RESEARCH PAPER

Physiological basis of genetic variation in leaf photosynthesis among rice (Oryza sativa L.) introgression lines under drought and well-watered conditions

Junfei Gu1, Xinyou Yin1*, Tjeerd-Jan Stomph1, Huaiqi Wang2 and Paul C. Struik1

1 Centre for Crop Systems Analysis, Department of Plant Sciences, Wageningen University, PO Box 430, 6700 AK Wageningen, The Netherlands
2 Plant Breeding & Genetics, China Agricultural University, 100193 Beijing, P.R. China

* To whom correspondence should be addressed. E-mail: Xinyou.Yin@wur.nl

Received 28 February 2012; Revised 11 May 2012; Accepted 14 May 2012

Abstract

To understand the physiological basis of genetic variation and resulting quantitative trait loci (QTLs) for photosynthesis in a rice (Oryza sativa L.) introgression line population, 13 lines were studied under drought and well-watered conditions, at flowering and grain filling. Simultaneous gas exchange and chlorophyll fluorescence measurements were conducted at various levels of incident irradiance and ambient CO2 to estimate parameters of a model that dissects photosynthesis into stomatal conductance (gs), mesophyll conductance (gm), electron transport capacity (Jmax), and Rubisco carboxylation capacity (Vcmax). Significant genetic variation in these parameters was found, although drought and leaf age accounted for larger proportions of the total variation. Genetic variation in light-saturated photosynthesis and transpiration efficiency (TE) were mainly associated with variation in gs and gm. One previously mapped major QTL of photosynthesis was associated with variation in gs and gm, but also in Jmax and Vcmax at flowering. Thus, gs and gm, which were demonstrated in the literature to be responsible for environmental variation in photosynthesis, were found also to be associated with genetic variation in photosynthesis. Furthermore, relationships between these parameters and leaf nitrogen or dry matter per unit area, which were previously found across environmental treatments, were shown to be valid for variation across genotypes. Finally, the extent to which photosynthesis rate and TE can be improved was evaluated. Virtual ideotypes were estimated to have 17.0% higher photosynthesis and 25.1% higher TE compared with the best genotype investigated. This analysis using introgression lines highlights possibilities of improving both photosynthesis and TE within the same genetic background.

Key words: drought, genetic variation, mesophyll conductance, modelling, Oryza sativa L., photosynthesis, rice, stomatal conductance.

Abbreviations: A, Net photosynthesis rate (µmol m–2 s–1); Amax, light saturated net photosynthesis at ambient CO2 and O2 level (µmol m–2 s–1); A0, Rubisco activity limited net photosynthesis rate (µmol m–2 s–1); Ae, electron transport limited net photosynthesis rate (µmol m–2 s–1); C0, ambient air CO2 concentration (µmol mol–1) (refers to leaf-surface CO2 level for model analyses, with boundary conditions already considered; –6.78 µmol m–2 s–1, from Li-Cor manual version 6.1); Ci, intercellular CO2 concentration (µmol mol–1); gs, stomatal conductance for CO2 (mol m–2 s–1); gms, mesophyll conductance (mol m–2 s–1); gmr, residual mesophyll conductance in the gms model (mol m–2 s–1); gm, estimated by the NRH-A method (mol m–2 s–1); gm, diffusion conductance from ambient air to the site of carboxylation (mol m–2 s–1); gms, residual diffusion conductance in the gms model (mol m–2 s–1); J, e– transport rate through photosystem II (PSII) used for NADP+ reduction (µmol e– m–2 s–1); Jmax, maximum value of J under saturated light (µmol e– m–2 s–1); Kc, Michaelis–Menten constant of Rubisco for CO2 (µbar); Ks, Michaelis–Menten constant of Rubisco for O2 (µbar); O2, oxygen partial pressure (µbar); Rs, day respiration (µmol CO2 released in the light other than by photosynthesis) (µmol m–2 s–1); Rg, stomatal conductance for CO2 (µmol m–2 s–1); s, lumped parameter; Sc/o, relative CO2/O2 specificity factor for Rubisco (µbar µmol–1); Vm, maximum rate of Rubisco activity-limited carboxylation (µmol m–2 s–1); A0, A0 parameter in the gm model; defining C4:C3 ratio at saturating light; Ci, parameter in the gm model, defining C4:C3 ratio at saturating light; C3, C4 parameter in the gm model, defining C4:C3 ratio at saturating light; Ci, value of conversion efficiency of incident light into J at the strictly limiting light (µmol photo–1 µmol e–1); δe, convexity factor for response of J to Lmax; Φ, apparent quantum efficiency of PSII e– flow on PSII-absorbed light basis [mol e– (µmol photon–1)]; Φ, apparent quantum efficiency of PSII e– flow on PSII-absorbed light basis [mol e– (µmol photon–1)]; N, leaf nitrogen per unit area (g N m–2 leaf); G, C4-based CO2 compensation point in the absence of Rs (µbar); FS, flowering stage and drought-stressed treatment; FW, flowering stage and well-watered treatment; GS, grain-filling stage and drought-stressed treatment; GW, grain-filling stage and well-watered treatment; LMA, leaf mass per area (g m–2 leaf); TE, transpiration efficiency (mmol m–2 h–1).

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Introduction

The response of leaf photosynthesis to drought involves interactions between physical and metabolic mechanisms (Kramer & Boyer, 1995; Pinheiro & Chaves, 2011). The understanding of these physiological mechanisms is necessary to improve physiological dissection of the complexity of leaf photosynthesis in response to drought (Serraj et al., 2008).

In general, the relationships among leaf photosynthesis ($A$), stomatal conductance ($g_s$), and transpiration are well understood, as $g_s$ has been studied most when investigating photosynthetic responses to drought (reviewed by Israelsson et al., 2006; Casson & Hetherington, 2010; Lawson et al., 2011). However, $g_s$ is not the only component of CO$_2$ diffusion in leaves. Mesophyll conductance ($g_{m}$), the conductance from substomatal cavities to the site of carboxylation, limits photosynthesis significantly as well, meaning that the CO$_2$ concentration in the chloroplast (C$_c$) is lower than in the intercellular space (C$_i$) (Lloyd et al., 1992; Warren et al., 2004, 2008; Flexas et al., 2008). Ignoring $g_{m}$ would erroneously attribute the decreased photosynthesis under drought to metabolic impairment (Delfine et al., 1998; Flexas et al., 2004; Centritto et al., 2009).

The value of $g_{m}$ is influenced by leaf traits such as leaf dry matter per unit area (LMA; Flexas et al., 2008; Galmés et al., 2011), but also by environmental variables, including water status (Delfine et al., 1998; Galmés et al., 2007; Niinemets et al., 2009), temperature (Bernacchi et al., 2002; Scafaro et al., 2011), and nutrient supply (Warren, 2004). There is increasing evidence that $g_s$ and $g_{m}$ are tightly correlated (e.g. Evans, 1999; Flexas et al., 2007a; Warren, 2008; Yin et al., 2009; Barbour et al., 2010; Douthe et al., 2011) and follow the same pattern of variation: declining in response to short-term increases in CO$_2$ partial pressure and increasing with increases in irradiance. Thus, the relationship between $g_s$ and $g_{m}$ is worth exploring further when assessing genetic variation in leaf photosynthesis. Genetic variation in the $g_{m}/g_s$ ratio will allow breeding for high transpiration efficiency (TE) (Galmés et al., 2011).

Photosynthesis is affected not only by diffusion components ($g_s$ and $g_{m}$) but also by various biochemical capacities of protein complexes. The potential activity of Rubisco ($V_{\text{max}}$) limits photosynthesis at low C$_c$. As C$_c$ increases, the chloroplastic electron transport capacity ($J_{\text{max}}$) can limit photosynthesis (Farquhar et al., 1980). Both $V_{\text{max}}$ and $J_{\text{max}}$ are closely related to the amount of leaf nitrogen per unit area ($N_l$) (Makino et al., 1984, 1985; Evans, 1989; Harley et al., 1992a).

Whilst most studies have focused on photosynthetic responses to environmental factors, significant genotypic variation of $A$ has long been reported among species of Oryza and among progeny plants derived from crosses between varieties. For example, variation was observed among varieties of japonica rice (Sasaki & Ishii, 1992; Ishii, 1995), and among varieties including indica and japonica rice and wild rice species (Cook & Evans, 1983; Dingkuhn et al., 1989; Yeo et al., 1994; Peng et al., 1998; Masumoto et al., 2004; Teng et al., 2004). Moreover, quantitative trait loci (QTLs) responsible for the different photosynthetic parameters have been mapped successfully (Zhao et al., 2008; Takai et al., 2009; Xu et al., 2009; Adachi et al., 2011). Recently, Gu et al. (2012), using a population of introgression lines (ILs) from a cross between upland rice and lowland rice, identified QTLs for light-saturated gas exchange and chlorophyll fluorescence parameters under both well-watered and drought conditions in the field. QTLs affecting these parameters tended to cluster in the same genomic regions, suggesting a common genetic basis and inherent physiological connections of photosynthesis parameters.

Few studies have investigated the physiological basis for these reported genetic variations and QTLs of leaf photosynthesis. Taylaran et al. (2011) showed that the higher $N_l$ and higher $g_s$ in indica cultivars could be the reason for higher $A$ than observed in a japonica variety. Similarly, Adachi et al. (2011) reported that two mapped QTLs of net photosynthesis actually arose from an increased $N_l$ and $g_s$. Scafaro et al. (2011) compared a cultivar of Oryza sativa with two wild Oryza relatives and found that the difference in mesophyll cell-wall thickness was responsible for differences in $g_{m}$, which resulted in substantial variation in $A$ between the cultivated and the wild rice. Masle et al. (2005) isolated a TE-regulating gene, ERECTA, from a population of Arabidopsis and found that $V_{\text{max}}, J_{\text{max}},$ stomatal density, and mesophyll development caused the genetic variation in TE and $A$.

As a follow-up of our QTL mapping study (Gu et al., 2012), the present study aimed to investigate the physiological basis of genetic variation and resulting QTLs identified for our IL population. Therefore, a model was used to analyse experimental data for complete curves of photosynthetic responses to CO$_2$ and to light measured on leaves in a representative subset of the ILs. Such a model analysis allowed us: (i) to identify the genetic variation in each biophysical and biochemical component; (ii) to analyse the physiological basis for the genetic variation in photosynthesis; and (iii) to evaluate the potential of utilizing the genetic variation in these components for improving $A$ and TE under contrasting drought stress. The information obtained could have an important implication for developing drought-tolerant varieties.

Materials and methods

Plant growth conditions, treatments, and experimental design

A greenhouse experiment was conducted at the research facility UNIFARM, Wageningen, The Netherlands. Physiological dissection of photosynthesis requires complete curves of responses to various CO$_2$ and light levels, and it was practically infeasible to obtain these curves experimentally for all individual genotypes of the IL population of Gu et al. (2012). Eleven lines (IL7, IL37, IL42, IL69, IL84, IL100, IL130, IL157, IL159, IL161, and IL164) and two parents [Shennong265, japonica; Haogelao, indica-japonica intermediate] were therefore selected. The selection was based on two criteria: (i) the ILs should carry many QTLs to reflect as much as possible the genetic variation of the population; and (ii) the ILs should contain as few chromosome segments from the donor parent as possible, to remove the background noise (see also Eshed & Zamir, 1995). These 11 ILs had on average 6.5% of the genome introgressed. Their graphical genotypes are shown in Fig. 1.

The temperature in the greenhouse was set at 26 °C for the 12h light period and at 23 °C for the 12h dark period. The CO$_2$ level was about 380 µmol mol$^{-1}$, the relative humidity was set at 65%, and extra SON-T light was switched on when global solar radiation intensity outside the greenhouse was <400 W m$^{-2}$ and then switched off once it exceeded 500 W m$^{-2}$. Pre-germinated seeds of the 13 genotypes were sown on sand beds twice (on 8 and 15 June 2010, respectively), to extend flowering and grain-filling
periods for a long enough time window of measurement. Seedlings were then transferred to containers (40 cm long × 30 cm wide × 20 cm high) in hydroponic culture using half-strength Hoagland’s solution, according to a completely randomized block design. Sixteen plants of each genotype were grown with 7.5 × 7.5 cm2 spaces between the plants. One week before flowering, eight plants per genotype were exposed to a moderate water stress [comparable to the stress level in the field experiment of Gu et al. (2012)], induced by adding 12.5% polyethylene glycol (PEG-8000) to the growth solution (Money, 1989). The stress was imposed continuously on plants until all measurements were completed. The remaining eight plants per genotype were maintained under non-stressed conditions. The flowering period of the 13 genotypes of the two sowings lasted from the 15 August until 3 September. Measurements were conducted at flowering and at grain filling (<14 days after flowering). Therefore, there were four stage × treatment combinations, namely flowering/drought-stressed treatment (FS), flowering/well-watered treatment (FW), grain filling/drought-stress treatment (GS), and grain filling/well-watered treatment (GW), for the measurements, as described below.

Gas exchange and chlorophyll fluorescence measurements

The flag leaves on the main stems of four representative plants (out of eight) per treatment of each genotype were used for measurements (except for IL42 at FS, because of a labour peak, as flowering of the late IL42 coincided with grain filling of some of the earlier genotypes). We used an open gas exchange system (Li-Cor 6400; Li-Cor Inc., Lincoln, NE, USA) and an integrated fluorescence chamber head (Li-Cor 6400-42 coincided with grain filling of some of the earlier genotypes). We use the model of Farquhar, von Caemmerer and Berry (1980) (FvCB model). The net CO2 assimilation (A) is expressed as the minimum of the Rubisco limited rate (A5) and the electron transport limited rate (A6):

\[
A = \min(A_5, A_6)
\]

(1)

A5 is described, following Michaelis–Menten kinetics, as:

\[
A_5 = \frac{(C_i - R_g) V_{\text{max}}}{C_i K_{\text{m}} + O K_{\text{mo}}} - R_g
\]

(2)

where \(C_i\) and \(O\) are the CO2 and O2 levels at the carboxylation sites of Rubisco, \(V_{\text{max}}\) is the maximum rate of carboxylation, \(K_{\text{m}}\) and \(K_{\text{mo}}\) are...
Michaels–Menten constants of Rubisco for CO₂ and O₂, respectively, and \( \Gamma_s \) is the CO₂ compensation point in the absence of day respiration \((R_d)\). In the model, \( \Gamma_s = 0.5O_2/S_{IO} \). As the constants \( K_m \) and \( K_{mo} \) are generally conservative for C₃ plants (von Caemmerer, 2000), their values were taken from Bernacchi et al. (2002).

\( A_i \) is described by:

\[
A_i = \frac{(C_i - \Gamma_s)J}{4C_i + 8\Gamma_s - R_d} \tag{3}
\]

where \( J \) is the potential PSII e⁻ transport rate that is used for CO₂ fixation and photorespiration, and can be described by the following (Ögren and Evans, 1993; von Caemmerer, 2000; Yin et al., 2009):

\[
J = K_{2IL}I_{\text{inc}} + J_{\text{max}} - \sqrt{K_{2IL}I_{\text{inc}} + J_{\text{max}}^2 - 4 \theta J_{\text{max}}K_{2IL}I_{\text{inc}}} \tag{4}
\]

where \( K_{2IL} \) is the conversion efficiency of incident light into \( J \) at strictly limiting light, \( J_{\text{max}} \) is the maximum value of \( J \) under saturated light, and \( \theta \) is the convexity factor.

Model parameters were estimated according to the procedure described by Yin et al. (2009). Specifically, using data of the e⁻ transport-limited range under non-photorespiratory conditions (i.e. the irradiance response curve at 2% O₂ combined with 1000 \( \mu \text{mol} \text{ mol}^{-1} \) \( C_o \)), a simple linear regression can be performed for the observed \( J \) against \((I_m, \Phi_s)\). The slope of the regression yields the estimate of a lumped parameter \( s \), and the intercept gives an estimate of \( R_d \) (Yin et al., 2009, 2011). This allowed the actual rate of linear electron transport to be calculated as:

\[
J = sI_{\text{inc}} \Phi_s \tag{5}
\]

The parameters \( J_{\text{max}}, K_{2IL}, \) and \( \theta \) were estimated by fitting Equation (4) to the calculated \( J \).

\( S_{IO} \) was calculated by following the procedure described by Yin et al. (2009); see their Equation 10). Once all the above parameters were estimated, their values were used as input to the model described below, upon which \( V_{\text{max}} \), and coefficients related to diffusional conductances were estimated.

**Modelling of \( g_m \) and \( g_s \)**

To examine any variation in mesophyll conductance \((g_m)\) in response to \( C_i \) and irradiance (at 21% O₂), the variable \( J \) method (Harley et al., 1992b) was first applied:

\[
g_m = \frac{A}{C_i - \Gamma_s(J + 8(A + R_d))} \tag{6}
\]

where \( A \) and \( C_i \) were taken from gas exchange measurements and \( J \) was calculated by Equation (5). This first analysis showed that \( g_m \) was variable (see Results). We therefore used a phenomenological equation of Yin et al. (2009) to model \( g_m \):

\[
g_m = g_{mo} + \frac{\delta_s (A + R_d)}{C_i - \Gamma_s} \tag{7}
\]

where \( g_{mo} \) is the minimum mesophyll conductance if the irradiance approaches zero, \( \delta_s \) is the coefficient that defines the \( C_i/C_o \) relationship under saturating light as: \((C_i-\Gamma_s)/(C_o-\Gamma_s)=1/(1+\delta_s)\) (Yin et al., 2009).

Combining Equation (7) with Equations (2) and (3) and replacing \( C_i \) with \((C_i-A/g_m)\) yields (Yin et al., 2009):

\[
A_i or A_j = \frac{-b - \sqrt{b^2 - 4ac}}{2a} \tag{8}
\]
The rationale for this method and the choice of data has been discussed fully by Yin and Struik (2009). This estimate using the NRH-A method should represent the average value of \( g_m \) within its lower range of the variation.

**Ideotype design**

The 13 lines used in this study were selected based on the QTLs detected by single-point analysis (Gu et al., 2012). In order to quantify the additive effect of the QTLs on each parameter in our model, a statistical covariant model was used, in which the value of a parameter \( X \) of introgression line \( k \), containing \( N \) QTLs (as represented by the nearest marker loci), for a specific stage \((S) \times \) treatment \((T) \) combination was presented as:

\[
X_{ik} = \mu + S_i + T_j + \sum_{m} a_m \times M_{ik} + e_{ik}
\]

(14)

where \( \mu \) is the intercept; \( S_i \) is growth stage effect, which stands for either of the two stages (flowering or grain filling); \( T_j \) is the treatment effect, which stands for either well watered or drought stressed; \( a_m \) is the additive effect of the \( m \)th QTL; \( M_{ik} \) is the genetic QTL scores of the individual introgression line \( k \) that take the value either –1 (allele coming from Shennong265) or 1 (Haogeliou allele present), and \( e_{ik} \) is an error term.

For the ideotype design, only QTLs with significant enhancing additive effects \((P < 0.05)\) were kept in Equation (14). For example, an ideotype for improved photosynthesis was the virtual genotype of parameter values were estimated as the sum of the allele effects that enhanced \( A \) for all QTLs of each FvCB model component. To construct the \( A \) response of ideotype to irradiance, estimated parameters were used as inputs in Equation (8). To calculate the TE response to irradiance, the following equation (Farquhar and Richards, 1984) was used:

\[
TE = \left( \frac{C_i - C_o}{1.6(1 - c_o)} \right)
\]

(15)

where \((c_i-c_o)\) is leaf-to-air VPD and \( C_i \) is calculated from our model using \( A \) and estimates of the parameters \( \delta_m \) and \( \delta_s \).

**Statistics and curve fitting**

A three-way analysis of variance of genotype \( \times \) treatment \( \times \) growth stage for the photosynthesis parameters was calculated. Non-linear fitting was carried out using the GAUSS method in PROC NLIN, and multiple linear regression fitting for Equation (14) was performed using PROC GLM of SAS (SAS Institute Inc., Cary, NC, USA).

**Results**

**Estimates of photosynthesis parameters**

The estimated values for \( S_{c0} \) did not differ among genotypes, nor among treatment 3 stage combinations; so a single value for \( S_{c0} \) was obtained from the pooled data \((\approx 3.02 \pm 0.03 \text{ mbar mbar}^{-1})\). As reported by Yin et al. (2009), the estimated values for \( R_d \) did not differ between 21 and 2% \( O_2 \) levels, and a common \( R_d \) across the \( O_2 \) levels was obtained. However, the estimated values for \( R_d \) and \( s \) were genotype, treatment, and stage specific (Supplementary material Table S1 at JXB online), and the values of \( R_d \) were generally lower than those of \( R_{ak} \) (Table S1). After parameter \( s \) was estimated, \( J \) was obtained from Equation (5) and \( J_{max} \times \kappa_{2LL} \) and \( \theta \) were then estimated by fitting Equation (4) (see Table S1).

Once values of \( J, S_{c0} \) and \( R_d \) were known, we used Equation (6) to evaluate the effects of variations of CO\(_2\) concentration and light intensity on \( g_m \) for four stage \( \times \) treatment combinations (FS, FW, GS, and GW) of each genotype. In general, \( g_m \) strongly declined with an increase in \( C_i \) and increased with an increase in light intensity, following the same response as \( g_s \) to CO\(_2\) concentration and light intensity; thus, a proportional relationship between \( g_m \) and \( g_s \) was obtained (Fig. S1 at JXB online). The slope of the proportional relationship, indicating the average \( g_m/g_s \) ratio, differed among the stage \( \times \) treatment combinations and was higher for drought-stressed plants than for well-watered plants, and for flowering than for grain filling.

The variation of \( g_m \) across \( I_{inc} \) and across \( C_i \) levels was confirmed by the curve-fitting based on Equation (8), as the value of parameter \( g_{fno} \) in the equation was found to be close to zero, whereas \( \delta_m \) was found to vary from 0.452 to 1.571 (a zero \( g_{fno} \) combined with a non-zero \( \delta_m \) would mean that \( g_m \) varies with \( C_i \) and \( I_{inc} \); see Yin et al., 2009). This method allowed solving of \( \delta_m \) and \( V_{max} \) simultaneously (Table S1; Fig. 2), when using the earlier estimated \( S_{c0}, R_d, J_{max}, \theta \) and \( \kappa_{2LL} \) as inputs. In this method, a universal parameter, \( \delta_m \) (rather than specific \( g_m \) values), across whole photosynthesis light- and CO\(_2\)-response curves was estimated. A further analysis based on Equation (9) also showed that parameter \( g_m \) did not differ significantly from zero \((P > 0.05)\). Therefore, an overall \( g_m/g_s \) ratio (Equation 10) was calculated for each introgression line at each stage \( \times \) treatment combination (Fig. 2D). The overall average \( g_m/g_s \) ratio obtained from this method for most of the stage \( \times \) treatment combinations (Table S1) was slightly higher than those values shown in Fig. S1, probably because the variable \( J \) method assumes no alternative e– transport, whereas the curve-fitting method does account for any alternative e– transport (Yin et al., 2009).

**Components of variation in and correlations among photosynthetic parameters**

The variation in each estimated photosynthetic parameter can be statistically partitioned into genetic, environmental (stress versus non-stress), and developmental (i.e. flowering versus grain filling) components, and their two-way interactions. However, as most interactions were not significant \((P > 0.05\); results not shown\), we omitted all interaction terms (Table 1). Significant genetic differences were found for \( R_d, \kappa_{2LL}, J_{max}, \theta, \) and \( V_{max} \) as well as for \( \delta_m \) and the \( g_m/g_s \) ratio \((P < 0.05; \) Table 1, Fig. 2), although environmental and developmental components contributed most to the variation in most parameters (Table 1).

**Physiological basis of the genetic variation**

Significant genetic differences of some model parameters \((P < 0.05; \) Table 1, Fig. 2) hinted a physiological basis for genetic
variation in $A$ found earlier by Gu et al. (2012), who identified QTLs for light-saturated $A$ ($A_{\text{max}}$) under field conditions.

Using our model approach, $A_{\text{max}}$ can be dissected into four physiological components: $g_s$, $g_m$, electron transport, and Rubisco activity. To quantitatively analyse the effects of each component, $A_{\text{max}}$ (at 380 $\mu$mol mol$^{-1}$ CO$_2$, 1500 $\mu$mol m$^{-2}$ s$^{-1}$ irradiance, 25 °C, and 1.5 kPa VPD) was first plotted against each component here. Within each stage $\times$ treatment combination, the correlation between $A_{\text{max}}$ and each component ($g_s$, $g_m$, $J_{\text{max}}$, and $V_{\text{cmax}}$) could be observed (Fig. S2 at JXB online), providing the evidence about where genetic differences in $A_{\text{max}}$ possibly came about. In order to quantify the main sources of genetic variation in $A_{\text{max}}$, a multiple regression analysis was carried out (Table 2). For each stage $\times$ treatment combination, the genetic variation in $g_s$ and $g_m$ had the largest impact on the genetic variation in $A_{\text{max}}$. Under well-watered treatment, $g_m$ caused more genetic variation in $A_{\text{max}}$ than $g_s$ did, while under drought-stressed treatment, $g_s$ accounted for more genetic variation.

We also analysed TE under the same measurement conditions (Fig. S3 at JXB online). When we inspected the relationship within each stage $\times$ treatment combination, the correlation appeared very weak, except for $g_s$ (Fig. S3A) and $g_m$ (Fig. 3). Multiple regression analysis (Table 2) also showed that genetic variation in $g_s$ and $g_m$, relative to that in $V_{\text{cmax}}$ and $J_{\text{max}}$, contributed more to TE in this genetic background, and, not surprisingly, $g_m$ and $g_s$ affected TE in the opposite direction.

**Physiological basis of a major photosynthesis QTL**

Of the ILs used, IL161 is unique in that it has the background of the recurrent parent Shennong265 except for a single introgression segment on chromosome 9 from the donor parent (Fig. 1). Compared with the recurrent parent, IL161 significantly increased $A_{\text{max}}$ across stages and treatments; thus, a major QTL was consistently detected for $A_{\text{max}}$ on chromosome 9 (Gu et al., 2012). CO$_2$ and light response curves measured in the present study indicated that the QTL contributed to a higher photosynthesis rate across all irradiance and CO$_2$ levels (Fig. 4). Through our analysis, seven parameters of both IL161 and Shennong265 were estimated for each stage $\times$ treatment combination (Table 3). There was no significant difference between them for $R_d$, $\kappa_{\text{LL1}}$, and $\theta$ ($P > 0.05$). At flowering, IL161 showed significantly higher $g_m$, $g_s$, $V_{\text{cmax}}$ and $J_{\text{max}}$ than Shennong265 across the two treatments. At grain filling, however, only higher diffusional conductance (larger $g_m$ and $g_s$) could be the reason for higher $A$, as $V_{\text{cmax}}$ was even lower in IL161 than in Shennong265 for the stress treatment (Table 3). Therefore, there was a greater difference between IL161 and Shennong265 at flowering than at grain filling (Fig. 4). Our whole-curve measurements are consistent with the results of Gu et al. (2012) that larger additive effects of the QTL on $A_{\text{max}}$ were obtained at flowering than at grain filling.

Calculating $g_m$ and $g_s$-related photosynthesis parameters:

Calculated by the NRH-A method of Yin & Struik (2009): (D) mesophyll conductance: stomatal conductance ratio ($g_m/g_s$), calculated by Equation (10); (E) ratio of $J_{\text{max}}/V_{\text{cmax}}$. 

![Fig. 2. Values of photosynthesis parameters estimated for flag leaves of introgression lines, including two parents, Haogelao (H) and Shennong265 (S), at four stage $\times$ treatment combinations: FS (filled bars); FW, striped bars); GS, open bars); GW, hatched bars). (A) maximum rate of Rubisco activity-limited carboxylation ($V_{\text{cmax}}$); (B) maximum value of electron transport rate used for NADP$^+$ reduction ($J_{\text{max}}$); (C) mesophyll conductance [$g_m/(\text{NRH-A})$], calculated by Equation (10); (D) ratio of $J_{\text{max}}/V_{\text{cmax}}$.](http://jxb.oxfordjournals.org/DownloadedFromWageningenURLibraryOnOctober32012)}
Table 1. A three-way analysis of variance of genetic effect versus treatment versus growth stage for the estimated photosynthesis parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>$F$ value (probability of significance)</th>
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<tbody>
<tr>
<td>Genetic effect</td>
<td>Treatment</td>
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<tr>
<td>Primary parameters of the model</td>
<td>$R_d$</td>
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<tr>
<td></td>
<td>$k_{211}$</td>
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<td>$J_{\text{max}}$</td>
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<td>$V_{\text{cmax}}$</td>
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<td>Other parameters</td>
<td>$\delta_s$</td>
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<td>$g_{N_{\text{RH-A}}}$</td>
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<td>$g_{\text{FW}}$</td>
</tr>
<tr>
<td></td>
<td>$g_{\text{FS}}$</td>
</tr>
<tr>
<td></td>
<td>$J_{\text{max}}V_{\text{cmax}}$</td>
</tr>
</tbody>
</table>

$F$ and $P$ values significant at a level of $P < 0.05$ are shown in bold.

Table 2. Multiple linear regression analysis of light-saturated photosynthesis ($A_{\text{max}}$) or TE as a function of $g_s$, $g_m$, $J_{\text{max}}$, and $V_{\text{cmax}}$ (i.e., $A_{\text{max}}$ or TE $= b_0 + b_1g_s + b_2g_m + b_3J_{\text{max}} + b_4V_{\text{cmax}}$), based on data of 11 introgression lines and their parents, for each stage×treatment combination

<table>
<thead>
<tr>
<th>Trait</th>
<th>Stage × treatment</th>
<th>Intercept ($b_0$)</th>
<th>Regression coefficient (probability of significance)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$A_{\text{max}}$</td>
<td>FS</td>
<td>1.21</td>
<td>46.99 (4.4 × 10$^{-5}$)$^1$</td>
</tr>
<tr>
<td></td>
<td>FW</td>
<td>1.26</td>
<td>31.19 (1.3 × 10$^{-3}$)$^3$</td>
</tr>
<tr>
<td></td>
<td>GS</td>
<td>0.63</td>
<td>30.85 (7.0 × 10$^{-5}$)$^1$</td>
</tr>
<tr>
<td></td>
<td>GW</td>
<td>1.39</td>
<td>22.45 (7.7 × 10$^{-5}$)$^1$</td>
</tr>
<tr>
<td>TE</td>
<td>FS</td>
<td>6.02</td>
<td>-20.70 (6.7 × 10$^{-5}$)$^1$</td>
</tr>
<tr>
<td></td>
<td>FW</td>
<td>4.29</td>
<td>-15.77 (1.1 × 10$^{-4}$)$^1$</td>
</tr>
<tr>
<td></td>
<td>GS</td>
<td>4.84</td>
<td>-21.31 (1.0 × 10$^{-4}$)$^1$</td>
</tr>
<tr>
<td></td>
<td>GW</td>
<td>4.47</td>
<td>-13.21 (3.2 × 10$^{-4}$)$^1$</td>
</tr>
</tbody>
</table>

$^1, 2, 3, 4$ The comparative importance of each parameter, determined from the level of significance. Coefficient values significant at a level of $P < 0.05$ are in bold.

Relationships between photosynthesis parameters and leaf morpho-physiological characteristics

The variation in $g_m$($N_{\text{RH-A}}$), either across genotypes or across treatments, was negatively correlated with LMA (Fig. 5A). Similar relationships were found between LMA and $g_m$ or $g_s$ calculated for the condition of measuring $A_{\text{max}}$, despite lower $r^2$ values (results not shown). As expected, drought stress induced thicker leaves (increased LMA, Fig. 5A) and the increased LMA led to an increased $N_a$ ($r^2=0.40$). However, there was a poor correlation between $g_m$($N_{\text{RH-A}}$) and $N_a$ ($r^2=0.08$; Fig. 5B). Instead, the variation in $J_{\text{max}}$ and $V_{\text{cmax}}$, either across genotypes or across water-supply treatments, was found to be positively correlated with $N_a$ (Fig. 6A, 6B), but less correlated with LMA (results not shown). Analysis with an $F$ test demonstrated that generally there was no significant difference between well-watered and drought-stressed plants at grain filling on the relationships shown in Figs 5 and 6 ($P > 0.05$), although the slope of the relationship between $J_{\text{max}}$ and $N_a$ was significantly lower ($P=0.012$) for plants under drought conditions.
Ideotype design based on physiological understanding

Given the significant genetic difference in each of the model component traits (Table 1, Fig. 2) and their significant effects on $A_{\text{max}}$ and TE (Table 2, Fig. S2 and S3), it was considered worthwhile exploring the potential to improve $A$ and TE using the genetic variation observed. We therefore estimated the additive effects of individual genome loci, based on Equation (14). Of the loci differing among the ILs, seven loci were identified as significantly affecting the seven primary model parameters (Fig. 1, Table 4). These seven loci were also identified or in close proximity to those mapped for $A_{\text{max}}$ using the whole IL population (Gu et al., 2012), suggesting that our selected 11 ILs did represent the population well. There was no one-to-one locus–parameter relationship. Instead, each model parameter was controlled by one to three loci, and most loci had an effect on multiple parameters (Table 4), providing a genetic basis of significant correlations between model parameters in Table S2.

The ideotype for high $A$ requires high $g_s$ and $g_m$ and improved photosynthetic efficiency ($\kappa_{\text{LL}}$ and $\theta$) and capacities ($V_{\text{cmax}}$ and $J_{\text{max}}$), while the ideotype for high TE requires low $g_s$, high $g_m$, and improved photosynthetic efficiency and capacities. Thus, the

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**Fig. 4.** Photosynthesis response curves of IL161 (squares) and Shennong265 (circles) under 21% O$_2$ at four stage × treatment combinations: FS (A, B), FW (C, D), GS (E, F), and GW (G, H). The curves are drawn from the model using fitted parameter values: dashed lines for IL161 and solid lines for Shennong265. Left panels (A, C, E, and G) show the response of net photosynthesis $A$ to ambient CO$_2$ ($C_a$) under a light intensity of 1000 µmol m$^{-2}$ s$^{-1}$. Right panels (B, D, F, and H) show the response of photosynthesis $A$ to light intensity under 380 µmol mol$^{-1}$ CO$_2$. Values are means ± SD ($n=4$).
Table 3. Parameter values (±SE of the estimate) of the photosynthesis model, estimated for IL161 and Shennong265 (S) that differ in a single introgression region on chromosome 9 (see Gu et al., 2012), for the four stage-treatment combinations

<table>
<thead>
<tr>
<th>Environment</th>
<th>Genotype</th>
<th>Model parameters</th>
<th>Other derived parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$R_d$ $\kappa$ $J_{\text{max}}$ $\theta$ $V_{\text{max}}$ $\delta_m$ $\delta_l$</td>
<td>$g_a^a$ $g_m^c$</td>
</tr>
<tr>
<td>FS</td>
<td>IL161</td>
<td>0.669±0.193 0.344±0.044 214.8±14.9 $213.3±12.0^*$ 0.524±0.019 0.267±0.008</td>
<td>0.145 0.140</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>0.539±0.177 0.321±0.029 174.6±7.6 0.806±0.098 173.3±14.5 0.572±0.031 0.279±0.013</td>
<td>0.120 0.125</td>
</tr>
<tr>
<td>FW</td>
<td>IL161</td>
<td>0.474±0.175 0.332±0.033 222.7±13.5 $204.0±15.4^*$ 0.697±0.036 0.401±0.016</td>
<td>0.221 0.163</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>0.407±0.139 0.326±0.038 151.1±8.1 0.764±0.143 120.7±7.2 1.044±0.103 0.574±0.032</td>
<td>0.190 0.156</td>
</tr>
<tr>
<td>GS</td>
<td>IL161</td>
<td>0.317±0.147 0.322±0.053 153.2±10.4 0.863±0.137 133.4±4.6 $0.767±0.039^*$ 0.450±0.016</td>
<td>0.186 0.130</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>0.365±0.124 0.316±0.020 151.4±3.9 0.852±0.054 141.1±5.5 0.694±0.035 0.376±0.012</td>
<td>0.146 0.123</td>
</tr>
<tr>
<td>GW</td>
<td>IL161</td>
<td>0.059±0.235 0.297±0.031 143.9±6.0 0.884±0.078 101.5±4.7 1.340±0.158 0.723±0.026</td>
<td>0.200 0.171</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>-0.147±0.172 0.312±0.050 142.2±9.7 0.825±0.160 102.0±4.9 1.211±0.139 0.635±0.021</td>
<td>0.173 0.158</td>
</tr>
</tbody>
</table>

$^a g_a$ and $^c g_m$ were derived from the fitted model at saturated light of 1500 µmol m$^{-2}$ s$^{-1}$, CO$_2$ concentration of 380 µmol mol$^{-1}$.

$^*$ Statistically significantly different between IL161 and the recurrent parent Shennong265 (S) ($P < 0.05$).
Fig. 5. Relationship between (A) mesophyll conductance \( g_{m(NRH-A)} \) calculated by the non-rectangular hyperbolic method (Yin & Struik, 2009) and LMA; (B) \( g_{m(NRH-A)} \) and leaf nitrogen per unit area \( (N_a) \). Values are means ±SD of four replicates. Linear regressions were fitted for overall data (solid grey lines) and each stage x treatment combination: GS (triangles and dashed lines) and GW (diamonds and dotted-dashed lines). The significance of each correlation is indicated: * \( P < 0.05; ** P < 0.01 \).

Fig. 6. Relationship between (A) electron transport capacity \( \Delta j_{\text{max}} \) and leaf nitrogen content \( (N_a) \), and (B) Rubisco carboxylation capacity \( \Delta V_{\text{carx}} \) and \( N_a \). Symbols and significance levels are as described in Fig. 5.
Physiological basis of genetic variation in rice photosynthesis

The ideotype of high $A$ carried the alleles having positive effects on $\kappa_{2LL}$, $J_{\text{max}}$, $\theta$, $\delta_m$, $\delta_s$, and $V_{\text{cmax}}$, and negative effects on $R_d$, whereas the ideotype of high TE carried the alleles having positive effects on $\kappa_{2LL}$, $J_{\text{max}}$, $\theta$, $\delta_m$, and $V_{\text{cmax}}$, and negative effects on $\delta_s$ and $R_d$.

The ideotype of high $A$ showed an increase in $A$ of 15.2% (FS), 15.5% (FW), 20.6% (GS), and 17.1% (GW) compared with the mean $A$ of the 13 ILs (Fig. 7, solid lines), while the ideotype of high TE showed an increase of 32.2% (FS), 14.8% (FW), 26.1% (GS), and 17.3% (GW) compared with the mean TE of the 13 ILs (Fig. 8, solid lines).

The above estimated improvement in $A$ or TE was moderate, because the same alleles at some loci had contradicted effects on different photosynthesis parameters (Table 4). Assuming that these contradicted effects were not due to pleiotropy, but rather to tight gene linkage that could be broken through further rounds of introgression and a higher density marker map to develop near-isogenic lines carrying fine-mapped QTLs, we evaluated virtual ideotypes for $A$ and TE that contained only positive effects in all the photosynthesis parameters. For $A$, this virtual ideotype showed an average improvement of 29.9% (FS), 29.3% (FW), 36.4% (GS), and 34.5% (GW) compared with the mean $A$ of the 13 ILs (Fig. 7, dotted lines). For TE, the virtual ideotype showed an average improvement of 46.9% (FS), 28.2% (FW), 42.0% (GS), and 31.6% (GW) when compared with the mean TE of the 13 ILs (Fig. 8, dotted lines). When compared with the best genotype we investigated in each stage × treatment combination, for $A$, the virtual ideotype showed an improvement of 11.0% (FS), 9.6% (FW), 18.7% (GS), and 28.5% (GW); for TE, the virtual ideotype showed an improvement of 38.3% (FS), 12.3% (FW), 33.9% (GS), and 15.8% (GW).

The above analysis examined the ideotypes for $A$ and TE separately. To explore the potential of selecting a genotype with both improved TE and photosynthesis, $A_{\text{max}}$ was plotted against TE (Fig. 9). There were negative correlations for all the stage × treatment combinations, and the negative correlations were more significant under drought conditions than in a well-watered environment. These relationships suggested that simultaneous improvement of $A$ and TE is difficult, especially under drought conditions. The opportunities for simultaneous selection for improved $A$ and TE are discussed below.

Discussion

Physiological basis of genetic variation in photosynthesis

Our model approach allowed to quantitatively dissect photosynthesis into different physiological components: $g_s$, $g_m$, and biochemical efficiency ($\kappa_{2LL}$, $\theta$) and biochemical capacity ($J_{\text{max}}$ and $V_{\text{cmax}}$). In our analysis, most of the model parameters showed significant genetic differences (Table 1). For example, parameters $\kappa_{2LL}$ and $\theta$ both affected the electron transport efficiency under limited light. Thus, the genetic variation in $\kappa_{2LL}$ and $\theta$ (Table 1).

![Fig. 7. Constructed response curve of net photosynthetic rate ($A$) to light intensity at ambient CO$_2$ concentration (380 µmol mol$^{-1}$) at four stage × treatment combinations: FS (A), FW (B), GS (C), and GW (D). The rate of photosynthesis of the 13 lines (circles, values are means ±SD of the 13 lines) were calculated from the model using fitted parameter values. The ideotype response (solid lines) and the potential virtual ideotype curves (dotted lines) of photosynthesis were drawn using parameter values, which were calculated by methods described in the Materials and methods and Results sections.](http://jxb.oxfordjournals.org/
could potentially be used to improve photosynthetic efficiency before light intensity reaches saturation.

As our previous analysis identified QTLs for \( A_{\text{max}} \) (Gu et al., 2012), we specifically analysed the relative contribution of photosynthesis parameters (\( g_s, g_m, V_{\text{cmax}}, \) and \( J_{\text{max}}; \) Table 2) relevant for the condition under which \( A_{\text{max}} \) was measured. The value of \( g_s \) was found to be most associated with genetic variation in \( A_{\text{max}} \) in our IL population (Table 2, Fig. S2A) under drought conditions. This was in line with reported results showing that mapped QTLs of net photosynthesis (Adachi et al., 2011) were related to \( g_s \). These results are not surprising, given that \( g_s \) controls diffusion of CO\(_2\) from ambient air into intercellular airspace and that stomata have evolved into physiological control mechanisms to maximize carbon gain while minimizing water loss (Lawson et al., 2011). However, \( g_m \) was also important for the expression of genetic variation in \( A_{\text{max}} \) (Table 2, Fig. S2B). In fact, under well-watered conditions, \( g_m \) contributed most to the genetic variation in \( A_{\text{max}} \) (Table 2).

We found that \( V_{\text{cmax}} \) and \( J_{\text{max}} \) contributed comparatively less to genetic variation in \( A_{\text{max}} \) in each stage × treatment combination (Table 2). This is surprising, given that \( V_{\text{cmax}} \) and \( J_{\text{max}} \) reflect fitted for all the stage × treatment combinations, when forcing the regression line to go through the origin. This dashed line shows the trend line for both high TE and \( A_{\text{max}} \). (This figure is available in colour at JXB online.)
Rubisco carboxylation and e- transport capacities, respectively. The weak correlation between biochemical capacities and \(J_{\text{max}}\) within each stage × treatment (Fig. S2D,E) could be due to the small range of variation in \(V_{\text{cmax}}\) and \(J_{\text{max}}\). A comparison of IL161 versus Shengnong265 (whose difference was due to a single introgression on chromosome 9) showed (Table 3) that \(V_{\text{cmax}}\) and \(J_{\text{max}}\) together with \(g_{\text{w}}\) and \(g_{\text{s}}\) did explain the difference in photosynthesis light and CO₂ response curves (Fig. 4), at least for the flowering stage.

It is known that a long-term environmental adaptation results in a change in leaf morphology, and LMA as a morphological trait has a high plasticity in adjusting to environmental conditions (Westoby et al., 2002; Poorter et al., 2009). For example, Pons and Pearcy (1994) showed that plants that switched from a high-light environment to low light can substantially (30–50%) decrease LMA within days. The change in LMA was also shown in our data obtained after the grain-filling stage measurements, where the average LMA for drought-stressed leaves was higher than the average for non-stressed leaves (Fig. 5A). Our results agree with the literature (Flexas et al., 2008; Niinemets et al., 2009; Galmés et al., 2011) that \(g_{\text{m}}\) decreases with increasing LMA (Fig. 5A). Interestingly, this relationship also holds for the genetic variation across 13 lines within either stress or non-stress treatment, and the stress treatment did not change the relationship (Fig. 5A). This suggests that LMA plays an important role in the plant’s adaptation to environmental conditions as well as in the plant’s genotypic strategies within the same environment.

Similar to LMA, \(N_{\text{i}}\) also varied between treatments and among genotypes (Fig. 5B). \(V_{\text{cmax}}\) and \(J_{\text{max}}\), rather than \(g_{\text{m}}\) or \(g_{\text{s}}\), were linearly correlated with \(N_{\text{i}}\) (Figs 5 and 6). Furthermore, \(V_{\text{cmax}}\) and \(J_{\text{max}}\) were less correlated with LMA (results not shown). Again, water supply treatments hardly affected these relationships across the 13 genotypes. As \(V_{\text{cmax}}\) and \(J_{\text{max}}\) affected genetic variation of \(A_{\text{max}}\) (Table 2), especially at flowering stage (Table 3), the elevated capacity of nitrogen accumulation in the leaf should be a preferred trait for improving leaf photosynthetic capacity, as suggested in the literature (Peng et al., 1998; Shiratsuchi et al., 2006; Taylaran et al., 2011).

### Physiological basis of genetic variation in transpiration efficiency

TE is another important breeding target for drought tolerance (Condon et al., 2002, 2004). Our data showed that genetic variation in \(g_{\text{w}}\) was best correlated with genetic variation in TE in our genetic material (Table 2, Fig. S3A). This was in line with reported results that the gene for TE, *ERECTA*, is related to \(g_{\text{s}}\) (Masle et al., 2005).

From a theoretical perspective, however, Condon et al. (2004) indicated that, under certain environment conditions, TE could be improved not only by lowering \(g_{\text{w}}\) but also by higher photosynthetic potential, or a combination of these two. In particular, a greater \(g_{\text{m}}/g_{\text{s}}\) ratio results in a higher TE without a negative impact on carboxylation (Barbour et al., 2010; Galmés et al., 2011). We found significant genetic difference for the \(g_{\text{m}}/g_{\text{s}}\) ratio in this population (\(P < 0.01\); Table 1), and the genetic variation in TE was strongly correlated with the variation in this ratio (Fig. 3). A further improvement of TE may be achieved by improving biochemical activities, resulting in improved \(A\) with the same transpiration. An ideal plant in drylands would have low \(g_{\text{s}}\), high \(g_{\text{m}}\), and improved biochemical efficiency (Flexas et al., 2010). However, our data showed little association between TE and \(V_{\text{cmax}}\) or \(J_{\text{max}}\) (Table 2, Fig. S3).

### Potential of using genetic variation to improve photosynthesis and TE

Our model analysis revealed a strong physiological basis of the genetic variation in photosynthesis and in TE; therefore, the model was used to design ideotypes for an improved \(A\) or TE based on their physiological components. This kind of bottom-up approach has been successful in the past for yield component analysis. For example, more insights could be obtained from analysing QTLs or genes for yield components rather than for grain yield per se (Yin et al., 2002), and the component-trait QTLs could be explored to improve yields. Based on this ideotype idea, recent genomic studies have successfully identified genes for
one or a few yield components (reviewed by Miura et al., 2011; Xing and Zhang, 2011). However, very few studies have been performed using the same approach for photosynthesis.

Based on the genetic variation from our study, we could significantly improve $A$ and TE by manipulating alleles of loci influencing different physiological components of photosynthesis (Figs 7 and 8), suggesting that an understanding of the physiological basis of photosynthesis will benefit marker-assisted selection. Some gene linkage limited further improvement. For example, a locus from Shenong265 has positive effects on both $g^{'}_m$ and $g_e$, which will benefit breeding for high $A$, while it has a contradictory effect for high TE. High $g_w$ will increase photosynthesis at the expense of high transpiration. Any further improvement of these rice ideotypes of our IL background for higher photosynthetic performance and TE requires further steps of marker-assisted selection. For example, further backcrossing using markers is needed to reduce the size of introgression segments and develop near-isogenic lines carrying line-mapped QTLs to break any gene linkage. Through this approach, a potential improved ideotype could be achieved as shown by the dotted lines in Figs 7 and 8.

Can $A$ and TE be improved simultaneously?

As expected from existing physiological understanding, our data showed general negative correlations between $A_{\text{max}}$ and TE among ILs in each stage × treatment combination (Fig. 9). This agrees with the observation that selection for higher TE often hampers plant growth and results in smaller plants (Blum, 2005). The negative correlations were stronger under drought conditions than in a well-watered environment (Fig. 9). As expected from existing physiological understanding, our data showed general negative correlations between $A_{\text{max}}$ and TE among ILs in each stage × treatment combination (Fig. 9). The ageing decreased $g_w$ (as shown by the correlation between $\delta_m$ and $\delta_l$ in Table S2, and co-location of QTLs of $\delta_m$ and $\delta_l$ in Table 4 and Fig. 1) could be broken (reflected by the dotted lines in Fig. 8), the best virtual ideotype could have both improved RED (red lines) and $A$ (green lines) compared with the average of ILs (dotted lines versus circles in Fig. 8). Similar results were given by Barbour et al., (2010) for barley varieties, in which variety Dasher with a higher $g_m$ and comparatively lower $g_e$ resulted in the highest $A$ and TE across the six varieties examined. Our analysis using ILs highlights the possibility of improving both $A$ and TE within the same genetic background.

Concluding remarks

In this study, combined gas exchange and chlorophyll fluorescence data of CO$_2$ and light response curves of photosynthesis were measured for two stages on leaves of 13 ILs under moderate drought and well-watered conditions. These curves showed that our previously reported QTLs, especially the major QTL on chromosome 9 (Fig. 4), identified for the condition of $A_{\text{max}}$ measurements (Gu et al., 2012), also affected $A$ across all irradiance and CO$_2$ levels. Using these curves, we estimated seven parameters of a combined conductance–FvCB model as proposed by Yin et al. (2009). We then quantitatively dissected photosynthesis into different physiological components: stomatal conductance, mesophyll conductance, and biochemical efficiency and capacity. Our model method, Equation (10), presents a novel approach to quantitatively analyse an overall relative limitation of stomatal versus mesophyll diffusion on photosynthesis of a genotype under a given condition.

Our data and analysis confirmed the literature reports in several areas. Firstly, we confirmed that $g^{'}_m$ strongly declined with an increase in $C_i$ and increased with an increase in light intensity, a response to CO$_2$ concentration and light intensity similar to that of $g_e$. (Centritto et al., 2003; Flexas et al., 2007a; Yin et al., 2009; Douthe et al., 2011). Therefore, there was strict proportionality (Fig. S1), although independence of $g_m$ on $I_{\text{inc}}$ and $C_i$ levels was also found (Tazoe et al., 2009, 2011). Secondly, our results confirmed that there was little significant influence of drought on $V_{\text{cmax}}$ and $J_{\text{max}}$ ($P > 0.01$), suggesting that no metabolic impairment but increased diffusion resistances happened under moderate drought (Centritto et al., 2003; Grassi & Magnani, 2005; Galmés et al., 2007). Our result of a decrease in $J_{\text{max}}/V_{\text{cmax}}$ under drought is in line with that of Galle et al. (2011), suggesting that drought stress could cause downregulation of linear electron transport (Kohzuma et al., 2009). Thirdly, we confirmed the decrease in photosynthetic parameters with leaf ageing (e.g. Harley et al., 1992a; Ethier et al., 2006; Flexas et al., 2007c). The ageing decreased $g^{'}_m$, $R_{\text{g}}, k_{\text{SSL}}, J_{\text{max}}, V_{\text{cmax}},$ and $g^{'}\delta_m\delta_l$, and increased $\theta$. These changes of parameters may be associated with leaf nitrogen loss through protein degradation as a result of retranslocation of nitrogen to the grains.

However, the main aims of our study were to analyse the effect of genotypes arising from segregation of photosynthetic QTLs detected by Gu et al. (2012) and to identify the physiological basis of genetic variation and the QTLs. Although the effects of leaf stage and water supply on photosynthesis were predominant, the effect of genotype was significant enough to allow examination of the physiological basis of the genetic variation by use of the combined conductance–FvCB model. Genetic variation in $A_{\text{max}}$ as well as in TE was mainly caused by genetic variation in $g_e$ and $g^{'}_m$ (Table 2), in line with significant stomatal and mesophyll limitations when plants face environmental stress (e.g. drought stress; Flexas et al., 2004; Grassi & Magnani, 2005). Thus, more efforts should be focused on $g_e$ and $g^{'}_m$ in breeding programmes for improving photosynthesis and TE. Furthermore, the relationships between photosynthetic parameters ($g^{'}_m$, $V_{\text{cmax}}$, and $J_{\text{max}}$) and morpho-physiological measurements (LMA, and $N_e$), which
were usually found across environmental treatments (e.g. Harley et al., 1992a; Flexas et al., 2008; Galmés et al., 2011), were shown here, for the first time, to be valid for the variation across genotypes of the same genetic background (Figs. 5A and 6). Therefore, variation in photosynthesis due to environmental conditions and the variation in photosynthesis due to genetic variation within the same environment may share common physiological mechanisms.

Based on the genetic variation of physiological components underlying A and TE, we explored the ideotype design by constituting alleles that contained loci influencing different components of the physiological process of photosynthesis. The suggested virtual ideotypes could be obtained by more rounds of introgression to break any gene linkage within the genome segments of our present ILs. Model calculation showed that these ideotypes could potentially improve A and TE by 17.0 and 25.1%, respectively, compared with the best genotype we investigated. In addition, our analysis using ILs highlights the possibility of improving both A and TE simultaneously within the same genetic background. Further experimental data with more ILs, especially under field conditions, can strengthen this conclusion. Of course, improvements in A and TE could also be achieved by broadening the genetic background. Recent advances in genome-wide association studies (e.g. Huang et al., 2010) will enhance this approach.

**Supplementary data**

Supplementary data are available at *JXB* online.

**Supplementary Table S1.** List of photosynthesis parameter values estimated for the ILs.

**Supplementary Table S2.** Simple correlation coefficients among seven parameters of the photosynthesis model at four stage × treatment combinations.

**Supplementary Fig. S1.** Relationships between stomatal conductance (g_s) and mesophyll conductance (g_m).

**Supplementary Fig. S2.** Relationships between light-saturated net photosynthesis rate (A_max) and (A) stomatal conductance to CO_2 (g_s), (B) mesophyll conductance to CO_2 (g_m), (C) total diffusion conductance to CO_2, including g_s and g_m (g_total), (D) electron transport capacity (J_max), and (E) Rubisco carboxylation capacity (V_cmax).

**Supplementary Fig. S3.** Relationships between transpiration efficiency (TE) and (A) stomatal conductance to CO_2 (g_s); (B) mesophyll conductance to CO_2 (g_m); (C) electron transport capacity (J_max); and (D) Rubisco carboxylation capacity (V_cmax).

**Acknowledgements**

J.G. is grateful to the China Scholarship Council for granting him a PhD scholarship. We thank Mr P.E.L. van der Putten for supporting the greenhouse experiment. This work was supported by the research programme ‘BioSolar Cells’.

**References**


