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Single leaves photosynthetic characteristics of two insect-resistant transgenic cotton (*Gossypium hirsutum* L.) varieties in response to light

C.X. SUN[*,+], H. QI[**], J.J. HAO[*], L. MIAO[*], J. WANG[*], Y. WANG[*], M. LIU[**], and L.J. CHEN[***]

Department of Biology, Science College, Northeastern University, P.O. Box 325, 110004, Shenyang, P. R. China[*]
Agronomy College, Agricultural University of Shenyang, 110016 Shenyang, P. R. China[**]
Institute of Applied Ecology, Chinese Academy of Sciences, 110006 Shenyang, P. R. China[***]

Abstract

How the photosynthetic characteristics of insect-resistant transgenic cotton (*Gossypium hirsutum* L.) respond to light or whether this genetic transformation could result in unintended effects on their photosynthetic and physiological processes is not well known. Two experiments were conducted to investigate the shapes of net photosynthetic rate (P_N), stomatal conductance (g_s), apparent light use efficiency (LUE_{app}) and water use efficiency (WUE) light-response curves for single leaves of *Bt* (*Bacillus thuringiensis*) and *Bt*+CpTI (cowpea trypsin inhibitor) transgenic cotton plants and their non-transgenic counterparts, respectively. Results showed that the significant difference in response of P_N and WUE to light between transgenic cotton and non-transgenic cotton occured but not always throughout the growing season or in different experiments or for all transgenic cotton lines. It was highly dependent on growth stage, culture condition and variety, but no obvious difference between any transgenic cotton and non-transgenic cotton in the shapes of g_s and LUE_{app} light-response curves was observed in two experiments at different growth stages. In the field experiments, transgenic *Bt*+CpTI cotton was less sensitive to response of P_N to high irradiance at the boll-opening stage. In pot experiments, WUE light-response curves of both *Bt* transgenic cotton and *Bt*+CpTI transgenic cotton progressively decreased whereas non-transgenic cotton slowly reached a maximum at high irradiance at boll-opening stage. We supposed that culture environment could affect the photosynthesis of transgenic cotton both directly and indirectly through influencing either foreign genes expression or growth and physiological processes.

Additional key words: apparent light use efficiency; *Bacillus thuringiensis*; light-response curve; net photosynthetic rate; stomatal conductance; transgenic cotton; trypsin inhibitor; water use efficiency.

Introduction

The production of insect-resistant transgenic cotton is supposed to bring significant economic benefits and result in good ecological benefits (Qaim and Zilberman 2003). Since 1997, China has formally approved commercial production of transgenic cotton, and in 2007, the total planting area of insect-resistant transgenic cotton reached 380 million hectares, accounting for 69 % of the total planting area of cotton in our country (Mo 2007).

Photosynthesis is the physiological basis of crop growth and production, and a determining factor of crop yield. On one hand, stomata are the joining point between carbon and water circles in ecological systems, on the other hand, stomata are the pathway that permits the entrance of CO_2 and simultaneous loss of water vapor and then controls the balance between H_2O lost and CO_2 assimilated (Wullschleger and Oosterhuis 1989, Yu *et al.* 2001). Studies have been conducted looking at the response of transgenic insect-resistant cotton in terms of gas exchange properties. Dong *et al.* (2006) reported that three *Bt* cotton varieties had showed different curvilinear changes in the diurnal course of leaf photosynthetic rate. Hebbar *et al.* (2007) pointed out that the stomatal

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[+]Author for correspondence; fax: +86-24-23128449 , e-mail: suncaixia@hotmail.com

Abbreviations: α – the apparent quantum yield for CO_2 assimilation; *Bt* – *Bacillus thuringiensis*; C_i – intercellular CO_2 concentration; CPTI – cowpea trypsin inhibitor; E_m – the rate of transpiration; g_s – stomatal conductance; LUE_{app} – apparent light use efficiency; $P_{max,i}$ – the maximum net photosynthetic rate at 400 μmol mol^{-1} of CO_2; P_N – the net photosynthetic rate; PPFD – photosynthetic photon flux density; R_D – the apparent dark respiration rate; WUE – water use efficiency.

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conductance rates of transpiration and photosynthesis did not differ significantly between *Bt* and non-*Bt* counterparts up to 80 days after sowing. Our former results also showed that the changes in g_s, transpiration rate (E_m) and intercellular CO_2 concentration (C_i) in the leaves of *Bt* and *Bt*+CpTI transgenic cotton were not significantly different to non-transgenic cotton. However, the difference of P_N between *Bt* transgenic cotton and non-*Bt* cotton was significant at the seedling stage (Sun *et al.* 2007). Light plays a key role in photosynthesis and productivity of crops by providing the energy needed for assimilatory power, activating enzymes concerned with photosynthesis, promoting the opening of stomata, and regulating the development of the photosynthetic apparatus (Xu 2002). Among environmental factors, photosynthetic photon flux density (PPFD) is particularly subjected to a rapid and marked fluctuation in the field. This may require a rapid and efficient response of plant physiological processes to light, and thus limitation of these processes by light could potentially be minimized (Yu *et al.* 2001). However, how these physiological processes or characteristics of insect-resistant transgenic cotton response to light, are not well known.

Therefore, the objectives of the present study were to investigate responses of P_N, g_s, LUE_{app} and WUE of *Bt* and *Bt*+CpTI transgenic cotton to light, and to describe any unintended effects of transgene insertion on the transgenic cotton in photosynthetic physiological terms. This information would be valuable in discussion on the use of transgenic cotton.

Materials and methods

Cotton culture: The pot and field experiments were conducted at the Experimental Station of Shenyang Agricultural University (SAU), Shenyang (123°4′E, 41°8′N), Liaoning. Two types of indigenous Chinese commercial insect-resistant transgenic cotton including the *Bt* transgenic cotton Z30, the *Bt*+CpTI transgenic cotton SGK321, and their non-transgenic parental counterparts Z16 and SY321 were used in these experiments, respectively. Acid-delinted seeds of each variety were kindly provided by the Germ Plasma Resources Centre, Institute of Cotton, Chinese Academy of Agricultural Sciences (Anyang, Henan).

Cotton seeds were sown in pots containing 15 kg brunisolic soil obtained from the plough layer in the field at the Experimental Station of SAU in an outside growing area at the Experimental Station of SAU in mid May 2006. The soil in two experiments is a brunisolic soil having pH 5.72, organic matter 2.52 g kg^{-1}, total N 1.22 g kg^{-1}, total P (P_2O_5) 1.12 g kg^{-1}, total K (K_2O) 24.24 g kg^{-1}. Six pots were used for each variety and the plant population was thinned with three plants maintained per pot two weeks after emergence. Water stress was minimized with timely irrigation and insecticides were applied as needed during the season.

In 2007, the field experiments were arranged in a randomized complete block design with three replications. Each plot was formed by five rows with row length of 8 m and plant population density was 4.5 plants m^{-2}. Cotton seedlings were transplanted in early May. Fertilizer consisted of 225–82.5–187.5 kg ha^{-1} of $N–P_2O_5–K_2O$ incorporated before planting. Side-dressing with 90 kg(N) ha^{-1} was conducted 10 weeks after planting. Furrow irrigation provided a well-watered environment and insecticides were applied as needed during the season. Intensive management in cotton fields was carried out according to local agronomic practices unless otherwise indicated.

Photosynthetic characteristics measurements: P_N, g_s, and E_m of single leaves were measured on the second young fully mature leaf on the main stem at squaring and boll-opening stages in 2006 (a pot experiment) and 2007 (a field experiment) with a portable photosynthesis system *LI-6400* (*LI-COR*, Lincoln, NE, USA). During the measurements of light response curves of photosynthetic characteristics, PPFD was 0, 10, 20, 40, 60, 80, 100, 200, 400, 600, 800, 1000, 1200, 1400, 1600, 1800 and 2000 μmol(photon) m^{-2} s^{-1}, adjusted automatically by a red:blue light source (*LI-6400-02BL ED; LI-COR*). The leaves were held at each PPFD for a minimum of 30 min prior to determination to allow equilibration of the photosynthetic system to that PPFD. The temperature, relative air humidity and CO_2 concentration in leaf chamber were kept at 30 °C, 60 % and 400 μmol mol^{-1}, respectively. All readings were made between 9:00 and 11:00 hours on cloudless days.

Model fitting and data analysis: The light response curves of P_N were fitted to a Michaelis-Menten model based on measurement of P_N and PPFD (Thornley 1976).

$$P_N = \frac{\alpha\,\mathrm{PPFD}\,P_{\max,i}}{\alpha\,\mathrm{PPFD} + P_{\max,i}} - R_D\,,$$

where α is the apparent quantum yield for CO_2 assimilation, $P_{\max,i}$ is the maximum net photosynthetic rate at 400 μmol mol^{-1} of CO_2, and R_D is the apparent dark respiration rate. These parameters were estimated using Nonlinear Regression in *SPSS 11.0* based on Michaelis-Menten model.

LUE_{app} was calculated by using the equation:

$$\mathrm{LUE}_{\mathrm{app}} = \frac{P_N}{\mathrm{PPFD}}$$ (Long *et al.* 1993).

WUE was calculated by using the equation:

$$\mathrm{WUE} = \frac{P_\mathrm{N}}{E_\mathrm{m}} \quad \text{(Nijs } et\ al. \text{ 1997).}$$

Data were statistically analyzed by the *ANOVA* procedures in *SPSS 11.0* (Chicago, USA). All measurements were recorded from six replications at each sampling date.

Results

***P*$_\mathrm{N}$**: A non-rectangular hyperbolic curve has been widely used to describe photosynthetic light-response curves. P_N of all test cotton varieties during their whole growth season fitted the non-rectangular hyperbolic equation well. Parameters α, $P_{\mathrm{max,I}}$ and R_D, defining the fitted curves, were summarized in Table 1.

Fig. 1 shows the light-response curve for P_N, constructed using the estimated value calculated by the parameters in Table 1. On the whole, at low irradiance (below 200 μmol m^{-2} s^{-1}), all transgenic insect-resistant cotton had similar shapes of photosynthetic light-response curve as their non-transgenic counterparts. However, the difference between transgenic insect-resistant cotton and non-transgenic cotton in the shape of photosynthetic light-response curve broadened with the increase in irradiance (Fig. 1).

Table 1. Parameters of photosynthesis in response to light intensity between two transgenic insect-resistant cotton (SGK321, Z30) and their non-transgenic counterparts (SY321, Z16) at squaring and boll opening stages in 2006 (pot experiment) and 2007 (field experiment). Values in each row followed by the same letters are not significantly different ($p<0.05$) according to Duncan's multiple range test. α – the apparent quantum yield; $P_{\mathrm{max,i}}$ – the maximum net photosynthetic rate; R_D – the apparent dark respiration rate. Means ($n = 6$).

Year	Stage	Parameter	Variety			
			SY321	SGK321	Z16	Z30
2006	Squaring stage	α	0.067a	0.060a	0.068a	0.070a
		$P_{\mathrm{max,i}}$ [μmol CO_2 m^{-2} s^{-1}]	36ab	33a	42b	34a
		R_D[μmol CO_2 m^{-2} s^{-1}]	3.0a	3.2a	4.0b	4.3b
		r^2	0.9979	0.9975	0.9983	0.9984
	Boll opening stage	α	0.059a	0.063a	0.089a	0.069a
		$P_{\mathrm{max,i}}$ [μmol CO_2 m^{-2} s^{-1}]	22a	18a	17a	16a
		R_D [μmol CO_2 m^{-2} s^{-1}]	1.8bc	1.4ab	2.0c	1.2a
		r^2	0.9984	0.9982	0.9731	0.9963
2007	Squaring stage	α	0.080a	0.071a	0.072a	0.072a
		$P_{\mathrm{max,i}}$ [μmol CO_2 m^{-2} s^{-1}]	30a	23a	35a	33a
		R_D [μmol CO_2 m^{-2} s^{-1}]	2.6a	2.2a	3.1a	2.5a
		r^2	0.9894	0.9946	0.9951	0.9978
	Boll opening stage	α	0.075a	0.070a	0.086a	0.070a
		$P_{\mathrm{max,i}}$ [μmol CO_2 m^{-2} s^{-1}]	35b	23a	28ab	21a
		R_D [μmol CO_2 m^{-2} s^{-1}]	3.0b	1.8a	2.7ab	2.0ab
		r^2	0.9984	0.9982	0.9731	0.9963

In the pot experiments, at squaring stage, P_N of two varieties of transgenic insect-resistant cotton increased over the entire course of the light-response curve, and the difference in P_N between transgenic *Bt* cotton Z30 and its non-transgenic counterpart Z16 was more distinct than that of transgenic *Bt*+CpTI cotton SGK321 compared to non-transgenic counterpart SY321 (Fig. 1*A*). On the other hand, parameters α and R_D did not significantly vary between any transgenic cotton and their non-transgenic counterpart, however, $P_{\mathrm{max,i}}$ of *Bt* cotton Z30 decreased 19 % more than its non-transgenic counterpart Z16 and the difference was significant. Thus, the difference between transgenic *Bt* cotton Z30 and non-transgenic cotton Z16 in the response of P_N to light at the squaring stage in the pot experiments was due to a change in $P_{\mathrm{max,i}}$ ($p<0.05$) but not in the parameter α and R_D, implying a change in high light use efficiency (LUE) (Stirling *et al.* 1993). The difference between transgenic cotton and non-transgenic cotton in the shape of the photosynthetic light-response curve at boll-opening stage was less obvious than that at squaring stage, especially for transgenic *Bt* cotton Z30 with a similar shape of curve as non-transgenic cotton Z16 at high irradiance range (Fig. 1*B*). Moreover, parameters α and $P_{\mathrm{max,i}}$ were not significantly different between any transgenic cotton and their non-transgenic counterparts at boll-opening stage, but R_D of *Bt* cotton Z30 decreased 40 % more than its non-transgenic counterpart Z16 and the difference was significant in the pot experiments. R_D change was usually related to changes in C_i, enzymatic activity, dark CO_2 fixation rate,

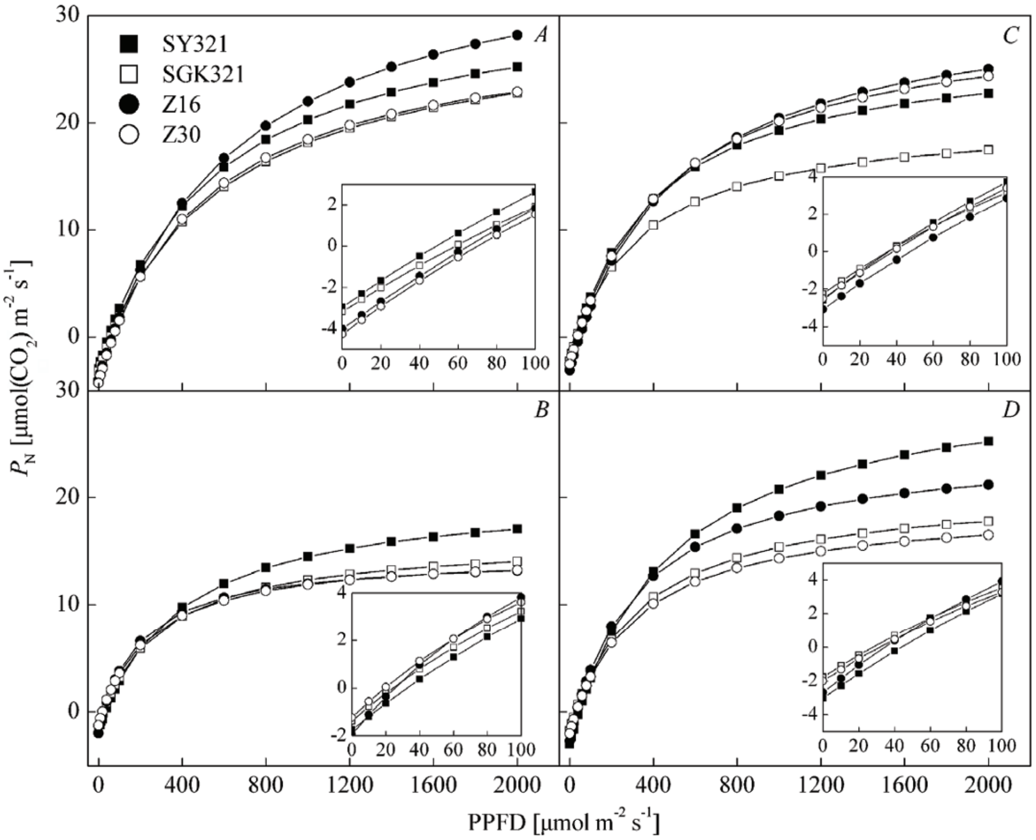

Fig. 1. Response of P_N to light intensity of the second young fully mature leaves on main stem between two transgenic insect-resistant cotton (□ SGK321, ○ Z30) and their non-transgenic counterparts (■ SY321, ● Z16) at squaring (*A*,*C*) and boll opening stages (*B*,*D*) in 2006 (*A*,*B*, pot experiment) and 2007 (*C*,*D*, field experiment). Each data point represents estimated value using Michaelis-Menten model, in which adopted value of α, $P_{max,i}$ and R_D are shown in Table 1, respectively. Means (n = 6).

or nonstructural saccharides (Shaish *et al*. 1989, Qiao *et al*. 2007). Low R_D underlined the low metabolic activity of transgenic *Bt* cotton Z30 during the later growing season compared with non-transgenic cotton Z16 (Gratani *et al*. 2007).

In the field experiments, P_N of transgenic *Bt*+CpTI cotton SGK321 increased in a distinctly different way over the entire course of the light response curve compared to non-transgenic SY321 at the boll-opening stage, but there were no statistically significant differences in parameters between any transgenic insect-resistant cotton and their non-transgenic counterpart at the squaring stage (Fig. 1*C*). Likewise, no statistically significant differences in any parameters of transgenic *Bt* cotton Z30 were observed compared to non-transgenic cotton Z16 at the boll-opening stage. However, both $P_{max,i}$ and R_D of transgenic *Bt*+CpTI cotton SGK321 were decreased significantly accompanied by the inhibition of P_N under high irradiance conditions while non-transgenic cotton SY321 could maintain a fairly high rate of photosynthesis (Fig. 1*D*). In this case, in the lower PPFD range (below 200 μmol m^{-2} s^{-1}), light plays a dominant limiting role in photosynthesis, apparent quantum yield of SGK321 observed from the light response curve did not change significantly (Table 1). In the period of P_N curvilinear increase, SGK321 exhibited lower P_N than SY321 caused possibly by either poor capacity to activate Rubisco that is a key enzyme in the process of carbon fixation, or poor capacity to provide energy to form assimilatory power, or poor capacity to regulate the stomata opening, meaning inadequate absorbtion of CO_2 (Xu 2002). $P_{max,i}$ of transgenic *Bt*+CpTI cotton SGK321 decreased significantly in comparison with SY321 indicating that photoinhibition occurred at exposure to high irradiance caused by excessive light energy absorption (Ögren and Evans 1993). The term photoinhibition has been used to describe light induced reduction of photosynthesis arising from either damage to the D1 protein of PSII reaction centers or increases in non-photochemical quenching of PSII excitation energy (Bradbury and Baker 1986). Chow (1994) has pointed out that plants could protect their photosynthetic apparatus from photodamage through several pathways by thermal dissipation. We deduced that decreases in the efficiency of electron transport and the content of photosynthetic key enzymes such as Rubisco could result in a reduction in photosynthesis in SGK321. On the other hand, decreased operation of protective thermal dissipation or

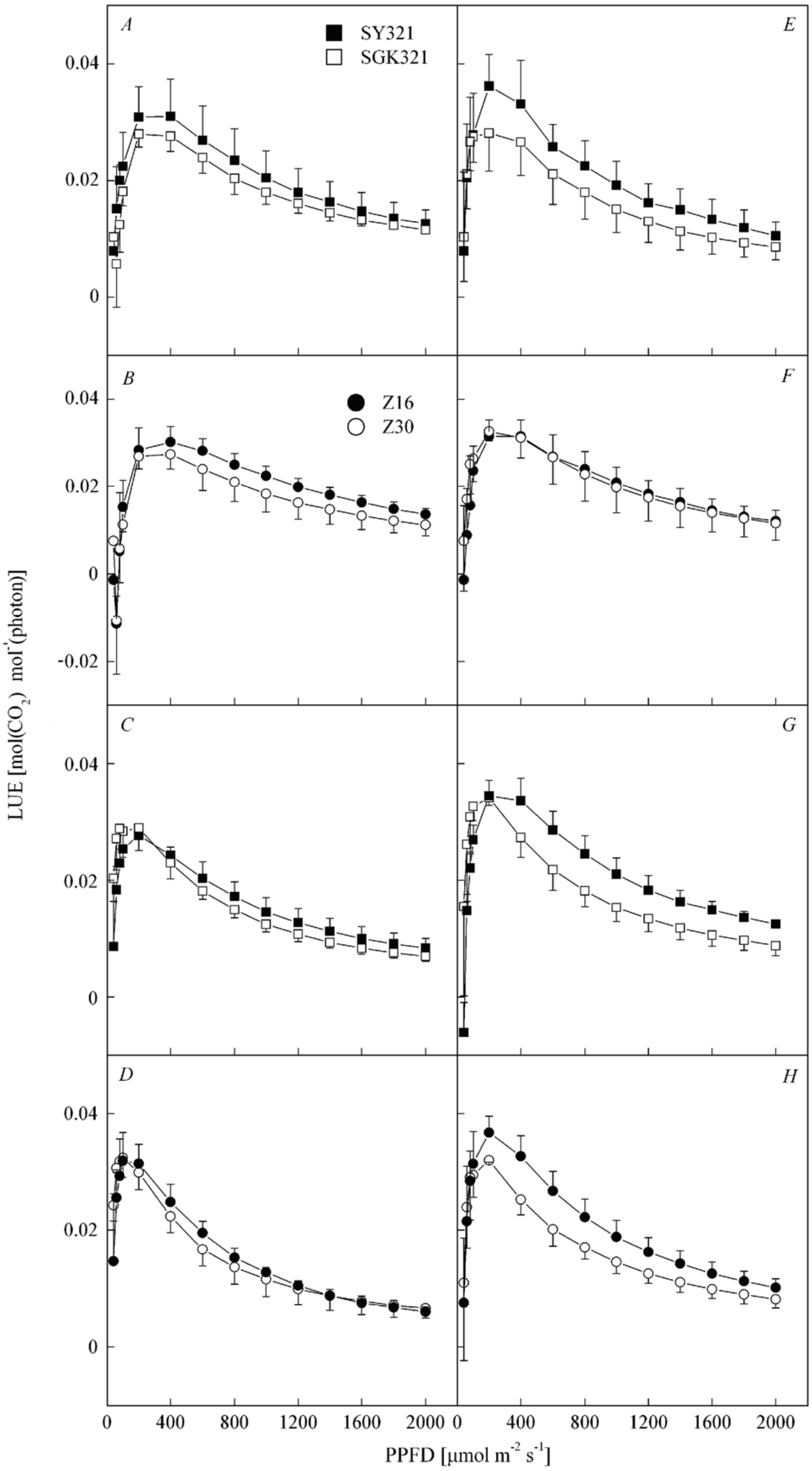

Fig. 2. Comparison of LUE versus light intensity curves of the second young fully mature leaves on main stem of two transgenic insect-resistant cotton (□ SGK321, ○ Z30) and their non-transgenic counterparts (■ SY321, ● Z16) at squaring (*A*,*B*,*E*,*F*) and boll-opening stages (*C*,*D*,*G*,*H*) in 2006 (*A*,*B*,*C*,*D*, pot experiment) and 2007 (*E*,*F*,*G*,*H*, field experiment). Means ±SD are shown ($n = 6$).

limitation of the removal of storage matter caused by a significant decline in R_D also might result in the photoinhibition of SGK321 at high irradiance (Niyogi 1999). Apparently, transgenic cotton SGK321 could not response to high light conditions rapidly and efficiently in field experiments.

LUE$_{app}$: The shapes of the light-response curves of LUE$_{app}$ for cotton studied in our research all exhibited two distinct phases; a rapid increase to maximum at low irradiance from 100 µmol m^{-2} s^{-1} to 400 µmol m^{-2} s^{-1}, and a period of linear decline to negligible LUE$_{app}$ at high irradiance (Fig. 2). In both pot and field experiments,

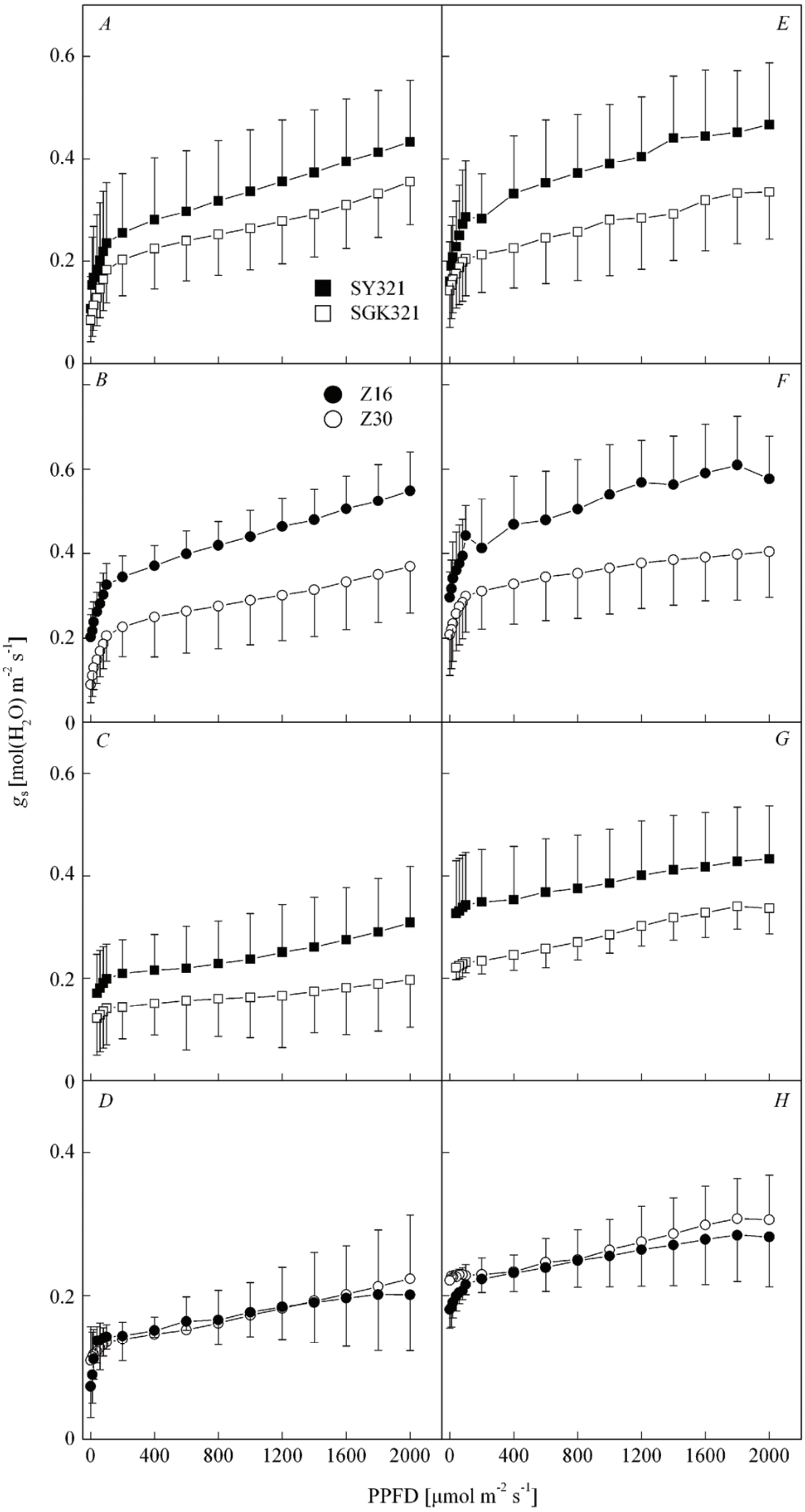

Fig. 3. Comparison of g_s versus light intensity curves of the second young fully mature leaves on main stem of two transgenic insect-resistant cotton (□ SGK321, ○ Z30) and their non-transgenic counterparts (■ SY321, ● Z16) at squaring (*A*,*B*,*E*,*F*) and boll-opening stages (*C*,*D*,*G*,*H*) in 2006 (*A*,*B*,*C*,*D*, pot experiment) and 2007 (*E*,*F*,*G*,*H*, field experiment). Means ±SD are shown (n = 6).

LUE_{app} of transgenic insect-resistant cotton reached a maximum with values slightly lower than, or similar to, the non-transgenic counterpart at a certain irradiance, and then declined much quickly than in the non-transgenic counterpart except for transgenic *Bt* cotton Z30 at squaring stage in the field experiments. In this case, no obvious difference between Z30 and Z16 in LUE_{app} was observed (Fig. 2*F*).

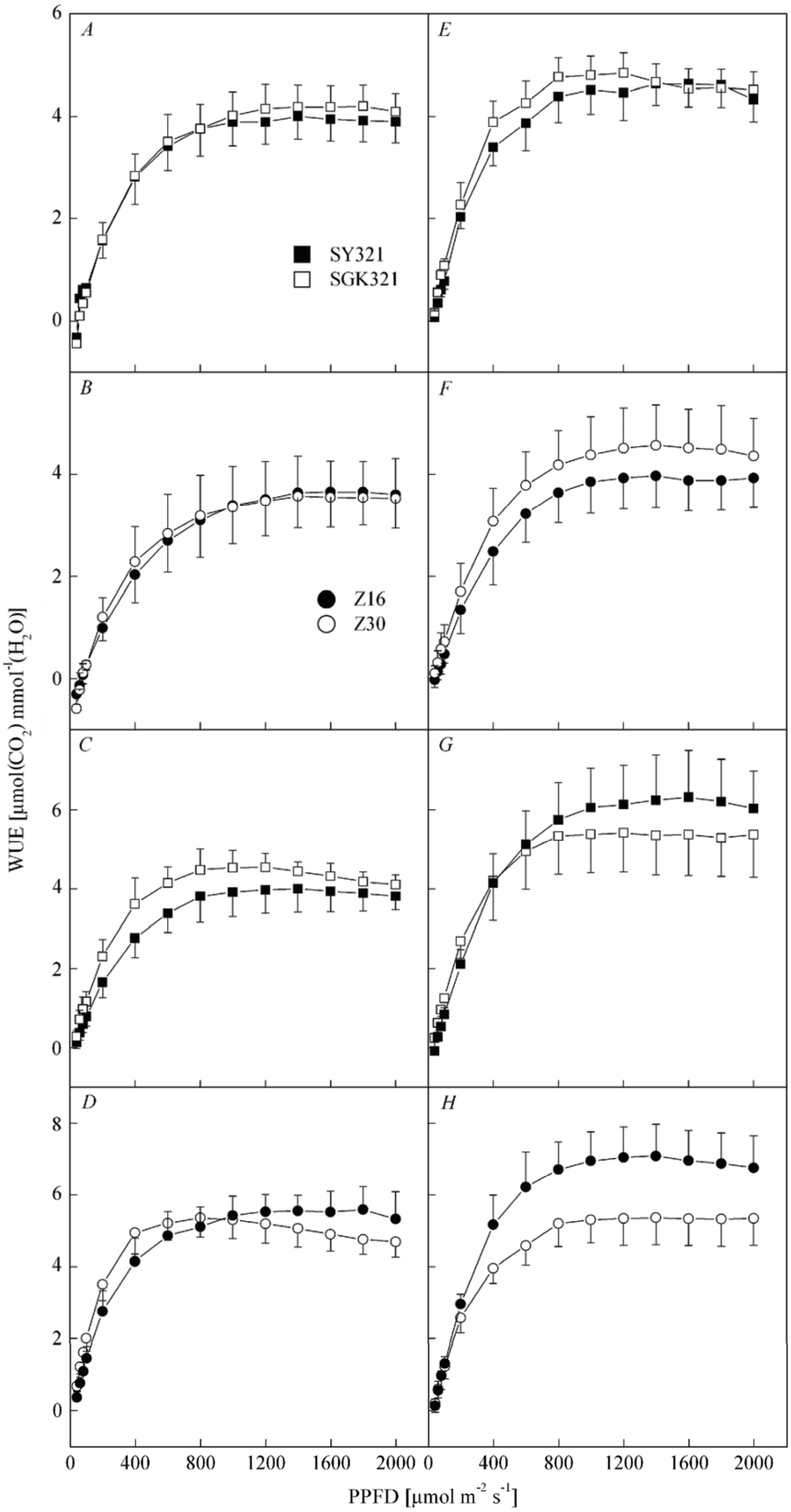

Fig. 4. Comparison of WUE versus light intensity curves of the second young fully mature leaves on main stem of two transgenic insect-resistant cotton (□ SGK321, ○ Z30) and their non-transgenic counterparts (■ SY321, ● Z16) at squaring (*A*,*B*,*E*,*F*) and boll-opening stages (*C*,*D*,*G*,*H*) in 2006 (*A*,*B*,*C*,*D*, pot experiment) and 2007 (*E*,*F*,*G*,*H*, field experiment). Means ±SD are shown ($n = 6$).

g_s: Although the data were somewhat scattered, results indicated that g_s of all cotton studied in this paper markedly increased with light over the entire course of the light response curve (Fig. 3). The increases in g_s of transgenic cotton were slighter than their non-transgenic counterpart, however, g_s of transgenic cotton Z30 at boll-opening stage exhibited the same shape of curves as its non-transgenic cotton Z16 both in pot and field experiments (Fig. 3*D*, *H*).

WUE: Water use efficiency is an often used parameter which relates gas exchange fluxes of carbon dioxide and water vapor and quantifies the total amount of CO_2 fixed per unit water lost (Wullschleger and Oosterhuis 1989). Overall, in low irradiance ranges from starting point to about 1000 μmol m^{-2} s^{-1} both in pot and field experiments, WUE of all cotton progressively increased with light to a maximum, whereas at high irradiance most cotton remained steadily at the maximum (Fig. 4).

In the pot experiments, at the squaring stage, the shape of light-response curves of WUE of two varieties of transgenic cotton were similar to their non-transgenic counterpart respectively (Fig. 4*A*,*B*). However, WUE of both transgenic *Bt* cotton Z30 and transgenic *Bt*+CpTI cotton SGK321 decreased slowly rather than remaining steady after reaching saturation at high irradiance at the boll-opening stage (Fig. 4*C*,*D*). These changes in WUE with PPFD could not be explained solely by variations in g_s since increases in g_s with PPFD were almost similar for all cotton varieties (Fig. 3). WUE of plants depends on photosynthesis coupled with transpiration through regulation of stomata opening. However, differing from transpiration, photosynthesis is also an intrinsic biochemical reaction and is inhibited by feedback of photosynthetic products and also reflects the heterogeneous character of diffusivity of CO_2 and H_2O (Yu *et al*. 2001). Since light probably has a more direct limit on the photochemical processes of P_N than on the physical processes controlling transpiration, WUE can be expected to rise with increases in PPFD at low irradiance (Wullschleger and Oosterhuis 1989). After incubation under low light the activation of photosynthetic enzymes is faster than simultaneous opening of stomata (Xu 2002).

In the field experiments, no obvious differences in WUE between any transgenic cotton and their non-transgenic counterpart were seen in the low irradiance range. On the other hand, the difference between transgenic insect-resistant cotton and non-transgenic cotton in the shape of WUE light-response curve broadened with the increase in irradiance (Fig. 4*E*,*F*,*G*,*H*).

Discussion

There has been a significant debate concerning the potential unintended effects of insertion of the foreign gene into transgenic crops (Conner and Jacobs 2000, Saxena and Stotzky 2001). Although the methods used to produce transgenic crops are being continually improved, it is not possible at present to control the exact stability, integration and expression of the inserted gene into the plant genomes, that is, it may alter the plant characteristics in physiology, anatomy and metabolism as a result of secondary or pleiotropic effects of the transgene expression and insertion (Cellini *et al*. 2004, Shrawat and Lörz 2006).

Our present data indicate that substantial differences did occur in the shape of P_N and WUE light-response curves between transgenic cotton and non-transgenic parental counterparts both grown in the field and pots, respectively (Table 1, Figs. 1*A,D*; 4*C*,*D*). However, the change in P_N with respect to PPFD suggested that leaves of transgenic cotton exposed to saturating light intensities were less capable of assimilating of CO_2 compared to non-transgenic cotton leaves either due to possible photoinhibition or other unintended effects of transgene insertion or the transformation process which were not studied in this paper (Cellini *et al*. 2004). It was even as Ashok and Horst (2006) reviewed that many factors could contribute to variation in transgene expression including tissue culture-induced variation or chimerism in the primary integration site (position effects), transgene copy number (dosage effects), transgene mutation and epigenetic gene silencing.

Wells (1988) has presented information that cotton leaves, which emerged during vegetative growth, had higher P_N levels than those presented in leaves, which emerged during periods of fruit development. Wullschleger and Oosterhuis (1990) have also pointed out that the response of P_N and g_s to incident PPFD conditions during canopy development was highly age-dependent. There were substantial adjustments in leaf physiology and morphology in response to the ambient light environment and this ability of leaves to alter the photosynthetic apparatus has also been recognized to depend closely on the developmental stage of the cotton tissue (Sassenrath-Cole *et al*. 1996, Dong *et al*. 2006). In agreement with these studies, our results also showed that a significant difference in response of P_N and WUE to light between transgenic cotton and non-transgenic cotton did not always occur throughout the growing season which was in agreement with our work showing that the responses of P_N and WUE to CO_2 were highly growth-stage-dependent (Sun *et al.* 2009).

Growth-stage variation in the response of P_N and WUE to light could be caused either by the expression mechanisms of photosynthetic regulation genes having spatial and temporal characteristics or by temporal specific expression of *Bt* and *Bt* coupled with CpTI (Sachs *et al*. 1998, Kang *et al*. 2005). Transgenic cotton had imperfections such as an imbalance between source and sink (Tian and Yang 1999), less capability utilizing photosynthetic products by cotton bolls (Zhao *et al*. 2002) *etc*. Hebbar *et al*. (2007) reported that premature senescence could impact on growth and physiological processes of transgenic *Bt* cotton. We speculated that disorder in nitrogen metabolism (Sassenrath-Cole *et al*. 1996) and an imbalance of source and sink (Fitt *et al*. 1994, Wright 2004) led to transgenic cotton responding to senescence in a different way, probably through a possible accelerated senescence phenomenon at the end of the growing season. The progressive loss of chloroplast membrane integrity coupled with increased leaf waxiness (Bondada and Oosterhuis 2002), breakdown of Rubisco protein (Jiang *et al.* 1993), decreases in levels of leaf nitrogen, soluble protein, chlorophyll, photosynthetic enzymes and RNA synthesis (Evans 1983, Wells 1988, Wullschleger and Oosterhuis 1990) may limit photosynthetic activities of cotton leaves during senescence. On the other hand, the structural and biochemical changes of the leaf could have effects on photosynthesis though variation in the partitioning of incoming radiation into reflectance, absorption and transmittance (Kakani *et al.* 2004). Since all cotton varieties showed similar changes in g_s and LUE_{app} at different stages, the differences in g_s and LUE_{app} response to light between transgenic cotton and non-transgenic parental counterpart could not be explained by any stage-related trend.

Our results showed that the responses of P_N and WUE to light observed in the pot experiment differed from those observed under field conditions. The reason for such discrepancies may be due, in part, to profound different effects of microclimate on cotton between transgenic varieties and non-transgenic varieties. P_N, g_s and various other photosynthetic characteristics are influenced by numerous environmental and physiological factors. Although these effects are often highly species-dependent, many studies also indicated that the conditions under which a plant develops could exert a significant influence on its photosynthetic characteristics (Bunce 1985, Schulze 1986, Wells 1988, Wullschleger and Oosterhuis 1990). For example, environmental factors can induce changes in leaf internal structure that are associated with a decrease in photosynthesis (Kakani *et al.* 2004). Variation of numerous environmental factors, such as temperature (Traore *et al.* 2000), CO_2 concentration (Coviella *et al.* 2002, Wu *et al.* 2007), water (Matzke *et al.* 1990, Traore *et al.* 2000), methods of fertilizer application, and cultivation management (Bruns and Abel 2003) could lead to change either in transgene expression or in growth and physiological processes within transgenic crops. We speculate that culture environment could affect photosynthesis of transgenic cotton both by a direct pathway and in an indirect manner through transgene expression. However, our study cannot distinguish these effects from canopy environment and the intrinsic metabolic processes of transgenic cotton.

The introduction of transgenic crops and accompanying changes in management practices may have potential effects on agroecosystems (Hoffman 1990, Trevors *et al.* 1994). It is obvious that environmental factors must be given full consideration in the safety assessment of transgenic crops. Optimisation of environmental factors and the cultivation practices of transgenic crops are expected to allow the achievement of maximal economic benefit and ecological benefit from transgenic crop production by identifying interactions between transgenic crops, environmental factors and cultivation practices.

Photosynthesis represents the final result of the complex interaction of numerous processes, any of which may be influenced by various environmental factors either directly or indirectly. It is worth mentioning that our research merely focused on photosynthetic changes based on an individual leaf throughout the growing season. Photosynthetic ability of the crop may also be affected by the structure of the crop canopy such as leaf structure, leaf shape, leaf area, plant type *etc.* (Heitholt 1994, Sassenrath-Cole 1995). When analyzing responses of photosynthetic characteristics to light at the whole plant or population level, it is also necessary to take into account possible effects due to canopy structure, consequences of changes in the light gradient within the leaf or differential acclimation of leaf surfaces to incident light (Terashima and Saeki 1985, Stirling *et al.* 1993), particularly for crops as morphologically complex as cotton with the indeterminate growth habit. Additional investigations are needed to examine these issues in more depth.

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