Journal of Photochemistry and Photobiology B: Biology 110 (2012) 1–8

Contents lists available at SciVerse ScienceDirect

Journal of Photochemistry and Photobiology B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol

Genotypic variations in photosynthetic and physiological adjustment to potassium deficiency in cotton (*Gossypium hirsutum*)

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ARTICLE INFO

Article history:
Received 29 October 2011
Received in revised form 13 January 2012
Accepted 7 February 2012
Available online 15 February 2012

Keywords:
Cotton
Potassium deficiency
Carbon metabolism
Nitrogen metabolism
Genotypic variation

ABSTRACT

A hydroponic culture experiment was conducted to determine genotypic variation in photosynthetic rate and the associated physiological changes in response to potassium (K) deficiency in cotton (*Gossypium hirsutum* L.) seedlings with contrasting two cotton cultivars in K efficiency. The K-efficient Liaomian18 produced 66.7% more biomass than the K-inefficient NuCOTN99^{B} under K deficiency, despite their similar biomass under K sufficiency. Compared with NuCOTN99^{B}, Liaomian18 showed 19.4% higher net photosynthetic rate (P_n, per unit leaf area) under K deficient solutions and this was associated with higher photochemical efficiency and faster export of soluble sugars from the phloem. The lower net P_n of NuCOTN99^{B} was attributed to higher capacity for nitrate assimilation and lower export of soluble sugars. Furthermore, NuCOTN99^{B} showed 38.4% greater ETR/P_n than Liaomian18 under K deficiency, indicating that more electrons were driven to other sinks. Higher superoxide dismutase (SOD) and lower catalase (CAT) and ascorbate peroxidase (APX) activities resulted in higher levels of reactive oxygen species (ROS; e.g. $O_2^-$and H_2O_2) in NuCOTN99^{B} relative to Liaomian18. Thus, the K inefficiency of NuCOTN99^{B}, indicated by lower biomass and net P_n under K deficiency, was associated with excessively high nitrogen assimilation, lower export of carbon assimilates, and greater ROS accumulation in the leaf.

1. Introduction

Potassium (K) is one of the macronutrients essential for plant growth and development, and plays an important role in a wide range of physiological processes, such as maintenance of electrical potential gradients across cell membranes, turgor generation, maintenance of anion–cation balances, activation of numerous enzymes, protein synthesis, and maintenance of photosynthesis and related processes [1]. However, compared with nitrogen (N), K is less applied worldwide. The N:K application ratio declined from 1:0.74 in the 1960s to 1:0.27 in the early 2000s [2], thus causing soil K mining. For example, China had a negative K balance (K application with potash fertilizers minus K removal by crops) of about $-60\ kg\ ha^{-1}\ y^{-1}$ in the late 1990s with an ongoing downward trend [3]. The lack of K application and soil K mining will cause unavoidable K deficiency in plants.

Cotton (*Gossypium hirsutum* L.) has a high requirement for K and shows a greater response to K fertilizer than does corn or soybean [4]. Widespread K deficiency in cotton crops has occurred in many countries [5,6], because of the negative K balance in the soil, adoption of modern cultivars characterized by faster fruit set and greater boll load [7], and popularization of transgenic Bt (*Bacillus thuringiensis* Berliner) cotton [6], which is more susceptible to K deficiency [8,9].

Plants have developed a wide range of adaptive or resistance mechanisms to maintain productivity under a variety of environmental stress conditions, including K deficiency. Genotypes showing high K efficiency usually have greater uptake or utilization efficiency [10,11]. Our previous work has indicated great genotypic differences in K efficiency among cotton genotypes [8,12]. For example, the larger root system and greater internal K utilization efficiency in Liaomian18 compared with NuCOTN99^{B} resulted in greater K efficiency [12]. We demonstrated that the greater internal K utilization efficiency of Liaomian18 was independent of certain biophysical functions of K; for example, the osmotic potential and relative water content in leaves of Liaomian18 were similar to those of NuCOTN99^{B} under K deficiency [12]. Consequently, it was necessary to study the biochemical and physiological functions of K (e.g., the activation of enzymes and related processes, and maintenance of photosynthesis), which would be mainly responsible for the genotypic variations in K utilization efficiency between these two cultivars.

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1011-1344/$ - see front matter
doi:10.1016/j.jphotobiol.2012.02.002

The objectives of the present study were to (i) investigate the effect of K on photosynthesis and related physiological processes such as chlorophyll (Chl) fluorescence, nitrogen metabolism and reactive oxygen species (ROS) accumulation in cotton seedlings and (ii) examine differences in the responses to K supply between a K-efficient cultivar, Liaomian18 and a K-inefficient cultivar, NuCOTN99^{B}. The data obtained will reveal the mechanisms underlying genotypic variations in internal K utilization efficiency, and provide a framework to identify breeding targets for improving the K use efficiency of cotton.

2. Materials and methods

2.1. Plant material

A K-efficient cotton cultivar, Liaomian18 (non-Bt cotton) developed by the Liaoning Cash Crops Research Institute, and one K-inefficient cotton cultivar NuCOTN99^{B} (Bt cotton) developed by Monsanto Company and distributed by the Hebei Jidai Cotton Seed Company in China, were used in the present study. The former cultivar produced the same biomass as the latter under K sufficiency (2.5 mM), but produced much higher biomass than the latter under K deficiency (0.03 mM) [12].

2.2. Growth conditions

Hydroponic culture experiments were conducted in a growth chamber with a 14 h photoperiod at 28/20 °C (day/night) temperatures, 70–80% relative humidity, and 450 μmol m^{-2} s^{-1} photosynthetically active radiation. Seeds were surface-sterilized with 9% H_2O_2 for 30 min and germinated in a K-free sand medium. After emergence (4 d after germination), uniform seedlings were cultured hydroponically by transferring to 35 cm × 27 cm × 12 cm plastic pots that contained 2.2 L half-strength modified Hoagland's solution containing 2.5 mM Ca $(NO_3)_2$, 1 mM $MgSO_4$, 0.5 mM $(NH_4)H_2PO_4$, 0.2 μM $CuSO_4$, 1 μM $ZnSO_4$, 0.1 mM Fe Na EDTA, 20 μM H_3BO_3, 5 pM $(NH_4)_6Mo_7O_{24}$ and 1 μM $MnSO_4$. The concentration of K in the form of potassium sulfate (K_2SO_4) in solutions was 0.03 mM for the K-deficient treatment and 2.5 mM for the K-sufficient treatment. Each pot contained six seedlings. All solutions were changed twice weekly; deionized water was added daily to replace the water lost by evapotranspiration. The solution pH was maintained at 6.5 by addition of concentrated NaOH solution and the solution was continuously aerated with an air pump to provide O_2 and achieve nutrient homogeneity. A completely randomized design was used with four replications (pots).

2.3. Carbon assimilation, chlorophyll a fluorescence and carbohydrate concentration

At the five-leaf stage (26 d after transfer [DAT] to nutrient solution), the gas exchange, including stomatal conductance (g_s), of the youngest fully-expanded main-stem leaf (the 4th leaf from apex) was measured three times per leaf between 9:00 and 11:30 with a Li-6400 (Li-COR, Lincoln, USA) at 27 °C, 60% relative humidity, 500 μmol mol^{-1} CO_2 concentration and 1000 μmol m^{-2} s^{-1} quantum flux. The value of photorespiration (PR) was determined as the difference in photosynthesis rate between low and normal oxygen content in the air [13]. During the same period, Chl*a* fluorescence of the same leaf was determined three times per leaf with a modulated fluorometer (PAM 2100, Walz, Effeltrich, Germany) following the method of Schreiber et al. [14]. The electron transport rate (ETR) was calculated with the following equation:

$$\mathrm{ETR} = f \times I \times a_{\mathrm{leaf}} \times [(F'_{\mathrm{m}} - F'_{\mathrm{s}})/F'_{\mathrm{m}}]$$

where f is the fraction of absorbed quanta used by photosystem II (PSII), typically assumed to be 0.5, I is the incident photon flux density (mmol m^{-2} s^{-1}), and a_{leaf} is the leaf absorbance assumed to be 0.84 [15], and F'_{m} and F'_{s} represent the maximum and steady-state Chl*a* fluorescence in light-adapted leaves.

After measurement of Chl*a* fluorescence and gas exchange, the youngest fully-expanded leaves were sampled and separated into two parts. One part was preserved at −80 °C for the determination of total Chl content, Rubisco (EC 4.1.1.39, IRA) activity and carbohydrate content, and the other portion was oven-dried at 80 °C for measurement of biomass and analysis of K with an atomic absorption spectrophotometer (SpectAA-50/55, Varian, Australia) [8]. Specific leaf area was calculated by dividing the leaf area by the dry weight of the same leaf. Concentrations of Chl were determined in 80% acetone extract [16]. IRA initial activity was assayed according to modified method of Cheng and Fuchigami [17], 50 μL of sample extract was added to 900 μL of assay solution, immediately followed by 50 μL of 10 mM RuBP. The assay solution consisted of 100 mM Hepes-KOH (pH 8.0), 25 mM $KHCO_3$, 20 mM $MgCl_2$, 3.5 mM ATP, 5 mM phosphocretaine, 5 units NAD-glyceraldehyde-3-phosphate dehydrogenase (NAD-GAPDH, EC1.2.1.12), 5 units 3-phosphoglyceric phospokinase (PCK, EC 2.7.2.3), 17.5 units creatine phosphokinase (EC2.7.3.2), and 0.25 mM NADH. The enzyme reaction was measured as the decrease in absorbance at 340 nm for 1 min. Starch, soluble sugars, sucrose and reducing sugars were analyzed as described in Cross et al. [18].

2.4. Nitrogen assimilation and recycling

Leaf samples were prepared using the same method as for determination of IRA. Nitrate reductase (EC 1.7.99.4, NR) activity and glutamine synthetase (EC 6.3.1.2, GS) activity were determined according to Ding et al. [19]. The concentration of soluble protein was determined with Coomassie brilliant blue G-250 [20], using bovine serum albumin (BSA) as a standard. Protease activity and peptidase activity were assayed as described in Setlow [21], and total free amino acid was measured using spectrophotometric analyses as described by Cross et al. [18].

2.5. Phloem export of soluble sugars and free amino acids

Phloem exudate was collected from detached youngest fully-expanded main-leaf leaves using the EDTA-promoted technique [22]. The cut ends of petioles were placed in 8 mL of 20 mM EDTA solution (pH 6). In order to avoid contamination with xylem exudates, the EDTA solution was discarded after 15 min in the dark, then the leaves were rinsed, transferred to fresh 8 mL EDTA solution, and kept in the dark for 5 h to avoid transpiration. The exudation solutions were stored at −20 °C for measurement of soluble sugar and free amino acid contents [18].

2.6. Reactive oxygen species metabolism

Leaf samples were prepared using the same method as for determination of IRA. Leaf tissues (0.5 g) were homogenized in 1 mL of 50 mM potassium phosphate (pH 7.0) and 1% (v/v) Triton X-100. The homogenate was centrifuged at 12,000*g* for 20 min at 4 °C and the supernatant was immediately used for enzyme assays. Superoxide dismutase (SOD; EC 1.15.1.1) activity was determined following the method of Stewart and Bewley [23]. Catalase (CAT; EC 1.11.1.6) and ascorbate peroxidase (APX; EC 1.11.1.11) activities were measured according to Patra et al. [24] and Nakano and Asada [25], respectively. Malondialdehyde (MDA) content was determined with thiobarbituric acid as described in Dhindsa et al. [26]. Superoxide anion (O_2^-) production was measured by monitoring nitrate formation from hydroxylamine in the presence of O_2^-

[27]. The H_2O_2 content was assayed according to Mukherjee and Choudhuri [28].

2.7. Statistical analysis

The experiment was repeated three times with results showing similar trends; hence the pooled data are presented here. Statistical analysis was performed with SPSS 10.0 (SPSS Inc. Chicago, USA) using one-way analysis of variance, and treatment means were compared using Duncan's multiple range test ($p < 0.05$). All data were tested for normality and homogeneity; non-normal data were transformed with an appropriate transformation method (e.g., log 10, $\log^{(1+x)}$ or LN).

3. Results

3.1. Biomass production and partitioning

There were no significant differences in biomass production and its partitioning to root, stem and leaf between Liaomian18 and NuCOTN99^B receiving sufficient K supply (Fig. 1). Potassium deficiency severely impeded seedling growth; the K-inefficient cultivar, NuCOTN99^B was more severely affected than the K-efficient cultivar, Liaomian18. For example, biomass production under K deficiency was only 22.9% of that in the K-sufficient treatment for NuCOTN99^B but 60.0% for Liaomian18 (Fig. 1A). Biomass partitioning to the stem (Liaomian18) or the root (NuCOTN99^B) was decreased by K deficiency in favor of the leaf (Fig. 1B).

3.2. Carbon assimilation

Under K sufficiency, the youngest fully-expanded leaf of two cultivars showed the same specific leaf area (SLA); the Chl content and stomatal conductance (g_s) of NuCOTN99^B were higher than those of Liaomian18, but total photosynthesis (P_n), both per unit area and per unit fresh weight (FW), did not differ significantly between the cultivars, possibly because of similarities in their intercellular CO_2 concentrations (C_i) and IRA (Table 1). The net P_n of NuCOTN99^B tended to be lower than that of Liaomian18, owing to its greater photorespiration (PR; Table 1).

Potassium deficiency caused a severe decrease in SLA, P_n and associated parameters (except PR of Liaomian18 and C_i; Table 1). Averaged for cultivars, the SLA, total and net P_n (per unit FW), Chl, g_s, and IRA decreased by 62.1, 22.1, 22.5, 56.7, 82.8 and 78.2%, respectively. The SLA, Chl and P_n in NuCOTN99^B were more affected than in Liaomian18 (Table 1), although both cultivars had the same leaf K concentration (about 0.3%) under K deficiency. In addition, K deficiency caused a 1.2-fold increase in PR per unit FW in Liaomian18 compared to K sufficiency, but caused a 1.1-fold decrease in NuCOTN99^B (Table 1).

Table 1
Effect of potassium (K) on concentrations of total chlorophyll (Chl), total photosynthesis rate (P_n), net photosynthesis rate (nP_n), specific leaf area (SLA), stomatal conductance (g_s), intercellular CO_2 concentration (C_i), Rubisco activity (IRA), and photorespiration (PR) in the youngest fully-expanded leaf of cotton seedlings at the five-leaf stage (26 d after transfer to hydroponic culture). Seedlings were grown in either K-deficient (0.03 mM; K1) or K-sufficient (2.5 mM; K2) solutions. Values are the means of four replications. Means within the same row followed by the same letter are not significantly different ($P \leqslant 0.05$) according to Duncan's multiple range test.

Parameters	Liaomian18		NuCOTN99^B	
	K1	K2	K1	K2
P_n (μmol CO_2 m^{-2} s^{-1})	8.65^b	10.48^a	5.75^c	10.83^a
PR (μmol CO_2 m^{-2} s^{-1})	3.33^a	1.84^b	1.28^c	3.43^a
nP_n (μmol CO_2 m^{-2} s^{-1})	5.32^b	8.63^a	4.46^c	7.40^a
P_n (μmol CO_2 g^{-1} FW s^{-1})	36.24^b	41.18^a	28.13^c	41.55^a
PR (μmol CO_2 g^{-1} FW s^{-1})	15.66^a	7.24^c	6.28 ^{c}	13.16^b
nP_n (μmol CO_2 g^{-1} FW s^{-1})	26.55^b	33.92^a	21.81^c	28.40^a
SLA (cm^2 g^{-1} DW)	83.8^b	169.7^a	42.5^c	160.3^a
Chl (mg g^{-1} FW)	2.69^c	5.33^b	2.22^d	6.13^a
g_s (μmol H_2O m^{-2} s^{-1})	32.3^c	175.2^b	38.4^c	241.3^a
C_i (μmol CO_2 mol^{-1})	390.8^{ab}	327.4^b	421.3^a	325.3^b
IRA (μmol g^{-1} FW min^{-1})	0.228^b	1.260^a	0.269^b	1.056^a

3.3. Chlorophyll fluorescence

There were no differences between Liaomian18 and NuCOTN99^B supplied with sufficient K in terms of maximum quantum yield of PSII (F_v/F_m), actual quantum yield of PSII (ϕPSII), non-photochemical quenching (qN), photochemical quenching (qP) and ETR in the youngest fully-expanded leaf (Table 2). Potassium deficiency significantly suppressed F_v/F_m, ϕPSII, qP and ETR, indicating destruction of the PSII reaction center and decreased photosynthesis occurred. Similar to Chl and P_n, Chl fluorescence was more strongly affected in NuCOTN99^B than in Liaomian18. The ETR/P_n (per unit area) ratio was increased greater in NuCOTN99^B than in Liaomian18 under K deficiency (Table 2). Non-photochemical quenching was depressed by K deficiency in both cultivars (Table 2).

3.4. Leaf carbohydrate content and soluble sugar export in phloem

There were no significant differences in the contents of sucrose, reducing sugars, and soluble sugars in the youngest fully-expanded leaves of Liaomian18 and NuCOTN99^B under K sufficiency. However, the starch concentration in the leaf of Liaomian18 was higher

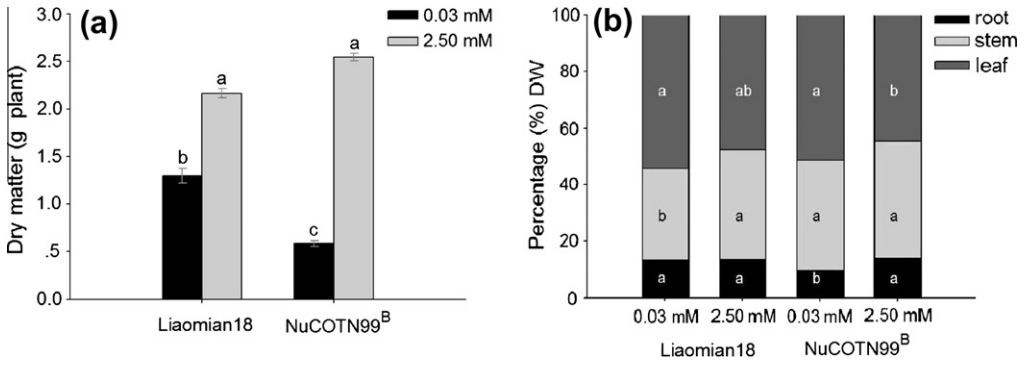

Fig. 1. Effect of potassium (K) supply on dry matter (a) and its partitioning to leaf, stem and root (b) of cotton seedlings at the five-leaf stage (26 d after transfer to hydroponic culture). Seedlings were grown in either K-deficient (0.03 mM) or K-sufficient (2.5 mM) solutions. The columns represent the mean of four replications. Columns with the same letter are not significantly different ($P \leqslant 0.05$) according to Duncan's multiple range test.

Table 2
Effect of potassium (K) on maximal efficiency of PSII photochemistry (F_v/F_m), non-photochemical quenching coefficient (qN), photochemical quenching (qP), photochemical quantum yield of photosystem (ϕPSII), electron transport rate (ETR) and ETR/P_n in the youngest fully-expanded leaf of cotton seedlings at the five-leaf stage (26 d after transfer to hydroponic culture). Seedlings were grown in either K-deficient (0.03 mM; K1) or K-sufficient (2.5 mM; K2) solutions. Values are means of four replications. Means within the same row followed by the same letter are not significantly different ($P \leqslant 0.05$) according to Duncan's multiple range test.

Trait	Liaomian18		NuCOTN99^B	
	K1	K2	K1	K2
F_v/F_m	0.738^b	0.791^a	0.669^c	0.801^a
qN	0.137^b	0.256^a	0.147^b	0.286^a
qP	0.882^b	0.942^a	0.829^c	0.947^a
ϕPSII	0.673^b	0.731^a	0.618^c	0.726^a
ETR (μmol m^{-2} s^{-1})	127.2^b	138.2^a	116.9^c	137.3^a
ETR/P_n	14.71^b	13.21^c	20.36^a	12.68^c

than that of NuCOTN99^B (Table 3). Potassium deficiency dramatically enhanced the accumulation of sucrose, reducing sugars and soluble sugars in leaf, especially for NuCOTN99^B (Table 3). The starch content under K deficiency decreased substantially in Liaomian18, but increased significantly in NuCOTN99^B (Table 3).

On the basis of a unit leaf (the youngest fully-expanded), NuCOTN99^B showed higher phloem export of soluble sugars under K sufficiency than Liaomian18. However, the reverse was observed on the basis of leaf FW, i.e. Liaomian18 exceeded NuCOTN99^B (Table 4). Under K deficiency, the export of soluble sugars was reduced considerably in both cultivars, but the actual level of export was higher in Liaomian18 than that in NuCOTN99^B, regardless of the unit of measurement (Table 4). When the export capacity of a leaf was evaluated based on the ratio of soluble sugars in the phloem to soluble sugars in the leaf (phloem:leaf ratio) per unit FW, the export was also more strongly impaired by K deficiency in NuCOTN99^B than in Liaomian18 (Table 4).

3.5. Nitrogen assimilation and recycling

Under K sufficiency, NuCOTN99^B showed 77.3% higher NR and 78.5% higher GS activities in the youngest fully-expanded leaf compared with Liaomian18 (Table 5). The concentration of soluble protein in the leaf of NuCOTN99^B was similar to that of Liaomian18 (Table 5). In contrast to NR and GS, protease activity of NuCOTN99^B was 72.2% lower than that of Liaomian18, and no difference in peptidase activity was observed (Table 5). The AA content in the leaf of NuCOTN99^B was 118.9% higher than that of Liaomian18, which perhaps resulted from the higher PR in NuCOTN99^B (Table 1) and the imbalance between nitrate assimilation and protein synthesis.

Potassium deficiency substantially depressed nitrogen assimilation, characterized by much lower NR activity and lower protein content, but greatly enhanced protein degradation, characterized by much higher protease and peptidase activities and higher AA content (Table 5). The activity of GS did not decline under K deficiency. Compared with the K-efficient cultivar, Liaomian18, the K-inefficient cultivar, NuCOTN99^B showed greater capacity for nitrogen assimilation and lower capacity for protein degradation under K deficiency, as indicated by higher NR activity, higher soluble protein content, and lower protease activity (Table 5). In addition, the AA content in the leaf of NuCOTN99^B was 55.4% higher than that of Liaomian18.

Table 3
Effect of potassium (K) on concentrations of starch, soluble sugar, sucrose and reducing sugars in the youngest fully-expanded leaf of cotton seedlings at the five-leaf stage (26 d after transfer to hydroponic culture). Seedlings were grown in either K-deficient (0.03 mM; K1) or K-sufficient (2.5 mM; K2) solutions. Values are means of four replications. Means within the same row followed by the same letter are not significantly different ($P \leqslant 0.05$) according to Duncan's multiple range test.

Trait	Liaomian18		NuCOTN99^B	
	K1	K2	K1	K2
Starch (mg g^{-1} FW)	8.83^c	11.78^a	10.84^b	9.54^c
Soluble sugar (mg g^{-1} FW)	10.93^b	6.40^c	12.41^a	5.73^c
Sucrose (mg g^{-1} FW)	2.90^b	2.74^d	4.00^a	2.45^d
Reducing sugars (mg g^{-1} FW)	7.60^b	2.88^c	9.05^a	2.86^c

Table 4
Effect of potassium (K) on phloem export of soluble sugar from the youngest fully-expanded leaf of cotton seedlings at the five-leaf stage (26 d after transfer to hydroponic culture). Phloem exudates were collected from detached leaves using the EDTA-promoted technique. Seedlings were grown in either K-deficient (0.03 mM; K1) or K-sufficient (2.5 mM; K2) solutions. Values are means of four replications. Means within the same row followed by the same letter are not significantly different ($P \leqslant 0.05$) according to Duncan's multiple range test.

Trait	Liaomian18		NuCOTN99^B	
	K1	K2	K1	K2
Export per leaf [1] (μg h^{-1} $leaf^{-1}$)	39.7^c	78.8^b	15.1^d	92.7^a
Export per unit FW [2] (μg g^{-1} FW h^{-1})	37.3^c	55.5^a	33.3^d	49.7^b
Phloem:leaf ratio [3] (100 μg g^{-1} FW h^{-1} μg g^{-1} FW)	0.341^b	0.867^a	0.268^c	0.867^a

[1] Exported soluble sugar per hour per leaf.
[2] Exported soluble sugar per hour per unit leaf fresh weight.
[3] Ratio of exported soluble sugar content in the phloem exudates to that in leaf per unit leaf fresh weight.

Table 5
Effect of potassium (K) on the activities of nitrate reductase (NR), glutamine synthetase (GS), protease and peptidase, and the concentrations of soluble protein and free amino acids (AA) in the youngest fully-expanded leaf of cotton seedlings at the five-leaf stage (26 d after transfer to hydroponic culture). Seedlings were grown in either K-deficient (0.03 mM; K1) or K-sufficient (2.5 mM; K2) solutions. Values are means of four replications. Means within the same row followed by the same letter are not significantly different ($P \leqslant 0.05$) according to Duncan's multiple range test.

Trait	Liaomian18		NuCOTN99^B	
	K1	K2	K1	K2
NR (μg g^{-1} FW h^{-1})	9.85^d	26.08^b	16.91^c	46.25^a
GS (μmol g^{-1} FW h^{-1})	413.4^a	212.3^b	411.0^a	407.6^a
Protease (μg g^{-1} FW h^{-1})	298.8^a	206.7^b	118.7^c	57.3^d
Peptidase (μg g^{-1} FW h^{-1})	14.96^a	2.70^b	13.15^a	1.64^b
Soluble protein (mg g^{-1} FW)	12.06^c	19.48^a	14.89^b	19.79^a
AA (mg g^{-1} FW)	1.607^b	0.443^d	2.498^a	0.970^c

3.6. Amino acid export in phloem

In accordance with the higher AA content in the leaf of NuCOTN99^B, this cultivar exported more phloem AA under K sufficiency, either on the basis of a unit leaf or the leaf FW (Table 6). However, the phloem:leaf ratio per unit FW indicated that the capacity for AA export in the leaf of NuCOTN99^B was lower than that of Liaomian18 (Table 6). Under K deficiency, amino acid export decreased dramatically, regardless of the unit of measurement (except the value per unit FW in NuCOTN99^B), and NuCOTN99^B showed greater export capability on the basis of leaf FW or phloem:leaf ratio (Table 6).

3.7. Reactive oxygen species metabolism

Under K sufficiency, no differences were found for O_2^- and H_2O_2 contents in the youngest fully-expanded leaves of Liaomian18 and NuCOTN99^B (Table 7). Consequently, the MDA content was

Table 6
Effect of potassium (K) on phloem export of free amino acids from the youngest fully-expanded leaf of cotton seedlings at the five-leaf stage (26 d after transfer to hydroponic culture). Phloem exudates were collected from detached leaves using the EDTA-promoted technique. Seedlings were grown in either K-deficient (0.03 mM; K1) or K-sufficient (2.5 mM; K2) solutions. Values are means of four replications. Means within the same row followed by the same letter are not significantly different ($P \leqslant 0.05$) according to Duncan's multiple range test.

Trait	Liaomian18		NuCOTN99^B	
	K1	K2	K1	K2
Export per leaf[1] ($\mu g\ h^{-1}\ leaf^{-1}$)	0.250^c	0.497^b	0.259^c	0.925^a
Export per unit FW[2] ($\mu g\ g^{-1}\ FW\ h^{-1}$)	0.235^d	0.351^c	0.572^a	0.496^b
Phloem:leaf ratio[3] ($100\ \mu g\ g^{-1}\ FW\ h^{-1}\ \mu g\ g^{-1}\ FW$)	0.014^d	0.079^a	0.022^c	0.059^b

[1] Exported free amino acids per hour per leaf.
[2] Exported free amino acids per hour per unit leaf fresh weight.
[3] Ratio of exported free amino acid content in phloem exudates to that in the leaf per unit leaf fresh weight.

Table 7
Effect of potassium (K) on concentrations of O_2^-, H_2O_2 and malondialdehyde (MDA), and activity of superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) in the youngest fully-expanded leaf of cotton seedlings at the five-leaf stage (26 d after transfer to hydroponic culture). Seedlings were grown in either K-deficient (0.03 mM; K1) or K-sufficient (2.5 mM; K2) solutions. Values are means of four replications. Means within the same row followed by the same letter are not significantly different ($P \leqslant 0.05$) according to Duncan's multiple range test.

Trait	Liaomian18		NuCOTN99^B	
	K1	K2	K1	K2
O_2^- ($nmol\ g^{-1}\ FW\ min^{-1}$)	4.59^b	3.91^b	10.19^a	4.39^b
H_2O_2 ($mmol\ g^{-1}\ FW$)	15.77^b	14.43^b	19.82^a	14.45^b
MDA ($\mu mol\ g^{-1}\ FW$)	4.34^b	2.94^c	5.03^a	3.45^c
SOD ($U\ g^{-1}\ FW$)	724.8^b	647.4^b	1090.8^a	760.0^b
APX ($mmol\ g^{-1}\ FW\ min^{-1}$)	3.98^a	2.38^c	2.87^b	1.56^d
CAT ($U\ g^{-1}\ FW\ min^{-1}$)	27.35^a	15.92^b	16.33^b	9.55^c

statistically similar in the two cultivars. Potassium deficiency resulted in accumulation of O_2^- and H_2O_2 in the leaf of NuCOTN99^B, but not in that of Liaomian18. However, the MDA content in the leaf of Liaomian18 was enhanced by K deficiency, albeit to a lower extent than that of NuCOTN99^B (Table 7), possibly because of increased levels of other undetermined ROS, such as singlet oxygen ($^1O_2$) in the leaf.

Several antioxidative enzymes play important roles in O_2^- and H_2O_2 removal. Liaomian18 showed similar SOD activity, but higher CAT and APX activities, under K sufficiency compared with NuCOTN99^B (Table 7). In response to K deficiency, both cultivars significantly increased SOD, CAT and APX activities (except SOD in Liaomian18; Table 7). With regard to the differences between the two cultivars, Liaomian18 had lower SOD activity, but greater CAT and APX activities than NuCOTN99^B under K deficiency (Table 7).

4. Discussion

4.1. K deficiency inhibits CO_2 and nitrate assimilation, restricts assimilate transport, and impairs ROS balance in cotton seedlings

In K-deficient plants, the loss of K^+ by guard cells results in decreased g_s and, thereby, depresses photosynthesis [29]. Also, non-stomatal reductions in photosynthesis of higher plants exposed to K-deficient environments are well known. For example, K deficiency has been shown to destroy Chl ultrastructure [30,31] and reduce Chl content [32], thus disrupting the photochemical reactions of photosynthesis, such as Hill reaction activity [33], and production rate of ATP and NADP [34]. The biochemical reactions of photosynthesis, such as Rubisco biosynthesis [35] and IRA are also impaired by K deficiency [36]. Furthermore, decreased photosynthesis in K-deficient plants is associated with restricted carbohydrate translocation [30,37], and the accumulated sucrose can affect gene expression related to photosynthesis [38].

The study of Bednarz et al. [39] on cotton indicated that during mild K deficiency, decreased g_s is the first response to result in decreased photosynthesis and, as K deficiency becomes more acute, biochemical factors also contribute. In the present study, cotton seedlings were grown in severely K-deficient solution (0.03 mM), in which both g_s and IAR (Table 1) declined dramatically compared with plants grown in K-sufficient solution (2.5 mM). However, because of the increase in C_i (albeit not significant in the leaf of Liaomian18; Table 1), it appears that non-stomatal factors play a more important role in decreasing photosynthesis in cotton seedlings under severe K deficiency.

Nitrate reductase is the key enzyme that catalyzes the rate-limiting step in the nitrate-assimilation pathway [40]. Glutamine synthetase functions as the major assimilatory enzyme for NH_4^+ incorporation into amino acids [41]. As earlier reported by other authors [1,42], nitrate assimilation and protein synthesis were strongly impeded by K deficiency. We found that mean NR activity and protein content in the leaf of K-deficient seedlings of the two cultivars declined by about 60% and 31.5%, respectively, whereas the content of amino acids increased 190.8% compared with K-sufficient seedlings (Table 5). The activity of GS did not decline under K deficiency, probably because its substrate, ammonia, is supplied from multiple pathways in addition to nitrate assimilation, such as PR and protein breakdown [43].

Increased concentrations of soluble sugars in leaves of K-deficient bean [32], cotton [30,37] and soybean [44] were considered to be related to restricted photosynthate transport from the source to sink organs [32]. Gerardeaux et al. [31] demonstrated that even before leaf photosynthesis was affected by K deficiency, assimilate export already declined. In the present study, we calculated the transport of soluble sugars using a different basis of measurement. Firstly, calculation on the basis of leaf FW as used by Cakmak et al. [32] makes sense when the export of assimilates among different K levels is compared. Secondly, comparison on the basis of a unit leaf is meaningful to investigate the supply capacity of a whole source leaf and therefore the available photosynthate obtained by sink organs. Lastly, the ratio of soluble sugars in phloem exudates to that in the leaf (phloem:leaf) per unit leaf FW indicates the proportion of total potential assimilate that are exported. The results of the present study indicated that K deficiency restricted assimilate export less (33% averaged across the two cultivars) when evaluated on the basis of leaf FW, but more when evaluated on the basis of a unit leaf (68% averaged across the two cultivars) due to the smaller leaves of K-deficient plants. Similarly, export based on the phloem:leaf ratio was reduced by 65% under K deficiency (Table 4). These results indicate that the application of different methods of calculation are useful in describing overall assimilate export.

Translocation of AA via the sieve tubes requires mass flow of sucrose driven by active sucrose transport [45]. Cakmak et al. [32]

found that the export of AA from a source leaf was decreased in K-deficient bean plants, which occurred simultaneously with the reduction in sucrose export. In the present study, amino acid transport from the source leaf, averaged across the two cultivars, declined only 4.6% under K deficiency when expressed on the basis of leaf FW. However, it decreased by 64 and 71% when expressed on the basis of a unit leaf and phloem:leaf ratio (Table 6), which were similar to that of soluble sugars.

Chloroplasts are the main organelles that produce ROS, such as O_2^-, H_2O_2 and $^1O_2$ during photosynthesis [46]. Under normal conditions, up to 20% of the total photosynthetic electron flux is distributed to form ROS [47]. However, under K deficiency, utilization of absorbed light energy during CO_2 fixation is limited, thus the electron flux to O_2 is intensified, resulting to an accumulation of large quantities of ROS in chloroplasts [48]. These findings are consistent with those obtained for NuCOTN99^B in the present study (Table 7). Although SOD (catalyzing the dismutation of O_2^- into O_2 and H_2O_2), CAT (catalyzing the decomposition of H_2O_2 to water and O_2) and APX (utilizing reductant in the form of ascorbate to detoxify H_2O_2) activities increased under K deficiency (Table 7), ROS were not scavenged completely and thus led to membrane damage characterized by MDA accumulation (Table 7) and Chl degradation characterized by lower Chl content (Table 1).

4.2. Why is Liaomian18 K efficient and NuCOTN99^B K inefficient?

The K-efficient cultivar, Liaomian18 showed greater capacity for carbon assimilation under K deficiency compared with the K-inefficient NuCOTN99^B, distinctly associated with the higher photochemical efficiency (Table 2), faster utilization of carbon assimilation (e.g. less soluble carbohydrates and starch in leaf; Table 3) and faster export of soluble sugars (Table 4). In addition, we assumed that the lower P_n in the leaf of NuCOTN99^B exposed to K deficiency was related to greater capacity for nitrate assimilation (Table 5). Because carbon and nitrogen metabolism must share organic carbon and energy supplied directly from photosynthetic electron transport and CO_2 fixation, up to 55% of net plant carbon is committed to nitrogen assimilation and metabolism in some tissues [49].

Chlorophyll fluorescence can be used to characterize energy utilization during photosynthetic electron transport [14]. In the present study, ETR/P_n in the leaf of NuCOTN99^B was significantly higher than that of Liaomian18 under K deficiency (Table 2), suggesting that more electrons were driven to other sinks including oxidation of molecular O_2 at the expense of reduced CO_2 assimilation. Moreover, a marked decline in CO_2 assimilation accompanied by a small decrease in PSII photochemical activity under abiotic stress will result in excessive excitation energy, since absorbed light energy exceeds the capacity of chloroplasts to use it in CO_2 fixation. Net P_n per unit area and ϕPSII in the leaf of the K-efficient Liaomian18 declined 17.4% and 7.9%, respectively, under K deficiency. However, in the leaf of the K-inefficient NuCOTN99^B these two traits decreased 46.9% and 14.9%, respectively, indicating more severe imbalance between absorbed light energy and utilization for carbon assimilation in NuCOTN99^B.

Plants have developed several mechanisms to cope with excessive excitation energy during photosynthesis, such as thermal dissipation characterized by qN [50], and PR [51] that functions as a 'safety valve' by preventing excess NADPH and ATP from reacting with oxygen and producing ROS. No difference was observed in qN between the two cultivars under either K sufficiency or deficiency, suggesting that thermal dissipation was not related to genotypic variation in the present study. Nevertheless, a strong difference in PR existed between Liaomian18 and NuCOTN99^B. For example, Liaomian18 showed lower PR under K sufficiency. When grown in K-deficient solution, PR in the leaf of Liaomian18 showed a 1.2-fold increase, but that of NuCOTN99^B showed a 1.1-fold decrease, and the actual PR value of Liaomian18 was about 1.5-fold greater than that of NuCOTN99^B (Table 1), indicating the former could eliminate excessive excitation energy more effectively. This kind of genotypic variation in PR under K deficiency is similar to differences among species reported in Yamada et al. [52] where K deficiency did not affect PR in wheat and soybean, but caused it to increase in sunflower. The increased PR of Liaomian18 under K deficiency also provided an explanation, at least partly, for the 44.8% higher GS activity in response to K deficiency, because the magnitude of the ammonium flux through the PR pathway was estimated to exceed that produced from nitrate reduction by five- to ten-fold in the leaves of C_3 plants [53].

With respect to antioxidant enzymes, NuCOTN99^B exposed to K deficiency showed higher SOD activity but lower APX and CAT activity relative to Liaomian18. Accompanied with the tendency to produce more excessive excitation energy and lower capability of PR protection in NuCOTN99^B under K deficiency (see above), this imbalance between SOD and CAT and APX resulted in greater accumulation of O_2^- and H_2O_2 in leaf of NuCOTN99^B compared with Liaomian18 (Table 7). Thus NuCOTN99^B suffered more severe oxidation degradation of Chl and membranes, as well as photoinhibition compared with Liaomian18, as indicated by its lower Chl content (Table 1), greater MDA content (Table 7), and lower F_v/F_m, respectively (Table 2).

Amino acids in the leaf originate from several pathways, such as NO_3^- assimilation, PR, and the hydrolysis of proteins and organic N [54]. In the present study, although both PR (Table 1) and protease activity (Table 5) in the leaf of NuCOTN99^B (Bt cotton) were less than 50% of those of Liaomian18 (non-Bt cotton) under K deficiency, NuCOTN99^B leaves accumulated 55.4% higher AA, which explicitly resulted from the 71.7% higher NR activity. In agreement with our results, Chen et al. [55] reported that Bt cotton cultivars showed higher AA content, higher NR activity, and lower protease activity in the leaf than the parental lines. Although it remains unclear why Bt cotton genotypes accumulate greater quantities of amino acids, we speculate it might be associated with Bt toxin biosynthesis or other processes related to Bt gene expression and toxin function.

5. Conclusions

The K-inefficient cotton cultivar, NuCOTN99^B (Bt cotton) showed similar biomass production and partitioning as the K-efficient cultivar, Liaomian18 (non-Bt cotton) under K sufficiency. However, NuCOTN99^B was affected much more by K deficiency than Liaomian18 in terms of biomass and photosynthesis. Potassium deficiency inhibited CO_2 and nitrate assimilation, restricted assimilates transport, and impaired the ROS balance in cotton seedlings. Factors contributing to the lower photosynthesis rate of NuCOTN99^B relative to Liaomian18 include: (1) higher excess excitation energy, such as greater ETR/P_n; (2) inefficient photoprotection, such as lower PR; (3) imbalance between SOD and CAT and APX activities; (4) higher ROS (O_2^- and H_2O_2) accumulation and therefore more severe photoinhibition (e.g. lower F_v/F_m); (5) greater capacity for nitrate assimilation; and (6) decreased export of soluble sugars.

These results shed light on the mechanisms underlying genotypic variations in internal K utilization efficiency in cotton. Future studies in this field need to address the issues of biochemical reaction related to photosynthesis such as Rubisco biosynthesis, dark respiration consumption, interaction between carbon and nitrogen metabolism, and effects of *Bt* gene introduction on carbon and nitrogen metabolism in cotton.

Abbreviations

AA	free amino acids
APX	ascorbate peroxidase
CAT	catalase
Chl	chlorophyll
Chl*a*	chlorophyll *a*
C_i	intercellular CO_2 concentration
ETR	electron transport rate
F_v/F_m	maximal efficiency of PSII photochemistry
g_s	stomatal conductance
GS	glutamine synthetase
IRA	initial Rubisco activity
MDA	malondialdehyde
NR	nitrate reductase
nP_n	net photosynthesis rate
P_n	total photosynthesis rate
PR	photorespiration
qN	non-photochemical quenching coefficient
qP	photochemical quenching
ϕPSII	photochemical quantum yield of photosystem
ROS	reactive oxygen species
SLA	specific leaf area
SOD	superoxide dismutase

Acknowledgments

This research was supported by the National Natural Science Foundation of China (Nos. 30571118 and 30971708). The authors thank Liaoning Cash Crops Research Institute, Liaoyang, Liaoning, China, and Hebei Jidai Cotton Seed Company Ltd., Shijiazhuang, Hebei, China, for providing cotton seeds.

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