Heterogeneity of photosynthesis within leaves is associated with alteration of leaf structural features and leaf N content per leaf area in rice

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Abstract. Increasing leaf photosynthesis rate ($A$) is considered an important strategy to increase C\textsubscript{3} crop yields. Leaf $A$ is usually represented by point measurements, but $A$ varies within each leaf, especially within large leaves. However, little is known about the effect of heterogeneity of $A$ within leaves on rice performance. Here we investigated the changes in gas-exchange parameters and leaf structural and chemical features along leaf blades in two rice cultivars. Stomatal and mesophyll conductance as well as leaf nitrogen (N), Rubisco and chlorophyll contents increased from base to apex; consequently, $A$ increased along leaves in both cultivars. The variation in $A$, leaf N content and Rubisco content within leaves was similar to the variations among cultivars, and the extent of $A$ heterogeneity within leaves varied between cultivars, leading to different efficiencies of biomass accumulation. Furthermore, variation of $A$ within leaves was closely associated with leaf structural and chemical features. Our findings emphasise that functional changes along leaf blades are associated with structural and chemical trait variation and that variation of $A$ within leaves should be considered to achieve progress in future breeding programs.

Additional keywords: biomass, CO\textsubscript{2} diffusion, leaf structure, leaf N content per leaf area.

Received 20 December 2014, accepted 11 April 2015, published online 20 May 2015

Introduction

Rice is one of the most important food crops in the world, directly feeding more people than any other crop (Mitchell and Sheehy 2006). To meet the demands of a rapidly growing population while arable land decreases, the improvement of rice productivity per area is an essential research goal. The development of modern semi-dwarf rice varieties with high harvest indices has dramatically improved rice yields since 1960s (Khush 2001). In modern agricultural systems, the rice harvest index is close to the theoretical maximum, and thus further improvement is very limited (Long et al. 2006). Therefore, increasing total biomass production per unit area is the key to continued increases in rice grain yield. As the fundamental process generating crop biomass and yield, the rate of photosynthesis ($A$) of individual leaves has become the focus of current efforts to increase rice production (Mitchell and Sheehy 2006). In most studies, leaf $A$ is usually represented by point measurements; however, $A$ is more likely varies with the position along leaves, especially, for large and narrow leaves.

In C\textsubscript{3} plants, $A$ is mainly determined by two factors: CO\textsubscript{2} concentration in chloroplasts and Rubisco activity (Farquhar et al. 1980). The diffusion of CO\textsubscript{2} into leaves is restricted by the stomata, and subsequent diffusion into chloroplasts is restricted by the intercellular airspaces and mesophyll cell structure (Flexas et al. 2008, 2012). The diffusion process of CO\textsubscript{2} from outside leaf into the intercellular airspaces is quantified as 'stomatal conductance' ($g_s$), and the further diffusion conductance of CO\textsubscript{2} from the intercellular airspaces to the chloroplasts is termed ‘mesophyll conductance’ ($g_m$). In general, $g_s$ responds physiologically within minutes to changes in the external environment (Creese et al. 2014), and the dynamic range of $g_s$ plays an important role in plant production under natural conditions. Under low evaporative demand and high light conditions, the upper limit of $g_s$ is determined by the opening and closing of stomata. When plants experience a high evaporative demand, leaves close their stomata to retain water within their highly water impermeable leaf cuticles thus minimising water loss. Greater stomatal conductance is associated with smaller stomatal sizes and higher stomatal density across broad geological time scales and diverse evolutionary lineages (Drake et al. 2013), which as well as within a single species across environmental gradients (Franks et al. 2009). Smaller stomata also open and close faster across varied environments (Drake et al. 2013; Lawson and Blatt 2014). Therefore, $g_s$ is...
primarily determined by stomata morphological features. Heterogeneity of $g_s$ within dicot leaves has been the focus of several recent studies, and heterogeneity of $g_s$ is thought to respond to local leaf water status (Mott 1995; Kamakura et al. 2011). However, the heterogeneity of $g_s$ along individual monocot leaf blades, and how leaf features affect that heterogeneity are still unclear.

It is recognised that $g_m$ is an important limiting process for photosynthesis, as it significantly affects the process of CO$_2$ diffusion from substomatal cavities to chloroplast stroma. Rapid changes in $g_m$ have been documented (Flexas et al. 2008; Warren 2008); however, these $g_m$ changes vary with leaf structural parameters such as cell packing, shape and wall thickness (Tosens et al. 2012; Tomás et al. 2013). The relationship between CO$_2$ diffusion processes and morpho-anatomical traits has been well documented, and many related trade-offs have been described (Ocheltree et al. 2012; Giuliani et al. 2013; Tanaka et al. 2013; Muir et al. 2014). However, most of these results were obtained across species, cultivars or genetic stocks. The relationship between morpho-anatomical traits and $g_m$ within individual leaves has received less attention. Nevertheless, results from studies on maize (Sasakawa et al. 1989) and sugarcane (Meinzer and Salindra 1997) leaves suggest that longitudinal variation in carbon isotope discrimination within individual leaves may equal or exceed the magnitude of genetic variable. Such results indicate that $g_m$ is varies along individual leaves.

Leaf nitrogen (N) content per leaf area in high plants is directly related to $A$, because stromal enzymes and thylakoid proteins account for the majority of leaf N (Terashima and Evans 1988; Evans 1989; Tazoe et al. 2006). Heterogeneity of leaf N within individual leaves, is considered an adaptation to light conditions, and was observed in sugarcane leaves (Meinzer and Salindra 1997). The relationship between leaf N content per leaf area and $A$ is principally affected by two factors: (i) N allocation between photosynthetic and non-photosynthetic N elements, and (ii) partitioning within photosynthetic apparatuses. As a limiting factor of photosynthetic capacity in full sunlight, ribulose-1, 5-bisphosphate carboxylase/oxygenase (Rubisco) is a particularly important photosynthetic enzyme (Brooks and Farquhar 1985), and it comprises ~50% of photosynthetic nitrogen (Evans 1989; Hikosaka and Terashima 1996). Yamori et al. (2011) suggested that N partitioning into Rubisco and cytochrome $f$ significantly affects the limiting step of $A$. Previous studies suggest that leaf N content per leaf area is also correlated with both $g_s$ and $g_m$ (Franks and Beerling 2009; Xiong et al. 2015). Incorporating variables such as leaf N content per leaf area will therefore improve our understanding of functional changes along leaf blades in rice.

Revealing the relationship among structural traits, chemical properties and $A$ in individual leaves may lead to the identification of structural and chemical features for enhancing crop productivity and as well as provide insight into plant adaptation. The aims of this study were to: (i) identify variation in gas exchange, morpho-anatomical traits and chemical properties along a leaf blade from base to apex in rice, (ii) investigate how leaf morpho-anatomical traits and chemical properties (leaf N status) influence heterogeneity of $A$ within leaves, and (iii) to examine whether plant biomass accumulation is impacted by heterogeneity of $A$ within leaves.

### Materials and methods

#### Plant Materials

Two rice cultivars Aus (*Oryza australiansis* L.) and Lat (*Oryza latifolia* L.) were investigated in a pot experiment at Huazhong Agricultural University, Wuhan, China. Plant materials were collected from the National Key Laboratory of Crop Genetic Improvement, Wuhan, China. Rice plants were grown in 15.0 L pots that filled with 13.0 kg soil with a density of three hills per pot and one seedling per hill. N, phosphorous (P) and potassium (K) were applied as basal fertilisers in amounts of 3.0, 1.95 and 1.95 g pot$^{-1}$ respectively. Six pots per cultivars were randomly distributed. Throughout their whole growth, plants were well watered and a water depth of at least 2 cm was maintained. Pests were controlled using chemical pesticides. Measurements were started at 50 days after planting.

#### Gas exchange measurements

Gas exchange was measured between 0930 and 1530 hours in an environment-controlled room with an air temperature of 28.0 $\pm$ 2.5°C, photosynthetic photon flux density (PPFD) at leaf surface of 1200 $\pm$ 50 $\mu$mol m$^{-2}$ s$^{-1}$ (artificial LED light source), and RH of 78.0% $\pm$ 5.0%. Measurements were performed on newly- and fully-expanded leaves of three plants of each cultivar after the plants were acclimated to the room environment for ~1.5 h. Each leaf was divided into 10 equal sections along leaf blades, and the middle of each section was labelled with a red marker. All measurements were centred on this midpoint (the first and last sections were excluded as accurate measurements for these sections were difficult). Gas exchange and chlorophyll fluorescence were simultaneously measured using two LI-6400XT portable photosynthesis systems equipped with 6400–40 leaf chamber (Li-Cor, Lincoln, NE, USA). Photosynthesis systems were zeroed before measurements were taken. The measurements were first made on the most basal section and then progressed sequentially along the length of the leaf blade towards the apex. Leaf temperature during measurements was maintained at 28°C. In the leaf chamber, PPFD was maintained at 1500 $\mu$mol m$^{-2}$ s$^{-1}$, leaf-to-air vapour pressure deficit (VPD) at 1.2–1.4 kPa, and CO$_2$ concentration at 400 $\mu$mol m$^{-2}$ s$^{-1}$ (maintained with a supplemental CO$_2$ mixture). After equilibration to a steady-state (usually occurring more than 20 min after clamping the leaf), the gas exchange parameters, steady-state fluorescence ($F_s$) and maximum fluorescence ($F_m$) were recorded. Actual photochemical efficiency of PSII ($\Phi_{PSII}$) was calculated as follows:

$$\Phi_{PSII} = \frac{F_m - F_s}{F_m}.$$

Electron transport rates (ETR) were computed as follows:

$$ETR = \Phi_{PSII} \cdot PPFD \cdot \alpha \cdot \beta,$$

where $\alpha$ is the leaf absorbance and $\beta$ represents the distribution of electrons between PSI and PSII.

After measurement of gas exchange and chlorophyll fluorescence, a light response curve was determined for the middle section of each leaf, under a low O$_2$ concentration.
(≤2%) by estimating α and β. After equilibration to a steady state, the gas-exchange system was immediately switched to a low O2 concentration (≤2%) without removing the leaves from the chamber. Light response curves and chlorophyll fluorescence were then simultaneously measured. During these measurements, chamber conditions were identical to those described above, except that PPFD was controlled across a gradient of 800, 600, 400, 200, 100 and 0 μmol m⁻² s⁻¹. After reaching a steady state, the parameters of gas exchange and chlorophyll fluorescence were simultaneously recorded. Under non-respiration conditions, the relationship between ETR calculated through gas exchange and chlorophyll fluorescence is expected to be linear because electron transport flow is primarily associated with Rubisco carboxylation and the α/β values do not change as light varies. Consequently, the slope of the relationship between ¼ΦPSII and the quantum efficiency of CO2 uptake (ΦCO2) is considered to be the value of α/β (Valentini et al., 1995; Long and Bernacchi, 2003).

The variable J method described by Harley et al. (1992) was used to calculate CO2 concentration in chloroplast (Cc) and mesophyll conductance of CO2 (gm). Cc was calculated as follows:

\[ C_c = \frac{\Gamma^* (ETR + 8(A + R_d))}{ETR - 4(A + R_d)}, \]

where \( \Gamma^* \) represents the CO2 compensation point in the absence of respiration. \( \Gamma^* \) is related to Rubisco specific factor and is relatively conserved under a given temperature condition. In the present study, a \( \Gamma^* \) value of 40 μmol mol⁻¹ and an \( R_d \) value of 1 μmol m⁻² s⁻¹ typically for rice plants were used (Yamori et al., 2011; Xiong et al., 2015). Then, \( g_m \) was calculated as follows:

\[ g_m = \frac{A}{C_i - C_c}, \]

where \( C_i \) represents the intercellular CO2 concentration.

**Measurements of leaf N, chlorophyll and Rubisco content per leaf area**

After measurement of gas exchange, leaf samples (longitudinal sections) were photo scanned and oven-dried separately at 80°C to constant weight. Leaf area was measured by using Image J software (National Institute of Health, Bethesda, MD, USA). The dry samples were digested by the micro-Kjeldahl method, and then the N concentration was measured with a discrete wet chemistry analyser (SmartChem 200, AMS-Westco, Rome, Italy). Absolute chlorophyll concentration was measured using a spectrophotometer (UV2102, Unico, Shanghai, China) and 95% (v/v) alcohol extracts of leaf tissue. Leaf tissue for these measurements was harvested using a circular punch that yields 0.5 cm diameter leaf discs.

Rubisco concentration was measured by the SDS–PAGE method (Makino et al., 1985). Leaf tissue was harvested using the aforementioned circular punch, and immersed in liquid nitrogen. The samples were stored at −80°C until Rubisco concentration assays were performed. The frozen leaf samples were ground in liquid nitrogen and homogenised on ice in an extraction buffer containing 50 mM Tris-HCl buffer (pH 8.0), 5 mmol β-mercaptoethanol, and 12.5% (v/v) glycerol. After centrifugation, SDS solution, β-mercaptoethanol, and glycerol were added to the supernatant fluid yielding final concentration of 2.0% (w/v), 4% (v/v) and 10% (v/v) respectively. This preparation is immediately treated at 100°C for 1 min, and then stored at −20°C until analysed by SDS–PAGE. The samples were loaded onto SDS–PAGE containing a 12.5% (w/v) polyacrylamide gel. After electrophoresis (DYY-11, Beijing Liuyi Instrument Factory, Beijing, China), the gels were washed with deionised water several times dried in 0.25%. Commassie blue staining solution for 9 h and decolourised until the background was colourless. The large and small subunits were transferred into a 10 mL cuvette with 2 mL of formamide and washed in a 50°C water bath for 8 h. The absorptions of washed solutions were measured at 595 nm (Infinite M200, Tecan Inc., Männedorf, Switzerland) using the background glue as blank and bovine serum albumin (BSA) as standard protein.

**Microscopic analysis**

Five small leaf discs (~10 × 10 mm) were removed from the middle of each leaf sections. For each cultivar, three leaves from different plants were measured. The leaf discs were infiltrated in a vacuum chamber (DZF-6050, Shanghai Hasuc Co. Ltd, Shanghai, China) with the fixative 2.5% glutaric aldehyde in 0.1 M phosphate buffer (pH = 7.6) at 4°C and then the samples were stored at 4°C until analysis. Images of the abaxial epidermal surfaces were captured under vacuum with a scanning electron microscopes (JSM-6390 LV, Tokyo, Japan). Stomatal size, stomatal density, guard cell length and the minor distance between the minor vein and stoma (Dv) on the abaxial lamina surface were calculated for each leaf based on 6–12 measurements.

**Biomass and leaf morphology**

Biomass samples were collected at 60 days after planting. The newly and fully expanded leaves were cut and the leaf width and length were measured quickly using a plastic ruler. After the leaf area was measured using a leaf area meter (LI-Cor 3000C, Li-Cor). For leaf area per plant, all green leaves per plant were collected and measured using the leaf area meter. Finally, the green and dead leaf blades, leaf sheaths and culms of each plant were collected, and the biomass determined by weighing the samples after drying for 5 days in an oven at 80°C.

**Statistical analysis**

One-way analysis of variance was calculated using SAS9.2 (SAS Institute Inc., Carey, NC, USA). The means of three replications were compared using the least significant difference (LSD) multiple comparison test (P < 0.05). Regression analyses were performed with mean values to test the correlations between parameters using SigmaPlot 12.5 (SPSS Inc., Chicago, IL, USA). All regressions were fitted by both linear and power models, and the model with higher regression coefficient was selected. Regression coefficients and significance are shown for P < 0.05.
Results

Growth characteristics and photosynthetic performance

There were significant differences in total biomass, tiller number, and leaf area between Lat and Aus (Table 1). Average tiller number, biomass, and leaf area per plant for Lat were 27.8, 127.5 and 83.9%, higher than those for Aus respectively. Both A and transpiration rate (E) increased from leaf blade base to apex for both cultivars (Fig. 1a, b). There were increases of ~105 and 51% of A, and ~80 and 70% of E along the leaf blade for Lat and Aus respectively. Compared with Aus, Lat had a lower value of A at its blade base and a higher value at its blade apex. However, E at both the blade base and apex in Lat were significantly higher than in Aus. Both gs and gm also increased from leaf blade base to apex in both cultivars (Fig. 1c, d). The two cultivars exhibited similar values of gs at their blade bases, but the gs values of Lat blade apexes were significantly higher than those of Aus. Conversely, Aus exhibited higher values of gm at its blade bases, but similar gm values at its blade apexes.

Leaf N, Rubisco and chlorophyll content per leaf area

Along leaf blades (from base to apex), the leaf N content per leaf area, chlorophyll content per leaf area, Rubisco content per leaf area, Rubisco to leaf N ratio, and Rubisco to chlorophyll ratio increased significantly in both Lat and Aus (Fig. 2). However, the ratio of chlorophyll to leaf N decreased from leaf base to apex. The two cultivars had similar leaf N content per leaf area and chlorophyll content per leaf area at their blade bases, but Aus had a higher leaf N content per leaf area and Lat had a higher chlorophyll content at their blade apexes. The increase of Rubisco content per leaf area along the leaf blades was greater in Lat (from 1.6 to 7.6 µmol m⁻²) than in Aus (from 4.1 to 7.0 µmol m⁻²), which resulted the high variability of Rubisco to leaf N ratio in Lat.

<p>| Table 1. Plants growth and leaf characteristics for the two investigated cultivars |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Tiller numbers</th>
<th>Leaf area (cm² plant⁻¹)</th>
<th>Biomass (g plant⁻¹)</th>
<th>Leaf area (cm²)</th>
<th>Leaf length (cm)</th>
<th>Leaf width (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lat</td>
<td>7.67 ± 1.15a</td>
<td>2883.3 ± 166.0a</td>
<td>33.67 ± 1.30a</td>
<td>134.98 ± 6.68a</td>
<td>76.83 ± 4.53a</td>
<td>2.24 ± 0.08a</td>
</tr>
<tr>
<td>Aus</td>
<td>6.00 ± 1.00b</td>
<td>1534.3 ± 187.5b</td>
<td>14.80 ± 1.07b</td>
<td>50.19 ± 8.04b</td>
<td>50.93 ± 3.76b</td>
<td>1.23 ± 0.11b</td>
</tr>
</tbody>
</table>

Fig. 1. Relationship of position along leaf blades (reported as the percentage of leaf length measured from ligule) with (a) photosynthetic rate (A), (b) transpiration (E), (c) stomatal conductance to CO₂ (gs), (d) mesophyll conductance to CO₂ (gm). The values shown are mean ± s.d. (significant differences are indicated: ***, P < 0.001).
Leaf anatomy

The leaf characteristics of plants analysed in this study are listed in Table 1. There were considerable differences between cultivars in leaf area, leaf length and leaf width. Leaf structure varied considerably along leaf blades (Fig. 3). The stomatal density increased considerably along leaf blades. However, the stomatal size decreased slightly, and the guard cell length was relatively constant along leaf blade in the two cultivars. The distance between stomata and the minor vein ($D_s$) considerably decreased within leaves of the both cultivars. At any given blade position, Lat had significantly higher stomatal densities than Aus; the stomatal densities ranged from 276 to $440\, mm^2$ for Lat and from $144$ to $328\, mm^2$ for Aus. However, the stomatal sizes and guard cell lengths were significantly lower for Lat than for Aus. The two cultivars exhibited similar $D_s$ at their blade bases; however, Aus had higher $D_s$ than Lat at its blade apexes.

Coordination of gas exchange, structural and chemical traits

There were significant correlations between $A$ and both $g_s$ (Aus: $R^2 = 0.93, P < 0.001$; Lat: $R^2 = 0.98, P < 0.001$), and $g_m$ (Aus: $R^2 = 0.98, P < 0.001$; Lat: $R^2 = 0.91, P < 0.001$) in the two rice cultivars (Fig. 4). We also observed a positive relationship between $g_s$ and stomatal density (Aus: $R^2 = 0.97, P < 0.001$; Lat: $R^2 = 0.96, P < 0.001$), but a negative relationship between $g_s$ and stomatal size in both cultivars (Aus: $R^2 = 0.87, P < 0.01$;...
Lat: $R^2 = 0.88$, $P<0.01$; Fig. 5). However, these correlation coefficients varied with cultivars. Furthermore, $g_s$ was closely correlated with $D_s$ in both cultivars (Fig. 5c).

Leaf N content per leaf area was correlated with $A$ in both rice cultivars (Fig. 6). The same pattern was observed between $A$ and Rubisco content per leaf area. Similarly, leaf N content per leaf area was correlated with the Rubisco to leaf N ratio. In addition, the changes of $g_s$ and $g_m$ along rice leaf blades were closely correlated with leaf N content per leaf area (Fig. 7).

**Discussion**

We found substantial heterogeneity of $A$ along rice leaf blades. In C3 plants, $A$ is mainly limited by CO2 concentration in the chloroplast or carboxylation, and is often quantified with the widely used FvB model (Farquhar et al. 1980). For many years, the limitation of Rubisco content and activity on $A$ has been confirmed, and CO2 concentrations in chloroplasts were considered to be restricted by both $g_s$ and $g_m$. The data presented here suggest that acropetal gradients of $A$ are related to changes...

**Fig. 3.** Relationship of position along leaf blades (reported as the percentage of leaf length measured from ligule) with (a) stomatal density, (b) stomatal size, (c) guard cell length, and (d) distance between stomata and minor veins ($D_s$). The values shown are mean ± s.d. (significant differences are indicated: ns, no significant ($P>0.05$); ***, $P<0.001$).

**Fig. 4.** Changes in photosynthesis ($A$) in relation to (a) stomatal CO2 conductance ($g_s$), (b) mesophyll CO2 conductance ($g_m$) and (c) total CO2 conductance ($g_t$) along leaves. The values shown are mean ± s.d. (significant differences are indicated: ***, $P<0.001$).
in both leaf biochemistry (carboxylation), CO₂ diffusion conductance and leaf structural features.

**Leaf biochemistry**

It is widely acknowledged that $A$ is closely related to leaf N content per leaf area in rice due to the close relationship between Rubisco content and leaf N content per leaf area (Evans 1989; Hikosaka and Terashima 1996). Here we observed that leaf N content, Rubisco content and chlorophyll content per leaf area are heterogeneous within individual leaf blades and the gradient of $A$ along a leaf blade is related to the distribution of N within a leaf (Fig. 6). The mechanism by which N is heterogeneously distributed is illustrated in Fig. 6.
distributed within leaf blades is possibly related to an adaptation and the developmental dynamic (Wang et al. 2014) to the growth form of grasses. Under natural growth conditions, the basal portion of rice leaves may be shaded by tillers and the upper portions of leaves (Song et al. 2013). Resources, for example, leaf N, would be optimally allocated to the apical regions of leaves, where light availability is typically higher, to maximise A. Leaf N content per leaf area and gas change measured in the present study agree with the view that rice leaves have adapted to a growth form in which light gradients exist along leaf blades. The second piece of evidence supporting acropetal increases in A as an adaptation to the growth form of rice is the N allocation among photosynthetic apparatuses. In the present study we observed that allocated leaf N to chlorophyll decreased along the leaf blade from the base to apex; however, the allocated leaf N to Rubisco increased (Fig. 2). Rubisco is linked to carboxylation capacity, whereas chlorophyll is related to light capture. The changes of Rubisco and chlorophyll content within leaves indicate that the limitation factor of A is variable in a single leaf. This result suggests that leaves optimise resources allocation within each leaf to maximise A based on light distribution on leaf surface. Similar relationships between light and N content per leaf area have been reported in a range of plants canopy; leaves exposed to higher irradiances have higher N contents per leaf area, ratios of N to leaf area and showed that g_m varied considerably within leaf blades and significantly limited A in rice (Fig. 4b). The g_m gradient within leaves is likely related to leaf anatomical and structural features. Mesophyll cell wall thickness, mesophyll cell wall surface area exposed to intercellular airspace per leaf area (S_m), and surface area of chloroplasts exposed to intercellular airspaces (S_c) are the most important structural components of a leaf that related to g_m (Flexas et al. 2008; Tanaka et al. 2013). Increased S_m and S_c resulting from increased mesophyll lobes and enlarged single chloroplast volume and/or chloroplast number per mesophyll cell under high leaf N content conditions have been previously suggested (Li et al. 2009). In the present study, the correlated increase of g_m and leaf N content per leaf area from leaf base to apex could be related to the gradients of S_m and S_c.

**Implications and conclusions**

Genetic variations in leaf anatomical and functional traits have been well documented. Xiong et al. (2015) investigated A, g_s, g_m and several morph-anatomical traits in 11 rice cultivars, and showed that A varied from 17.6 to 35.9 μmol m^{-2} s^{-1}. The present study shows that A varied from 17.3 to 34.4 μmol m^{-2} s^{-1} and 20.1 to 30.6 μmol m^{-2} s^{-1} along the leaf blade in Lat and Aus respectively (Fig. 1). This gradient of A within leaves may maximise net primary productivity by optimising the use efficiency of natural resources, especially, light. Our results imply that the variation of A within individual leaves is similar to variation among cultivars, and thus variation of A within individual leaves should be more thoroughly considered in future breeding programs.

The heterogeneity of A within Lat leaves was higher than that within Aus leaves. The high biomass accumulation in Lat plants, principally due to the high leaf area. However, it is unlike that 83.9% high of leaf area can lead to 127.5% high of biomass in Lat. Our results suggest that this elevated heterogeneity of A may increase for biomass accumulation under natural conditions. Our results also highlight that functions within leaves are closely associated with leaf structural and chemical features. The increase in A along leaves demands more CO_2 and Rubisco. On one hand, increased stomatal density and decreased D_s along leaf blades allows faster transport of water, thus permitting stomata to remain open by improving the availability of water. On the other hand, carboxylation capacity also increased due to the increased N content and the ratio of N allocated to Rubisco along leaf blades. In the present study, Lat showed larger variation in A, leaf structural (i.e. D_s) and leaf chemical (i.e. Rubisco and the ratio of Rubisco to leaf N) features than Aus, and this may have resulted in its higher plant biomass accumulation. The present study provides strong
evidence that the coordination of leaf structure, chemistry, and functions within leaves substantially affects the performance of plants.

Acknowledgements

This work was supported by the Major International Joint Research Project of the National Natural Science Foundation of China (31361140368), National Key Technology R & D Program of China (2012BAD04B12), Program for Changjiang Scholars and Innovative Research Team in University of China (IRT1247), and Fundamental Research Funds for the Central Universities (2012SC13).

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