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The cause of the difference in leaf net photosynthetic rate between two soybean cultivars

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Abstract

To explore the cause of difference in photosynthetic performance between different cultivars of crops, leaf net photosynthetic rate (P_N) and photosystem 2 (PS2) photochemical efficiency (F_v/F_m), apparent quantum yield of carbon assimilation (ϕ_c), electron transport rate, photophosphorylation activity, *etc.* were measured in two soybean cultivars, Heinong 42 and Heinong 37. At pod setting and filling, significant differences in P_N between them were observed. The former with a higher P_N (from 7 to 38 %) had a significantly higher leaf thickness, leaf dry mass/area (LMA), chlorophyll content, soluble protein content, apparent quantum yield of electron transport through PS2 (ϕ_e), carboxylation efficiency (CE), and ribulose-1,5-bisphosphate carboxylase (RuBPC) activity. The significantly higher P_N of Heinong 42 is mainly due to its higher content and activity of RuBPC.

Additional key words: carboxylation efficiency; chlorophyll fluorescence; electron transport; *Glycine max*; photochemical efficiency; photophosphorylation; photosystem 2; quantum yield; ribulose-1,5-bisphosphate carboxylase.

Introduction

The first green revolution has lost its edge in increasing crop yield, a new green revolution has begun (Mann 1999). The central objective is the improvement of photosynthetic efficiency of crops, and the sharpest tool is genetic engineering for the new green revolution (Xu and Shen 2001). The success of the new revolution depends on a comprehensive understanding of molecular mechanisms for the regulation of photosynthetic efficiency. Exploring the cause of differences in leaf photosynthetic rate among crop interspecies and/or intra-species is one of the important strategies to understand the underlying mechanisms.

There is evidence for the substantial inter- and intra-specific genetic variability in net photosynthetic rate, P_N (Nelson 1988, Joshi 1997). In the past several decades, many studies on this phenomenon have been made (Hesketh *et al.* 1981, Joseph *et al.* 1981, Johnson *et al.* 1987, LeCain *et al.* 1989, Morgan *et al.* 1990, Pettigrew *et al.* 1993, Holá *et al.* 1999, *etc.*). For example, in soybean cultivars P_N was positively correlated with stomatal conductance (g_s), leaf thickness, leaf dry mass per area (LMA), chlorophyll (Chl) content, soluble protein content, and ribulose-1,5-bisphosphate carboxylase (RuBPC) activity, and negatively correlated with leaf area (Dornhoff and Shibles 1976, Hesketh *et al.* 1981). There was a significant difference in photosynthetic electron transport activity and the difference was correlated with plastocyanin pool size among fifteen genotypes of soybean

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Abbreviations: CE – carboxylation efficiency; Chl – chlorophyll; C_i – intercellular CO_2 concentration; c-PSP – cyclic photophosphorylation; g_s – stomatal conductance; F_v/F_m – potential photochemical efficiency of PS2 measured with adequately dark-adapted leaves; $\Delta F/F_m'$ – actual photochemical efficiency of PS2 measured with irradiated leaves; I_c – compensation irradiance; LT – leaf thickness; nc-PSP – non-cyclic photophosphorylation; PPFD – photosynthetic photon flux density; P_N – net photosynthetic rate; PS – photosystem; PSP – photophosphorylation; q_N – non-photochemical quenching; R_D – dark respiration rate; R_P – photorespiration rate; RuBP – ribulose-1,5-bisphosphate; RuBPC – RuBP carboxylase; LMA – leaf dry mass/area; Γ – CO_2 compensation concentration; ϕ_c – apparent quantum efficiency of CO_2 assimilation; ϕ_e – apparent quantum efficiency of electron transport through PS2.
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(Burkey *et al.* 1996), but whether the difference was correlated with P_N is not known. Therefore, the cause of difference in leaf P_N among different soybean cultivars has yet to be elucidated.

In this study, P_N and the related parameters such as the activities of photosynthetic electron transport, photophosphorylation (PSP), and RuBP carboxylation of two soybean cultivars were measured to find the main reasons causing the difference in P_N among different cultivars.

Materials and methods

Plants: Seeds of soybean (*Glycine max* L. cv. Heinong 37 and Heinong 42) were sown in plastic pots containing garden soil on June 15, 2000. The pots were put in the field in the Institute of Plant Physiology and Ecology, Shanghai. The plants of two cultivars were grown in each pot. In order to obtain uniform plants, the seedlings of each cultivar were thinned from four to one per pot after the first trifoliate leaf appeared. Soybean cakes were used as basal fertiliser for the plants. Just after the second trifoliate leaf appeared, the plants were fertilised every other day with a nitrate-type Hoagland solution. Total nitrate concentration of the solution was 15 mM. The plants were watered twice a day. All measurements were performed with fully expanded, disease-free, and fully sunlight-irradiated leaves from July 30 to August 15, 2000.

Gas exchange measurements: P_N of attached leaves were measured by using a portable infrared gas analyser *CI-301* (*CID*, Vancouver, U.S.A.) alternately between the two cultivars during 11:00~12:00 (Beijing time) in laboratory. Irradiation was provided by a halogen lamp, and was allowed to pass through a flowing water layer between the lamp and the leaves to remove heat.

The measurements of P_N at saturating irradiance were performed with terminal leaflets at a PPFD of about 1 300 μmol m^{-2} s^{-1}, an air CO_2 concentration 380-420 cm^3 m^{-3}, and an air temperature 30-33 °C after the end of the induction period of photosynthesis. Then some of the terminal leaflets were used to measure the photosynthetic response to PPFD, apparent quantum yield of photosynthetic CO_2 assimilation (ϕ_c) (Xu *et al.* 1987), and carboxylation efficiency (CE) (Caemmerer and Farquhar 1981) at 30-33 °C. Compensation irradiance (I_c) and dark respiration rate (R_D) were obtained from the P_N *vs.* PPFD curve. CO_2 compensation concentration (Γ) and photorespiratory rate (R_P) were obtained from the P_N *vs.* C_i curve.

Leaf thickness, LMA, and Chl content were determined in some terminal leaflets following the P_N measurement. Every leaf was cut into 20 pieces and these pieces were piled up. Then the thickness of leaf was obtained by measuring the pile with a vernier calliper. Leaf segments with the same area were killed at 100 °C for an hour and then dried at 65 °C to constant mass to obtain LMA. Chl contents of leaves and chloroplast preparations were determined spectroscopically in 80 % (v/v) acetone according to Arnon (1949).

Chl *a* fluorescence was measured with a portable *PAM-2000* fluorometer (*H. Walz*, Effeltrich, Germany) with the standard settings at room temperature (about 28 °C). The potential photochemical efficiency of PS2 (F_v/F_m) was measured with fully dark-adapted leaves (through a whole night). Then the actual photochemical efficiency of PS2 ($\Delta F/F_m'$) and non-photochemical quenching (q_N) were measured after the leaves were exposed to an irradiance of PPFD about 1 200 μmol m^{-2} s^{-1} for about 60 min. The calculations were made according to Genty *et al.* (1989) and van Kooten and Snel (1990).

The apparent photosynthetic electron transport rate through PS2 was calculated by using the Chl fluorescence parameter $\Delta F/F_m'$ (Schreiber *et al.* 1994). The measurement of $\Delta F/F_m'$ and the calculation of apparent quantum efficiency of electron transport through PS2 (ϕ_e) were the same as described in Jiang and Xu (2000).

Chloroplasts were isolated according to Russell *et al.* (1995) with some modifications. After the end of induction period of photosynthesis, trifoliate leaves were immediately cut into pieces. They were then homogenised for 15 s in a triturator with 40 cm^3 of ice-cold isolation medium that contained 25 mM Tricine-KOH (pH7.8), 330 mM sorbitol, 5 mM $MgCl_2$, 10 mM NaCl, 5 mM EDTA, 10 mM Vc (vitamin *c*), 0.1 % bovine serum albumin (BSA, m/v), and 2 % soluble polyvinyl pyrrolidone (m/v). The homogenate was filtered rapidly through 4 layers of gauze and the filtrate was centrifuged at 100×*g* for 3 min. Then the supernatant was centrifuged at 9 000×*g* for 10 min. The chloroplast pellets were resuspended in 2 cm^3 of ice-cold re-suspension medium containing 25 mM Tricine-KOH (pH 7.8), 0.4 M sucrose, 10 mM NaCl, and 5 mM $MgCl_2$. The chloroplast preparation was stored in ice-bath before the measurement of electron transport rates and photophosphorylation activities.

Photosynthetic electron transport assay: The measurements of uncoupled electron transport rates were performed with NH_4Cl as an uncoupler using a Clark-type O_2 electrode. Oxygen evolution or uptake was measured at a saturating incident irradiance of about 1 500 μmol m^{-2} s^{-1} and 25 °C according to Zhang *et al.* (1988) with some modifications. PS2 electron transport rate was determined

with H_2O as the electron donor and with phenyl-benzoquinone (p-BQ) as the electron acceptor in 1 cm^3 of reaction mixture containing 25 mM Tricine-KOH (pH 7.8), 0.2 M sucrose, 5 mM NaCl, 5 mM $MgCl_2$, 0.5 mM p-BQ, and chloroplasts with about 10 μg Chl. Photosystem 1 (PS1) electron transport rate was determined with reduced 2,6-dichlorophenol-indophenol ($DCPIPH_2$) as the electron donor and with methyl viologen (MV) as the electron acceptor in 1 cm^3 of reaction mixture containing 25 mM Tricine-KOH (pH 7.8), 0.2 M sucrose, 5 mM NaCl, 5 mM $MgCl_2$, 5 mM Vc, 150 μM MV, 10 μM 3-(3',4'-dichlorophenyl)-1,1-dimethylurea (DCMU), 0.2 mM DCPIP, 2 mM NaN_3, and chloroplasts containing about 10 μg Chl. The whole chain (PS1+PS2) electron transport rate was determined with H_2O as the electron donor and with MV as the electron acceptor in 1 cm^3 of reaction mixture containing 25 mM Tricine-KOH (pH 7.8), 0.2 M sucrose, 5 mM NaCl, 5 mM $MgCl_2$, 2 mM MV, 1 mM NaN_3, and chloroplasts containing about 10 μg Chl.

Photophosphorylation (PSP) activity of chloroplasts was assayed by using the luciferin-luciferase method to measure the amount of ATP synthesised within 2 min at saturating irradiance of about 1 500 μmol m^{-2} s^{-1} and 25 °C according to Shen and Shen (1962) with some modifications. Cyclic photophosphorylation (c-PSP) activity was assayed in 1 cm^3 of reaction mixture containing 50 mM Tricine-KOH (pH 8.0), 2 mM $MgCl_2$, 1 mM ADP, 5 mM phosphate (P_i), 0.05 mM phenazine methosulfate (PMS), and chloroplasts containing about 10 μg Chl. Non-cyclic photophosphorylation (nc-PSP) activity was assayed similarly to c-PSP except that PMS was replaced by 1 mM potassium ferricyanide. By putting the test tubes for 3 min into boiling water the reactions were stopped.

RuBPC activity: RuBPC was extracted according to Chastain and Ogren (1985) with some modifications. After measurements of leaf P_N, 5 cm^2 segment of each terminal leaflet was cut down and put immediately into liquid N_2. The sample was ground in a mortar with 1.5 cm^3 of extraction buffer containing 100 mM Tris-HCl (pH 7.6), 0.25 mM EDTA, 10 mM $MgCl_2$, 10 mM mercaptoethanol, 0.1 % BSA, and 2 % unsolvable PVP (m/v). Special care was taken to ensure complete breakage of leaf tissues. Then the extract was centrifuged at 10 000×*g* and 4 °C for 10 min, and the supernatant (crude extract) was used for the activity assay. The total soluble protein content of the crude extract was measured according to the method of Bradford (1976) with crystalline BSA as standard.

RuBPC activity was assayed immediately after extraction according to the method of Chastain and Ogren (1985). It was initiated by adding 50 mm^3 of crude extract to the reaction mixture containing 100 mM CO_2-free Tris-HCl (pH8.2), 20 mM $MgCl_2$, 1 mM DTT, 0.4 mM ribulose-1,5-bisphosphate (RuBP), and 10 mM $Na^{14}HCO_3$ (0.2 mBq mol^{-1}) in a final volume of 500 mm^3. After incubation for 60 s at 25 °C, the assays were terminated by adding 200 mm^3 of 2 M HCl.

The amount of RuBPC protein in the crude extract was determined by SDS-PAGE according to Makino *et al.* (1986) with some modifications. Discontinuous PAGE of denatured soluble proteins of crude extract was made in a buffer containing 31 mM Tris-HCl (pH7.8), 0.19 M glycine, 0.1 % SDS (m/v) (pH 8.4) with stable current of 15 mA. The concentrations of stacking gel and resolving gel were 3.5 and 12.0 %, respectively. 8 μg protein of each sample was loaded. After electrophoresis the gels were stained with Coomassie brilliant blue *R250* and the RuBPC large subunit bands were scanned with a laser densitometer to determine their relative peak areas. Calibration curves were made with the RuBPC protein purified form tobacco leaves, and a linear relationship was found between the relative peak areas and the amount of the RuBPC protein loaded onto the gels.

All experimental data were tested for significance by a Student's *t*-test for the differences between the two cultivars. Two levels of significance tested were $p < 0.05$ and $p < 0.01$.

Results

P_N of soybean leaves: Statistically significant differences in P_N between the two soybean cultivars were observed during the period from pod setting to filling. Heinong 42 had a higher P_N and a higher g_s, but a lower C_i than Heinong 37 (Table 1).

The patterns of photosynthetic response to irradiance were similar in the two cultivars (Fig. 1). The saturating PPFDs for photosynthesis were about 1 200 and 900 μmol m^{-2} s^{-1} for Heinong 42 and Heinong 37, respectively. The latter had lower R_D [about –0.48 *vs.* –0.59 μmol(CO_2) m^{-2} s^{-1}] and compensation irradiance [about 7.8 *vs.* 8.3 μmol m^{-2} s^{-1}] than the former, but the differences were not significant (Table 2).

Apparent quantum yield of carbon assimilation (ϕ_c) and PS2 electron transport (ϕ_e), and PS2 photochemical efficiency: Heinong 42 had a slightly higher ϕ_c and significant higher ϕ_e than Heinong 37 (Table 2 and Fig. 2). The potential PS2 photochemical efficiency (F_v/F_m) of the former was slightly higher than that of the latter,

Table 1. Net photosynthetic rate, P_N [μmol(CO_2) m^{-2} s^{-1}], stomatal conductance, g_s [mol(H_2O) m^{-2} s^{-1}], and intercellular CO_2 concentration, C_i [cm^3 m^{-3}] of leaves in soybean cvs. Heinong 37 and Heinong 42 in the period of pod setting to filling. Each value is the mean ± SE of 3 leaves. The significant levels of difference between the two cultivars are $^*p < 0.05$, $^{**}p < 0.01$. The fully expanded leaves were measured by CID in laboratory during 11:00 ~ 12:00 from July 30 to Aug. 14, 2000. One plant of each cultivar was used in each measurement. After the end of the induction period of photosynthesis, P_N was measured at a saturating PPFD of about 1 300 μmol m^{-2} s^{-1}, an air CO_2 concentration of 380~420 cm^3 m^{-3}, and an air temperature 30~33 °C.

Date	Heinong 37			Heinong 42		
	P_N	g_s	C_i	P_N	g_s	C_i
July 30	28.0±0.3	0.275±0.014	254.8±6.8	35.8±0.9**	0.357±0.017*	250.1±12.4
July 31	24.9±0.9	0.275±0.010	255.3±11.3	30.1±0.8	0.304±0.048	230.0+24.4
Aug. 1	22.4±0.8	0.324±0.033	256.2±7.7	30.9±1.3**	0.356±0.040	220.8±14.0
Aug. 2	28.3±2.4	0.328±0.026	267.7±11.5	35.4±2.1	0.363±0.068	234.0±25.1
Aug. 3	25.3±0.3	0.328±0.003	262.6±2.7	32.4±0.8**	0.368±0.019	239.0±7.6*
Aug. 4	26.6±1.2	0.302±0.016	250.4±6.3	33.7±0.5**	0.357±0.040	230.0±17.3
Aug. 5	29.6±1.0	0.329±0.025	270.4±10.2	35.1±1.2*	0.328±0.02	267.0±6.7
Aug. 11	32.7±0.2	0.309±0.035	246.2±15.9	36.7±1.3*	0.408±0.015	260.3±9.2
Aug. 14	30.9±1.6	0.352±0.012	256.2±4.4	37.2±1.3*	0.444±0.018*	258.6±3.8

Table 2. Apparent quantum yield of carbon assimilation, ϕ_c [mol(CO_2) mol^{-1}(photon)], dark respiratory rate, R_D [μmol(CO_2) m^{-2} s^{-1}], compensation irradiance, I_c [μmol(photon) m^{-2} s^{-1}], carboxylation efficiency, CE [mol(CO_2) m^{-2} s^{-1}]. photorespiratory rate, R_P [μmol(CO_2) m^{-2} s^{-1}], CO_2 compensation concentration, Γ [cm^3 m^{-3}], initial activity of RuBPC [μmol(CO_2) m^{-2} s^{-1}], special activity of RuBPC [mmol(CO_2) s^{-1} kg^{-1}(protein)], relative amount of RuBPC [%], and total soluble protein content of leaf [g m^{-2}] in soybean cv. Heinong 37 and Heinong 42. Each value is the mean of 3-6 leaves, and SE is given in parentheses. The significant levels of difference between the two cultivars are $^*p < 0.05$, $^{**}p < 0.01$. All gas exchange measurements were performed in fully expanded leaves at 31~32 °C by CID in laboratory during July 30 – Aug. 1, 2000. RuBPC activity, relative amount of RuBPC (ratio of RuBPC protein/ total soluble protein), and total soluble protein content were measured with the leaves after their P_N was measured on Aug. 5, 2000.

	ϕ_c	R_D	I_c	CE	R_P	Γ	RuBPC			Soluble protein
							initial activity	specific activity	relative amount	
Heinong 37	0.0648	0.48	7.8	0.1048	5.60	53.4	33.78	9.146	48.40	76.5
	(0.0095)	(0.14)	(2.8)	(0.0014)	(0.34)	(2.6)	(1.26)	(0.800)	(2.41)	(1.3)
Heinong 42	0.0697	0.59	8.3	0.1657*	8.52*	51.8	44.00*	10.579	46.26	84.0**
	(0.0074)	(0.10)	(1.9)	(0.0165)	(0.60)	(2.8)	(2.71)	(1.150)	(2.56)	(1.4)

but the values of actual PS2 photochemical efficiency ($\Delta F/F_m'$) of the two cultivars were almost the same (Fig. 2). Furthermore, the non-photochemical quenching (q_N) of the former was slightly lower than that of the latter (Fig. 2).

Electron transport rates and photophosphorylation activities of isolated chloroplasts: The electron transport rates of PS2 and PS1 of Heinong 42 were significantly higher than those of Heinong 37 (Fig. 3). However, the whole chain electron transport rate of the former was not higher than that of the latter (Fig. 3). There was no significant difference between the two cultivars in nc-PSP or c-PSP activities (Fig. 3).

Carboxylation efficiency, activity and specific activity of RuBPC, leaf soluble protein content, and the amount of RuBPC: CE of Heinong 42 was much higher than that of Heinong 37, and the difference was significant (Table 2). The former also had a higher photorespiration rate (R_P) than the latter [8.52 *vs.* 5.60 μmol(CO_2) m^{-2} s^{-1}] and they had a similar Γ [51.8 *vs.* 53.4 cm^3 m^{-3}] (Table 2). Moreover, the activity of RuBPC of Heinong 42 was also significantly higher than that of Heinong 37. The specific activity of RuBPC of the former was also slightly greater than that of the latter, but the difference was not statistically significant (Table 2).

In consonance with its significantly higher carboxylation efficiency and RuBPC activity, Heinong 42 had a significantly higher soluble protein content of leaves than Heinong 37, but the percentages of RuBPC protein to total soluble protein in the two cultivars were the same (Table 2).

Leaf thickness, LMA, and leaf Chl content: There were significant differences in these parameters between Heinong 42 and Heinong 37, all being higher in the former (Fig. 4).

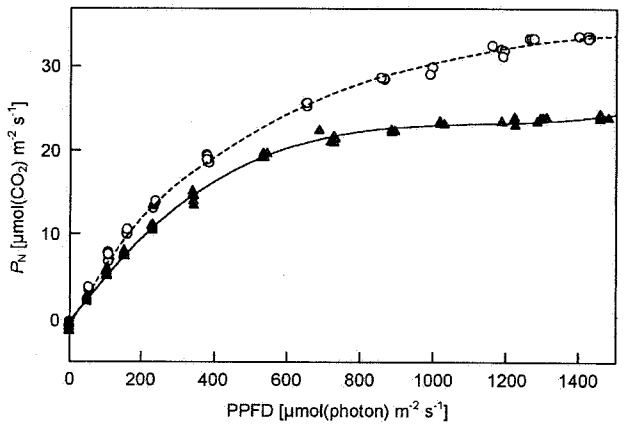

Fig. 1. Responses of net photosynthetic rate (P_N) to photosynthetic photon flux density (PPFD) in leaves of soybean cvs. Heinong 37 (▲) and Heinong 42 (○). P_N was measured by CID at *ca.* 390 $cm^3(CO_2)$ m^{-3} and 32 °C on 1 August, 2000. Only one of three repeats is shown here.

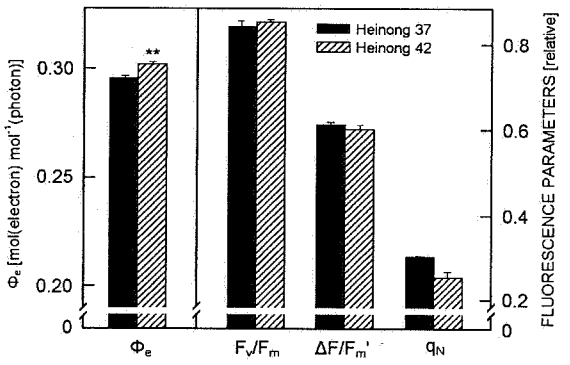

Fig. 2. Apparent quantum yield of electron transport through photosystem 2 (ϕ_c) and chlorophyll (Chl) fluorescence parameters of leaves of soybean cvs. Heinong 37 and Heinong 42. Means ± SE of 5-6 leaves. The significant level of difference between the two cultivars was $^*p<0.01$. All Chl fluorescence parameters were measured at room temperature (28 °C) by *PAM-2000* with the standard settings in laboratory. ϕ_c was calculated on the basis of Chl fluorescence parameter $\Delta F/F_m'$ on 14 August, 2000, while other parameters were measured on 1-10 August, 2000. The potential photochemical efficiency (F_v/F_m) was measured with fully dark-adapted leaves through whole night. Then the actual photochemical efficiency ($\Delta F/F_m'$) and non-photochemical quenching (q_N) were measured after the leaves were irradiated for about 60 min at an irradiance of 1 200 μmol(photon) m^{-2} s^{-1}.

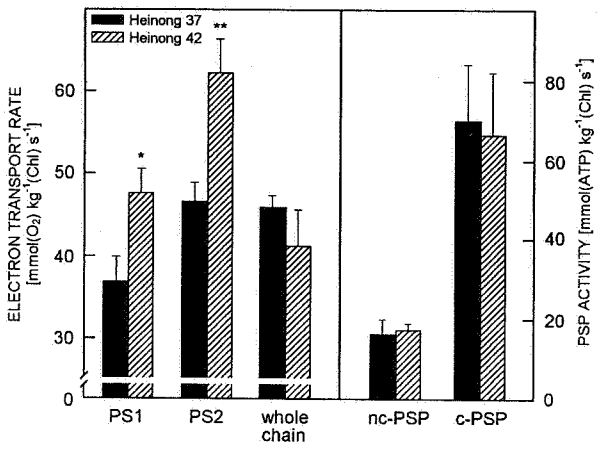

Fig. 3. Electron transport rates and photophosphorylation (PSP) activity of chloroplasts isolated from leaves of soybean cvs. Heinong 37 and Heinong 42. Means ± SE of 3-5 repeats. Significant leaves of difference between the two cultivars are indicated as $^*p<0.05$ and $^{**}p<0.01$. Electron transport rates were measured by oxygen electrode between 6-10 August, 2000 and PSP activity on 14 August, 2000. The chloroplasts were isolated from fully expanded leaves after the end of induction period of photosynthesis.

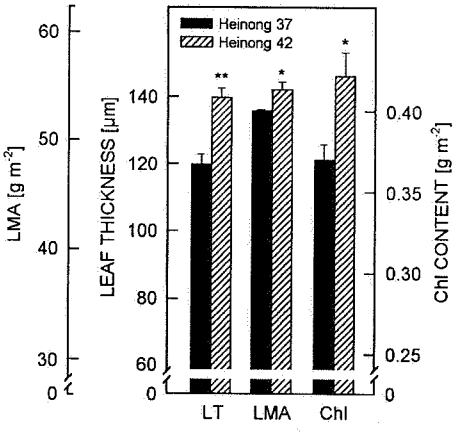

Fig. 4. Leaf thickness (LT), leaf dry mass per area (LMA), and leaf chlorophyll (Chl) content in soybean cvs. Heinong 37 and Heinong 42. Means ± SE of 3 leaves. Significant leaves of difference between the two cultivars are indicated as $^*p<0.05$ and $^{**}p<0.01$. All measurements were performed on 3 August, 2000.

Discussion

The significantly higher P_N in soybean cultivar Heinong 42 may be mainly attributed to a higher photosynthetic capacity of mesophyll cells rather than to higher g_s. In other words, the lower P_N in Heinong 37 can not be explained by lower g_s because Heinong 37 had a higher but not lower C_i (Table 1). This is similar to the results reported by Johnson *et al.* (1987), LeCain *et al.* (1989), and Jiang and Xu (2000).

We found that the higher photosynthetic capacity of mesophyll cells in Heinong 42 is not likely due to a higher activity of photosynthetic electron transport or PSP. Although the electron transport rates of PS1 and PS2, and ϕ_e were significant higher in Heinong 42 than in Heinong 37 (Figs. 2 and 3), the whole chain electron

transport rate was not so (Fig. 3). It is likely that the plastocyanin pool size is an important limiting factor to whole chain electron transport in soybean, as Burkey *et al.* (1996) suggested. Moreover, neither the nc-PSP nor c-PSP was significantly higher in Heinong 42 than in Heinong 37 (Fig. 3), nor was there any significant difference in apparent quantum yield of carbon assimilation (ϕ_c) or PS2 photochemical efficiency (F_v/F_m, $\Delta F/F_m'$) between the two cultivars (Table 2 and Fig. 2).

RuBPC is the key enzyme in photosynthetic carbon assimilation. *In vivo* CE (the slope of the initial linear portion of the P_N *vs.* C_i curve) is usually considered an index of the amount of activated RuBPC in leaf (Caemmerer and Farquhar 1981). Also, a strong positive correlation among P_N, CE, and activity/amount of RuBPC has been observed in many crops with cultivar difference in P_N, such as soybean, wheat, and rice (Hesketh *et al.* 1981, Joseph *et al.* 1981, Evans 1986, Nataraja and Jacob 1999). Moreover, under ambient air conditions the main limiting step of photosynthesis process may be in carbon assimilation rather than in energy conversion. After excluding the possibility that higher capacity of energy conversion is responsible for higher P_N in Heinong 42, the residual one is only higher carbon assimilation capacity in mesophyll cell. The possibility was confirmed by the following facts. The soybean cultivar Heinong 42 with significantly higher P_N had a significantly higher CE and activity of RuBPC per unit leaf area (Table 2). Although the percentage of the RuBPC protein to total soluble protein was similar between the two cultivars, Heinong 42 had a significantly higher total soluble protein content per unit leaf area than Heinong 37. Therefore, the former actually had a larger amount of RuBPC per unit leaf area (Table 2). In addition, some previous comparative studies on the RuBPC within C_3 plants indicated substantial interspecific and intraspecific variations in specific activity and *in vitro* kinetic properties (K_m and V_{max}) of the enzyme (Joseph *et al.* 1981, Evans 1986, Makino *et al.* 1988). Hesketh *et al.* (1981) found that P_N was positively correlated with RuBPC specific activity among soybean cultivars. Similarly, between the two soybean cultivars studied by us there was also a difference in the specific activity of RuBPC, being higher in Heinong 42, but it was not significant (Table 2). Therefore, mainly the higher amount or activity of RuBPC may explain higher P_N of Heinong 42.

In general, thicker leaves have higher P_N calculated per unit leaf area owing to their greater amounts of components of the photosynthetic apparatus. Dornhoff and Shibles (1976) and Pettigrew *et al.* (1993) reported a positive relationship between leaf thickness and P_N. A positive correlation between Chl content, soluble protein content, LMA, and P_N was also found in soybean (Hesketh *et al.* 1981), and other crops (Morgan *et al.* 1990, Pettigrew *et al.* 1993, Pettigrew and Meredith 1994). Our results about LT, LMA, leaf Chl content, and total soluble protein content (Table 2 and Fig. 4) are similar to those reported above.

The significant difference in P_N between the two soybean cultivars was not observed before pod setting stage (values not shown). An interesting question why the difference in P_N among cultivars appeared only in a certain period is worth further studying.

In summary, under non-stress conditions, a difference in g_s is usually not the main factor leading to significant cultivar difference in P_N. Although there are some cultivar differences in the energy conversion systems, the difference in P_N can be attributed chiefly to the differences in enzyme systems of carbon assimilation, especially the amount and/or activity of RuBPC. Selecting a higher amount and/or activity of RuBPC per unit leaf area during the reproductive period might be an important strategy for breeding good cultivars with higher photosynthetic efficiency.

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