest fully expanded leaf (4th leaf from the top of plant) grown at low K level (0.03 mM for standard and Y-grafts, and 0.01 mM for inverted Y-grafts) in growth chamber experiments. Each point represents the mean of a replication and linear regressions were fitted with Sigmaplot 11.0.

genotype. These contrasting results may be explained by several possible mechanisms. (1) The interaction of scion by rootstock varied with genotypes. For example, the rootstock effect on tomato salinity response depended on the shoot genotype (Santa-Cruz et al., 2002), and rootstock effects on several shoot variables (leaf area, g_s and [X-ACC] leaf) were dependent on the scion (Dodd et al., 2009). Therefore, the interaction between the late senescence line (K2, SCRC22) and the early senescence line (K1) could have been large in the study of Dong et al. (2008), whereas the interaction between SCRC22 and CCRI41 (early senescence) in the present study was nonexistent or small (Table 2). (2) The effect of the root on leaf senescence can depend on the shoot water and nutritional status (Dodd, 2005; Wilkinson and Hartung, 2009). It is possible that

the prevalence of these conditions in the K1 shoot (Dong et al., 2008) but not in the CCRI41 shoot permitted the SCRC22 root to alleviate senescence. (3) The signals and their sources, and sites of one signal pathway for one kind of phenotype are diverse (Matsumoto-Kitano et al., 2008). We consequently presumed that although CCRI41 in the present study and K1 in the study of Dong et al. (2008) had similar early senescence phenotypes, certain genes responsible for signal synthesis, transportation and perception associated with senescence are different between them, resulting in variable responses to the same graft partner (SCRC22). This may also be part of the mechanisms underlying the interaction between scion and root.

The results of a grafting study in soybean (Ookawa et al., 2001) indicated that the relative importance of root and shoot in regulation of leaf senescence varied with the growth stage. For example, the properties of the rootstock influenced the senescence of the scion at the early stage of ripening, but at the late stage of ripening, the scion played a major role. Nevertheless, we observed that the more important role of the shoot in the control of cotton leaf senescence was consistent from the seedling stage (Tables 2–4) to the late boll filling stage (Table 5), and to the mature stage (Table 6), thus suggesting that changes in either shoot demand or root function during the growth season does not affect the important role of shoot in regulation of leaf senescence.

Large boll load has been considered as one of the important factors causing premature senescence in cotton (Wright, 1999; Pettigrew, 2003). However, the early senescence cultivar, CCRI41 in the present study, did not have more bolls than the late senescence cultivar SCRC22 (Table 6), definitely indicating that the difference in K deficiency-induced senescence between CCRI41 scions and SCRC22 scions at maturity in the field was independent of sink demand. In addition, the K uptake of CCRI41 rootstock was enhanced by SCRC22 scion (Tables 2–4), indicating that potential root supply of K in CCRI41 is not the major cause for its premature senescence. Therefore, we concluded that factors beyond shoot demand and root supply in terms of K nutrition are responsible for the premature senescence of cotton plants in the present study. Considering that scions can regulate K uptake (Tables 2–4) and CKs delivery (unpublished data) in root, and K uptake and CKs delivery were positively related to leaf senescence (Fig. 2; Albacete et al., 2009), it appears that the shoot-to-root feedback signal(s) was probably involved in the role of shoot in regulating premature senescence of cotton plants by altering K uptake and CKs delivery, as found in pea (*Pisum sativum* L.) and Arabidopsis (*Arabidopsis thaliana*) (Beveridge et al., 1997; Foo et al., 2007).

The present grafting studies provides reliable evidence that the shoot plays a major role in K deficiency-induced senescence in cotton. We will continue to verify it using more genotypes, and to further explore the physiological and molecular mechanisms underlying this phenomenon, such as shoot-to root feedback signal(s) and their targeted processes, including K uptake and CKs delivery. This is not only of fundamental scientific interest, but is also of practical importance in the management of cotton affected by K deficiency.

Acknowledgments

This research was supported by the NSFC (National Natural Science Foundation of China, 30571118 and 30971708). We thank the Cotton Research Institute, Chinese Academy of Agricultural Sciences, Anyang, Henan and Cotton Research Center, Shandong Academy of Agricultural Sciences, Jinan, Shandong for providing cotton seeds. We are very grateful to Dr. J. Lynch of The Pennsylvania State University, State College, Pennsylvania, for discussion and technical reading of the manuscript.

References

Albacete A, Ghanem ME, Dodd IC, Pérez-Alfocea F. Principal component analysis of hormone profiling data suggests an important role for cytokinins in regulating leaf growth and senescence of salinized tomato. Plant Signal Behav 2010;5:45–8.
Albacete A, Martínez-Andújar C, Ghanem ME, Acosta M, Sánchez-Bravo J, Asins MJ, et al. 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 Environ 2009;32:928–38.
Anwar SA, McKenry M, Ramming D. A search for more durable grape rootstock resistance to root-knot nematode. Am J Enol Viticult 2002;53:19–23.
Bednarz CW, Oosterhuis DM. Plant potassium partitioning during progression of deficiency symptoms in cotton (*Gossypium hirsutum*), vol. 79. Better Crops. Potash and Phosphate Institute; 1995. pp. 12–14.
Beveridge CA, Murfet IC, Kerhoas L, Sotta B, Miginiac E, Rameau C. The shoot controls zeatin riboside export from pea roots. Evidence from the branching mutant rms4. Plant J 1997;11:339–45.
Beveridge CA. Long distance signalling and a mutational analysis of branching in pea. Plant Growth Regul 2000;32:193–203.
Bradford MM. A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein–dye-binding. Anal Biochem 1976;72:48–54.
Brouder SM, Cassman KG. Root development of two cotton cultivars in relation to potassium uptake and plant growth in a vermiculitic soil. Field Crop Res 1990;23:187–203.
Chen GX, Fu XP, Lips H, Sagi M. Control of plant growth resides in the shoot, and not in the root, in reciprocal grafts of flacca and wild-type tomato (*Lycopersiocon esculentum*), in the presence and absence of salinity stress. Plant Soil 2003;265:205–15.
Cornish K, Zeevaart JAD. Phenotypic expression of wild-type tomato and three wilty mutants in relation to abscisic acid accumulation in roots and leaflets of reciprocal grafts. Plant Physiol 1988;87:190–4.
Dodd IC, Theobald JC, Richer SK, Davies WJ. 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. J Exp Bot 2009;60:4029–39.
Dodd IC. Root-to-shoot signalling: assessing the roles of 'up' in the up and down world of long-distance signalling in planta. Plant Soil 2005;274:251–70.
Dong HZ, Li WJ, Tang W, Li ZH, Zhang DM. Effects of genotypes and plant density on yield, yield components and photosynthesis in Bt transgenic cotton. J Agron Crop Sci 2006;192:132–9.
Dong HZ, Niu YH, Li WJ, Zhang DM. Effects of cotton rootstock on endogenous cytokinins and abscisic acid in xylem sap and leaves in relation to leaf senescence. J Exp Bot 2008;59:1295–304.
Etehadnia M, Waterer D, Jong HD, Tanino KK. Scion and rootstock effects on ABA-mediated plant growth regulation and salt tolerance of acclimated and unacclimated potato genotypes. J Plant Growth Regul 2008;27:125–40.
Faiss M, Zalubìlová J, Strnad M, Schmülling T. Conditional transgenic expression of the ipt gene indicates a function for cytokinins in paracrine signaling in whole tobacco plants. Plant J 1997;12:401–15.
Foo E, Morris SE, Parmenter K, Young NM, Wang HT, Jones A, et al. Feedback regulation of xylem cytokinin content is conserved in pea and Arabidopsis. Plant Physiol 2007;143:1418–28.
Garrison FR, Brinker AM, Noodén LD. Relative activities of xylem-supplied cytokinins in retarding soybean leaf senescence and sustaining pod development. Plant Cell Physiol 1984;25:213–24.
Ghanem ME, Albacete A, Smigocki AC, Frébort I, Pospíšilová H, Martínez-Andújar C, et al. Root-synthesized cytokinins improve shoot growth and fruit yield in salinized tomato (*Solanum lycopersicum* L.) plants. J Exp Bot 2011a;62:125–40.
Ghanem ME, Hichri I, Smigocki AC, Albacete A, Fauconnier ML, Diatloff E, et al. Root-targeted biotechnology to mediate hormonal signalling and improve crop stress tolerance. Plant Cell Rep 2011b;30:807–23.
Holbrook NM, Shashidhar VR, James RA, Munns R. Stomatal control in tomato with ABA-deficient roots: response of grafted plants to soil drying. J Exp Bot 2002;53:1503–14.
James C. Global status of commercialized biotech/GM crops: 2009 ISAAA Brief No. 41. Ithaca, NY: ISAAA; 2009.
Jones TH, Cullis BR, Clingeleffer PR, Rühl EH. Effects of novel hybrid and traditional rootstocks on vigour and yield components of Shiraz grapevines. Aust J Grape Wine R 2009;15:284–92.
Li B, Wang CX, Zhang ZY, Duan LS, Li ZH, Tian XL. Three types of grafting technique available for research of root-shoot communication in cotton (*Gossypium hirsutum*) seedlings under low-potassium condition. Acta Agron Sin 2009;35:363–9.
Marschner H. Mineral nutrition of higher plants. London: Academic Press; 1995.
Matsumoto-Kitano M, Kusumoto T, Tarkowski P, Kinoshita-Tsujimura K, VáclavíkováK, Miyawaki K, et al. Cytokinins are central regulators of cambial activity. PNAS 2008;105:20027–31.
Ookawa T, Nishiyama M, Takahiro J, Ishihara K, Hirasawa T. Analysis of the factors causing differences in the leaf-senescence pattern between two soybean cultivars, Enrei and Tachinagaha. Plant Prod Sci 2001;4:3–8.
Pettigrew WT. Relationship between insufficient potassium and crop maturity in cotton. Agron J 2003;95:1323–9.
Qin SC, Zhang YS. Studies on the diagnosis of K-deficiency in cotton plant. Sci Agric Sin 1983;4:44–50.
Reddy KR, Hodges HF, Varco J. Potassium nutrition of cotton. Mississippi Agricultural and Forestry Experiment Station; 2000. pp. 1–10.

Rivero RM, Ruiz JM, Romero L. Iron metabolism in tomato and watermelon plants: influence of grafting. J Plant Nutr 2004;27:2221–34.
Rivero RM, Ruiz JM, Sanchez E, Romero L. Does grafting provide tomato plants an advantage against H_2O_2 production under conditions of thermal shock? Physiol Plant 2003;117:44–50.
Ruiz JM, Ríos JJ, Rosales MA, Rivero RM, Romero L. Grafting between tobacco plants to enhance salinity tolerance. J Plant Physiol 2006;163:1229–37.
Santa-Cruz A, Martinez-Rodriguez MM, Perez-Alfocea F, Romero-Aranda R, Bolarin MC. The rootstock effect on the tomato salinity response depends on the shoot genotype. Plant Sci 2002;162:825–31.
Sitton D, Itai C, Kende H. Decreased cytokinin production in the roots as a factor in shoot senescence. Planta 1967;73:296–300.
Tandonnet JP, Cookson SJ, Vivin P, Ollat N. Scion genotype controls biomass allocation and root development in grafted grapevine. Aust J Grape Wine R 2010;16:290–300.
Tian XL, Wang GW, Yang FQ, Yang PZ, Duan LS, Li ZH. Differences in tolerance to low-potassium supply among different types of cultivars in cotton (*Gossypium hirsutum*). Acta Agron Sin 2008;34:1770–80.
Van Norman JM, Frederick RL, Sieburth LE. BYPASS1 negatively regulates a root-derived signal that controls plant architecture. Curr Biol 2004;14:1739–46.
Werner T, Nehnevajova E, Köllmer I, Novák O, Strnad M, Krämer U, et al. Root-specific reduction of cytokinin causes enhanced root growth, drought tolerance, and leaf mineral enrichment in Arabidopsis and tobacco. Plant Cell 2010;22:3905–20.
Wilkinson S, Hartung W. Food production: reducing water consumption by manipulating long-distance chemical signalling in plants. J Exp Bot 2009;60:1885–91.
Wright PR. Premature senescence of cotton (*Gossypium hirsutum* L.): predominantly a potassium disorder caused by an imbalance of source and sink. Plant Soil 1999;211:231–9.
Yang FQ, Wang GW, Zhang ZY, Eneji AE, Duan LS, Li ZH, et al. Genotypic variations in potassium uptake and utilization in cotton. J Plant Nutr 2011;34:83–91.
Zhang ZY, Tian XL, Duan LS, Wang BM, He ZP, Li ZH. Differential responses of conventional and Bt-transgenic cotton (*Gossypium hirsutum* L.) to potassium deficiency. J Plant Nutr 2007;30:659–71.
Zhang ZY, Yang FQ, Li B, Eneji AE, Li JM, Duan LS, et al. Coronatine-induced lateral-root formation in cotton (*Gossypium hirsutum*) seedlings under potassium-sufficient and -deficient conditions in relation to auxin. J Plant Nutr Soil Sci 2009;172:435–44.
Zhao D, Oosterhuis DM, Bednarz CW. Influence of potassium deficiency on photosynthesis, chlorophyll content, and chloroplast ultrastructure of cotton plants. Photosynthetica 2001;39:103–9.