

Physiological basis of the difference in net photosynthetic rate of leaves between two maize strains

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Abstract

Significant differences in net photosynthetic rate (P_N) of leaves between two maize (*Zea mays* L.) strains (Shuang 105 and 40×44) grown in the field were observed. At several growth stages, P_N of 40×44 was higher than that of Shuang 105 (from 10.3 to 32.5 %). Moreover, the strain 40×44 had a higher plant height, larger leaf area, lower chlorophyll content, and higher photochemical efficiency of photosystem 2 (PS2) (F_v/F_m and $\Delta F/F_m'$) than strain Shuang 105. Shuang 105, which showed lower P_N , had lower stomatal conductances (g_s) but slightly higher intercellular CO₂ concentrations (C_i) than those of 40×44. Hence the differences in P_N between the two strains did not result from the difference in g_s , but probably from that in light reaction system.

Additional key words: chlorophyll content; intercellular CO₂ concentration; leaf area; photochemical efficiency; photosystem 2; plant height; quantum yield; stomatal conductance; *Zea mays*.

Introduction

Photosynthesis is the basis of the formation of crop yield. A positive correlation between leaf net photosynthetic rate (P_N) and crop yield is the reflection of the essential relationship between photosynthesis and crop yield, so high photosynthetic rate should be an important index for the selection of high-yielding strain (Xu and Shen, accepted). Evidence has suggested the existence of substantial interspecies and intraspecies genetic variability in the rate of photosynthesis (Nelson 1988, Joshi 1997). However, what makes the differences among intraspecies or interspecies, or what leads to a high P_N ? Many authors studied the factors inducing differences in photosynthesis. These include CO₂ conductance, leaf morphological and anatomical structure, the contents and activities of enzymes involved in carbon assimilation, and other relevant physiological processes, such as photorespiration and dark respiration (Randall *et al.* 1977, Hesketh *et al.* 1981, Austin *et al.* 1982, Johnson *et al.* 1987, Hobbs 1988, LeCain *et al.* 1989, Suresh *et al.* 1997, Courtois *et al.* 1999, Körnerová and Holá 1999, *etc.*). Although some positive or negative correlations were found between P_N and the factors studied in the

reports mentioned above, clear answers are still lacking. Such correlations do not necessarily imply that cause-effect relations between P_N and these factors exist. And some correlations in these studies were contradictory (Buttery and Buzzell 1977, Hesketh *et al.* 1981, Austin *et al.* 1982, Suresh *et al.* 1997). In addition, breeding achievements in increasing crop yield so far are the outcome of changes in plant development and morphology and not due to direct improvement in P_N (Feil 1992). However, the scope for optimising photosynthesis does exist, and thus it could be a useful strategy for enhancing yield of crops to genetically modify photosynthesis (Austin 1989, Joshi 1997). Consequently, to explore the internal reasons causing interspecies or intraspecies differences in P_N is of significance not only for revealing the regulatory mechanism of photosynthesis, but also for utilising the potentialities of plant photosynthesis.

In the present study some relevant photosynthetic parameters in two strains of maize grown in the field were measured and analysed.

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Abbreviations: Chl – chlorophyll; C_i – intercellular CO₂ concentration; 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; PPFD – photosynthetic photon flux density; P_N – net photosynthetic rate; PS – photosystem; Φ_e – apparent quantum efficiency of CO₂ assimilation; Φ_e – apparent quantum efficiency of electron transport through PS2.

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Materials and methods

Plants: Two strains (Shuang 105 and 40×44) of maize (*Zea mays* L.) were raised in the field of Shanghai Institute of Plant Physiology in 1998. The distance between rows was 70 cm, and that between plants in rows was 50 cm. Only the first and second fully expanded leaves counted from the tops of plants were used in the measurements of photosynthetic parameters at all development stages.

Measurements of leaf area, leaf thickness, and plant height: The maximum lengths and widths of leaves, which were different at different developmental stages, were measured. The shapes of leaves drawn on paper of uniform texture were cut out and their areas were got from their masses using the mass/area ratio of the paper. A regression equation between the leaf areas and the relevant product of their maximum lengths and widths was used for calculation of leaf area.

Every leaf was cut into 10 pieces and then these pieces were piled up. The thickness of leaf was obtained by measuring the pile with vernier calliper.

The height of the plant was taken as the height of uppermost visible leaf of plant above ground level before heading and as that of the top of the tassel after heading.

Chlorophyll (Chl) content was determined according to Arnon (1949).

Gas exchange measurement: P_N was measured using a portable infra-red gas analyser CI-301 (CID Company, U.S.A.) on attached leaves alternately between the two strains from 10:00 to 11:30 (Beijing time) on cloudless days. The CO_2 concentration of the air used in the measurements was about $400 \text{ cm}^3 \text{ m}^{-3}$. The leaves were then cut down under water for measurements in the laboratory of apparent quantum yield of photosynthetic

CO_2 assimilation (Φ_e) (Xu *et al.* 1987) and of electron transport through PS2 (Φ_e).

Chl *a* fluorescence was measured with a portable PAM-2000 Chl fluorometer (H. Walz, Effeltrich, Germany) with the standard settings. Photochemical efficiency of PS2 in adequately dark-adapted leaves (F_v/F_m) and irradiated leaves whose photosynthesis had been steady ($\Delta F/F_m'$) was measured in the field with attached leaves and in laboratory with detached leaves. The calculations were made according to the following equations (Genty *et al.* 1989):

$$F_v/F_m = (F_m - F_0)/F_m$$

$$\Delta F/F_m' = (F_m' - F_s)/F_m'$$

where F_0 , F_v , and F_m are the initial, variable, and maximal fluorescence, respectively. F_m' is the maximal fluorescence when leaf photosynthesis is in steady state.

Φ_e was calculated by using Chl fluorescence parameter $\Delta F/F_m'$. After the end of the induction period of photosynthesis, $\Delta F/F_m'$ was measured under photosynthetic photon flux density (PPFD) increasing from 0 to $50 \mu\text{mol m}^{-2} \text{ s}^{-1}$ with a step $10 \mu\text{mol m}^{-2} \text{ s}^{-1}$ and for 3 min at each irradiance. Apparent photosynthetic electron transport rate through PS2 (ETR) was calculated with the following equation (Schreiber *et al.* 1994):

$$\text{ETR} = \Delta F/F_m' \times \text{PPFD} \times 0.5 \times 0.84$$

where the factor 0.5 indicates that the transport of one electron requires the absorption of two photons by the two photosystems, and the factor 0.84 is an assumed absorption coefficient of the leaves to incident PPFD. Φ_e was calculated by the initial slope of ETR response curve to different PPFD.

Results

P_N of maize leaves: Significant differences in P_N between the two maize strains were observed at different growth stages, values of 40×44 being much higher than those of Shuang 105. Although Shuang 105 always had a lower g_s than 40×44, it always had a higher C_i than 40×44 (Fig. 1).

Chl content, leaf thickness, and plant height: There was no significant difference in leaf thickness and Chl content between Shuang 105 and 40×44 before heading, but after heading, the former had thicker leaves and higher Chl content than the latter, and the differences were significant (Fig. 2). At all growth stages, 40×44 had much larger leaves and much higher plant height than

Shuang 105 (Table 1). The total number of leaves per plant was similar and every leaf expanded at about the same time for the two strains.

Apparent quantum yield and the photochemical efficiency: Strain 40×44 showed higher Φ_e and Φ_e than Shuang 105, but the differences were not significant (Fig. 3). The potential PS2 photochemical efficiency (F_v/F_m) of 40×44 was higher than that of Shuang 105 in many measurements, and the differences were significant. However, the actual PS2 photochemical efficiency ($\Delta F/F_m'$) of 40×44 was only slightly higher than that of Shuang 105, and the differences were not significant (Table 2).

Discussion

There have been many reports about the positive relationship between photosynthetic rate and stomatal conductance (e.g., Austin *et al.* 1982, Johnson *et al.* 1987, Suresh *et al.* 1997). However, such positive correlation can not necessarily demonstrate which is the cause or effect of their changes. The change of another important parameter, intercellular CO_2 concentration, should be analysed before any conclusion is made about the cause-effect relation between changes of g_s and P_N (Farquhar and Sharkey 1982). The C_i is dependent on g_s and the CO_2 assimilation capacity of mesophyll cells. Johnson *et al.* (1987) reported that the variation in P_N among wheat accessions (*Triticum dicoccoides*) was attributable to non-stomatal factors to a significant extent, though higher P_N was correlated with higher g_s ($r = 0.86$, $p < 0.01$). At ambient CO_2 concentration of $330 \text{ cm}^3 \text{ m}^{-3}$, an accession PI 428042 with lower P_N had a much lower g_s but a slightly higher C_i compared with another accession PI 428109. PI 428109 had a steeper initial slope of the P_N vs. C_i curve and a higher CO_2 -saturated P_N . So they considered that the higher P_N was due to a higher capacity for mesophyll photosynthesis rather than a higher g_s . LeCain *et al.* (1989) got a similar conclusion in isogenic semidwarf and tall winter wheat strains. We observed that P_N of 40×44 was significantly higher than that of Shuang 105 (by about 10.3–32.5 %) at several growth stages. Although g_s of Shuang 105 was often lower (only in one time measurement an opposite result was obtained), its C_i was always higher than that of 40×44 (Fig. 1). Thus the relatively lower P_N of Shuang 105 was not caused by an inadequate supply of CO_2 or a lower g_s .

Table 1. The maximal single and total leaf areas [cm^2] and height [cm] of plants in two maize strains (Shuang 105 and 40×44) measured at different growth stages. The significant levels of difference between the two strains are indicated by asterisk * and ** for $p < 0.05$ and $p < 0.01$, respectively. Each value is the mean \pm SD of 5 leaves or plants. Plant height was measured from the ground level to the uppermost visible leaf or to the top of the tassel after heading.

Age [d]	Strain	Leaf area single	total	Height
52	Shuang	316.7 \pm 111.8	—	46.2 \pm 8.0
	40×44	629.5 \pm 90.3**	—	83.0 \pm 4.5**
58	Shuang	416.9 \pm 63.8	—	65.2 \pm 13.4
	40×44	723.6 \pm 90.6**	—	111.0 \pm 7.4**
74	Shuang	406.4 \pm 55.9	2273.8 \pm 500.8	105.4 \pm 9.3
	40×44	697.6 \pm 88.0**	5228.6 \pm 704.8**	198.2 \pm 10.5**

Under saturating PPFD for photosynthesis, changes in Chl content, in a certain range, have no obvious effect on P_N (Buttery and Buzzell 1977, Austin *et al.* 1982, Suresh *et al.* 1997). Furthermore, the Chl-deficient mutants did

not always show a lower P_N (Buttery and Buzzell 1977, Hesketh *et al.* 1981, Tan *et al.* 1996). Between Shuang 105 and 40×44 no significant difference in Chl content was observed before heading (Fig. 2), thus we presume that the difference in P_N between these two strains could not be attributed to that of Chl content. In general, thicker leaves have a higher P_N per unit leaf area owing to a greater amount of the photosynthetic components, especially ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) per unit leaf area. Dornhoff and Shibles

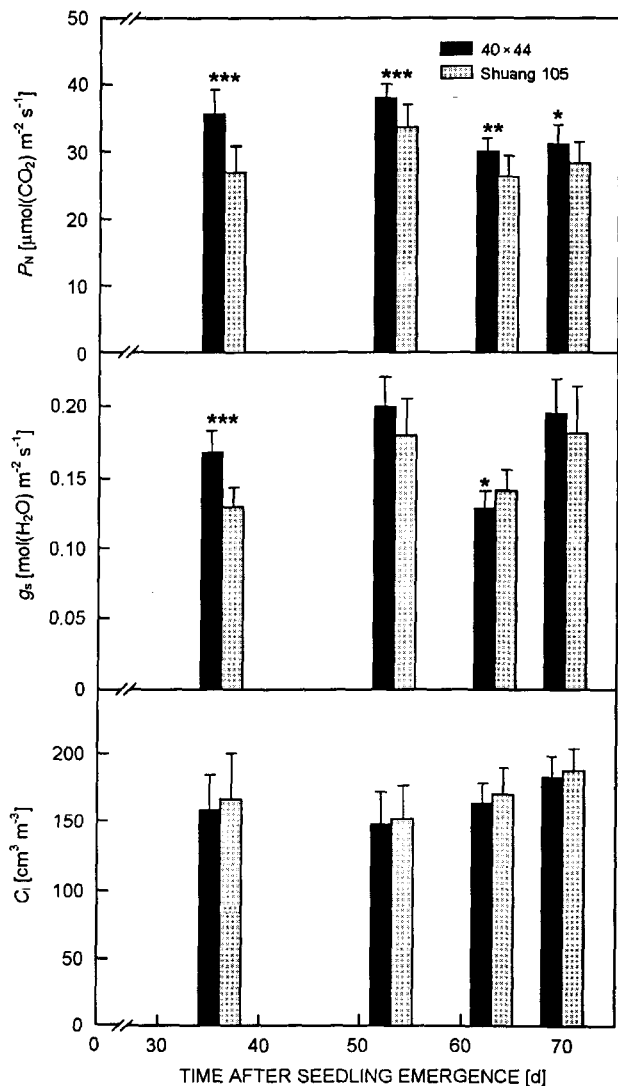


Fig. 1. Net photosynthetic rate (P_N), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i) in leaves of two maize strains (Shuang 105 and 40×44) measured at different growth stages. Each value is the mean \pm SD of 9–11 leaves, and the significant levels of difference between the two strains are indicated by asterisks: * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. All measurements were made during 10:00–11:30 (Beijing time) on clear days. The samples measured were the first and second fully expanded leaves counted from the top of plants.

(1976) and Pettigrew *et al.* (1993) reported a positive relationship between leaf thickness and P_N . We found that Shuang 105 had thicker leaves (Fig. 2), but much lower P_N values than those of 40×44 (Fig. 1). Thus, the difference in P_N reported here also can not be attributed to that in leaf thickness.

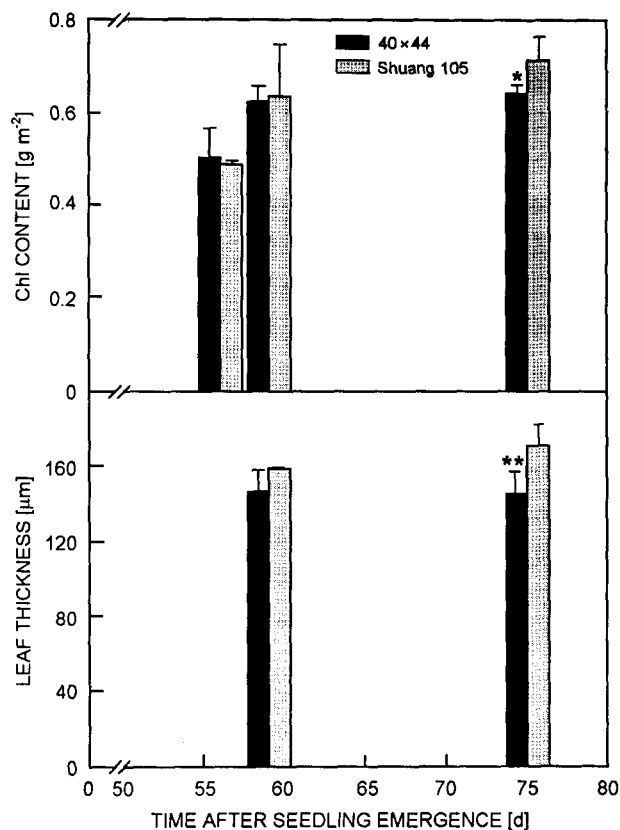


Fig. 2. Chlorophyll (Chl) content and thickness of leaves in two maize strains (Shuang 105 and 40×44) at different growth stages. The significant levels of difference between the two strains are indicated by asterisks: * $p < 0.05$, ** $p < 0.01$. Each value measured on the 75th day is the mean \pm SD of 5 leaves and the others are those of 3 leaves.

Genetic variation in photochemical activity has already been observed by Burkey *et al.* (1996) in soybean (*Glycine max.* L.), Synková *et al.* (1997) in tomato, and Krebs *et al.* (1996), Holá *et al.* (1999), and Körnerová and Holá (1999) in maize (*Zea mays* L.). Also, Burkey (1994) reported a genetic variation in photosynthetic electron transport in barley (*Hordeum* sp.). So, it is necessary to consider if the difference in the efficiency of the light reaction system can account

for the difference in P_N between the two maize strains. The maximal or potential PS2 photochemical efficiency (F_v/F_m) of 40×44, measured with attached or detached leaves, was always higher than that of Shuang 105. And the difference was significant in most measurements (Table 2). Moreover, the actual photochemical efficiency ($\Delta F/F_m'$) of 40×44 was slightly higher than that of Shuang 105 in five measurements with attached leaves, though only in one measurement the difference between them was significant (Table 2). From these results we deduce that the strain 40×44 has a more efficient light reaction system. The values of Φ_e and Φ_c are consistent with this deduction (Fig. 3). Therefore, the difference in P_N between the two strains was probably due to differences in capacity of absorbing, transporting, and converting radiant energy of the light reaction system. It is worth studying whether the differences in dark reaction system of the photosynthetic apparatus, especially RuBPCO content and/or activity, can account for the difference in P_N between these two maize strains.

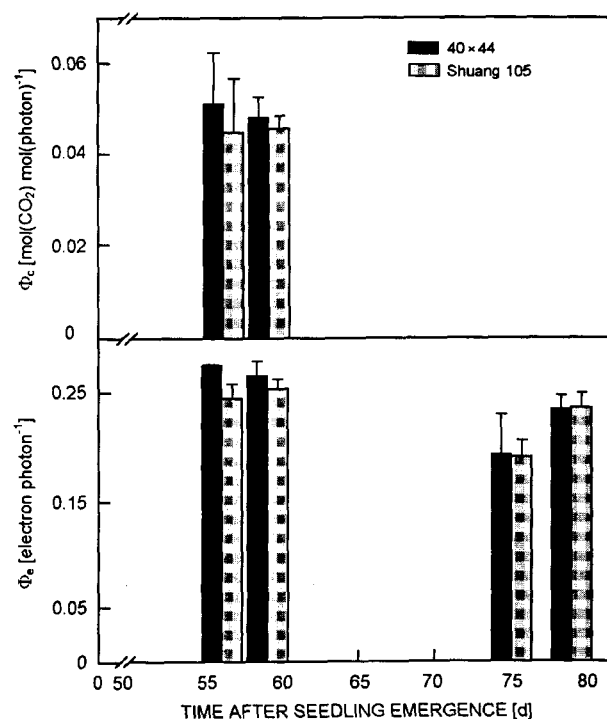


Fig. 3. Apparent quantum yield of photosynthetic CO_2 assimilation (Φ_c) and of electron transport through PS2 (Φ_e) in detached leaves of two maize strains (Shuang 105 and 40×44) measured at different growth stages. Each value is the mean \pm SD of 2-5 leaves.

Table 2. Photosystem 2 (PS2) photochemical efficiency of leaves in two maize strains (Shuang 105 and 40×44) measured at different growth stages. F_v/F_m of leaves was measured after adequately dark-adapted through whole night (about 12 h). $\Delta F/F_m'$ of the detached leaves was measured after irradiating at $1500 \mu\text{mol}(\text{photon}) \text{ m}^{-2} \text{ s}^{-1}$ for about 2 h in laboratory, and that of attached leaves was measured under different PPFD [700, 1200, and $1700 \mu\text{mol}(\text{photon}) \text{ m}^{-2} \text{ s}^{-1}$ for A, B, and C, respectively] in the field during 10:00~12:00 (Beijing time) on clear days. The significant levels of difference between the two strains are indicated by asterisk: * $p < 0.05$, ** $p < 0.01$. Each value is the mean \pm SD of 5-8 leaves. PA means plant age [d].

Strain	Attached leaves			Detached leaves		
	PA	F_v/F_m	$\Delta F/F_m'$	PA	F_v/F_m	$\Delta F/F_m'$
Shuang 105	53 (C)	0.774 ± 0.013	0.164 ± 0.064	45	0.809 ± 0.006	0.104 ± 0.047
40×44		$0.798 \pm 0.008^{**}$	$0.266 \pm 0.055^*$		0.817 ± 0.006	0.211 ± 0.068
Shuang 105	63 (C)	0.788 ± 0.010	0.173 ± 0.032	75	0.788 ± 0.010	—
40×44		$0.802 \pm 0.008^{**}$	0.200 ± 0.046		$0.808 \pm 0.005^{**}$	—
Shuang 105	52 (A)	—	0.370 ± 0.021	—	—	—
40×44		—	0.377 ± 0.037	—	—	—
Shuang 105	65 (B)	—	0.278 ± 0.048	—	—	—
40×44		—	0.295 ± 0.017	—	—	—
Shuang 105	68 (C)	—	0.179 ± 0.050	—	—	—
40×44		—	0.185 ± 0.240	—	—	—

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