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A Model Explaining Genotypic and Ontogenetic Variation of Leaf Photosynthetic Rate in Rice (*Oryza sativa*) Based on Leaf Nitrogen Content and Stomatal Conductance

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• *Backgrounds and Aims* Identification of physiological traits associated with leaf photosynthetic rate (P_n) is important for improving potential productivity of rice (*Oryza sativa*). The objectives of this study were to develop a model which can explain genotypic variation and ontogenetic change of P_n in rice under optimal conditions as a function of leaf nitrogen content per unit area (N) and stomatal conductance (g_s), and to quantify the effects of interaction between N and g_s on the variation of P_n.
• *Methods* P_n, N and g_s were measured at different developmental stages for the topmost fully expanded leaves in ten rice genotypes with diverse backgrounds grown in pots (2002) and in the field (2001 and 2002). A model of P_n that accounts for carboxylation and CO_2 diffusion processes, and assumes that the ratio of internal conductance to g_s is constant, was constructed, and its goodness of fit was examined.
• *Key Results* Considerable genotypic differences in P_n were evident for rice throughout development in both the pot and field experiments. The genotypic variation of P_n was correlated with that of g_s at a given stage, and the change of P_n with plant development was closely related to the change of N. The variation of g_s among genotypes was independent of that of N. The model explained well the variation in P_n of the ten genotypes grown under different conditions at different developmental stages.
• *Conclusions* The response of P_n to increased N differs with g_s, and the increase in P_n of genotypes with low g_s is smaller than that of genotypes with high g_s. Therefore, simultaneous improvements of these two traits are essential for an effective breeding of rice genotypes with increased P_n.

Key words: Model, leaf photosynthesis, genotypic and ontogenetic variation, rice (*Oryza sativa*), leaf nitrogen content, stomatal conductance, internal conductance.

INTRODUCTION

Breeding of rice genotypes with higher yield potential is required to meet the increasing demand for this staple cereal caused by a rapid increase of the human population in Asia. In situations where useful genes for improved plant type have already been utilized in the existing high-yielding rice, identification of physiological traits associated with higher yield is necessary for breeding of genotypes that break through the current plateau of yield potential. Since recently bred high-yielding cultivars commonly possess effective plant structures and sufficient leaf area index (LAI) for light interception after panicle initiation, high yield potential of rice is associated with higher leaf photosynthetic rate (P_n) during the late reproductive period (Horie *et al.*, 2003; Takai *et al.*, 2006) and the grain filling (Arjunan *et al.*, 1990; Kuroda and Kumura, 1990; Sasaki and Ishii, 1992). Therefore, identification of traits determining P_n is of primary importance for increased yield potential of rice.

Previous studies showed that a large genotypic variation exists in P_n of rice and its relatives (Cook and Evans, 1983; Yeo *et al.*, 1994; Horie *et al.*, 2003). P_n under current atmospheric CO_2 concentration is limited by carboxylation capacity that is determined by the amount of Rubisco and its kinetics (Farquhar and Sharkey, 1982; Makino *et al.*, 1985). While genotypic differences in Rubisco kinetic parameters are small in rice (Makino *et al.*, 1987), large genotypic variation has been reported for leaf nitrogen content, which correlates with the amount of Rubisco (Cook and Evans, 1983; Horie *et al.*, 2003). Thus, the genotypic variation of P_n in rice has been related to that of leaf nitrogen content per unit area (N) (Cook and Evans, 1983). However, N is not only a genetic trait but is also affected by plant ontogenetic development and nitrogen management (Hasegawa and Horie, 1996). Furthermore, a number of studies have indicated that stomatal conductance for CO_2 diffusion (g_s) limits P_n under ambient CO_2 concentrations (Kuroda and Kumura, 1990; Miah *et al.*, 1997). Although N and g_s have been suggested to be major factors limiting P_n, the interactive effects of these traits on the variation of rice P_n have not been quantitatively evaluated yet.

The primary objective of this study was to develop a model which can explain genotypic and ontogenetic variation of P_n in rice, based on experimental data, i.e. N content and gaseous diffusive conductance. P_n, N and g_s were measured at different developmental stages for ten rice genotypes grown in pots (2002) and in the field

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(2001 and 2002). By analysing the data thus obtained, a model was synthesized to explain genotypic and ontogenetic variation of P_n based on CO_2 diffusion equations. There are two assumptions concerning the nature of internal conductance (g_w) in the leaf. One was that g_w was mainly determined by the surface area of the chloroplasts facing the cell walls (S_c) and that S_c was proportional to N (von Caemmerer and Evans, 1991; Evans *et al.* 1994). The other was that the variation of g_w was proportional to that of g_s (Loreto *et al.*, 1992; Lauteri *et al.*, 1997). An examination was carried out into which assumption for g_w could better explain the genotypic and ontogenetic variation of P_n in rice grown under optimum conditions. Here, the results of the measurements, modelling and simulation of genotypic and ontogenetic variation in rice P_n are reported.

MATERIALS AND METHODS

Plant materials

Ten widely different rice cultivars were collected from diverse rice cultivating regions in the world. Four cultivars, 'Takanari', 'IR72', 'Shanguichao' and 'Ch86', are *indica* genotypes; three cultivars, 'Nipponbare', 'Takenari' and 'Koshihikari', are temperate *japonica* genotypes; the cultivar, 'Banten' is a tropical *japonica* genotype; IR65564-44-2-2 ('NPT') was bred by crossing tropical *japonica* and *indica*, and was named as a new plant type by the International Rice Research Institute (IRRI); WAB450-I-B-P-38-HB ('WAB') is an interspecific hybrid genotype between *O. sativa* ssp. *japonica* and *O. glaberrima*, and named NERICA (new rice for Africa) by the West Africa Rice Development Association (WARDA). 'Takenari', 'Ch86' and 'Banten' are traditional cultivars, and the others are improved ones. 'Takanari', 'IR72' and 'Shanguichao' are high-yielding cultivars among the improved cultivars (Horie *et al.*, 2003).

Cultivation conditions

In the pot experiment in 2002, each cultivar was sown on 30 April and two seedlings transplanted on 24 May into 3·8 L pots with 12 replicates. Plants were grown outdoors and under flooding at Kyoto, Japan (35°2′N, 135°47′E, 65 m altitude) and received full sunlight until the gas exchange measurements. Nitrogen, phosphorus and potassium were applied at 0·3 g per pot as basal dressing, and 0·1 g of nitrogen was top-dressed biweekly to maintain high plant nitrogen status throughout the entire growth period.

Field experiments were done at Kyoto, Japan in 2001 and 2002. The soil was alluvial loam soil classified into Haplaquept. The rice genotypes with three replicates were sown in seed beds on 2 May and 30 April, and transplanted into the main fields on 23 May and 25 May in 2002 and 2001, respectively. The experiment was a randomized block design. Each plot was $>20\ m^{-2}$, and hill spacing was $0{\cdot}15 \times 0{\cdot}3$ m (density: 22·2 hill m^{-2}) in both years. Each plot was fertilized with $4\ g\ m^{-2}$ of nitrogen and $12\ g\ m^{-2}$ of phosphorus and potassium as basal, and top-dressed with $2\ g\ m^{-2}$ of nitrogen every 20 d until 10 d after heading. For tall cultivars of 'Ch86' and 'Banten', the amounts of basal and top-dressing nitrogen fertilizer were reduced to half that of the other cultivars to avoid lodging.

Measurements of leaf photosynthetic rate and stomatal conductance

Pot experiment. The exchange rates of CO_2 and water vapour were measured in the topmost fully expanded leaves at panicle initiation (PI), heading and 3 weeks after heading (3 WAH) by an open gas exchange system devised in the authors' laboratory. From the afternoon of the day before gas exchange measurement, plants in the pots were kept in a black net (transmittance 60 %) to avoid high light and wind in order to maintain favourable leaf water status during gas exchange measurements. Four leaves from each cultivar were enclosed in four acrylic chambers ($30 \times 5{\cdot}5 \times 6{\cdot}5$ cm, length × width × height) in one measurement series. Two series of measurements were made for one cultivar, and thus eight leaves of one genotype were measured at one developmental stage. Concentrations of CO_2 and water vapour at the inlet and outlet of the chamber were simultaneously monitored by an infrared gas analyser (LI-7000, LI-COR, Lincoln, NE, USA). Photosynthetic photon flux (PPF) at the leaf surface, measured with a quantum sensor (LI-190SH, LI-COR), was first set to zero to obtain the dark respiration rate. Then, the leaves were irradiated at $1000\ \mu mol\ m^{-2}\ s^{-1}$ with halogen lamps (JD500W-M, IWASAKI, Tokyo, Japan) for 30 min and gas exchange rates measured at a PPF of $1500\ \mu mol\ m^{-2}\ s^{-1}$. The air around each leaf was stirred by four fans (DC Fine Ace 20, SANYO DENKI, Tokyo, Japan) installed inside the chamber to maximize boundary layer conductance ($1{\cdot}07\ mol\ CO_2\ m^{-2}\ s^{-1}$), which was measured with a wetted filter paper. Leaf temperature was $30 \pm 1{\cdot}2$ °C, measured with a copper–constantan thermocouple appressed to the lower leaf surface, vapour pressure deficit was $1{\cdot}0 \pm 0{\cdot}1$ kPa and CO_2 concentration was $349 \pm 0{\cdot}1\ \mu mol\ mol^{-1}$ at the leaf surface. Leaf and air temperatures were recorded with a data logger (DR232, YOKOGAWA, Tokyo, Japan). From these measurements, leaf gas exchange parameters were calculated according to von Caemmerer and Farquhar (1981).

Field experiment. P_n and g_s in the topmost fully expanded leaves of each genotype were measured with apparatus for photosynthesis and transpiration measurements (LI-6400, LI-COR). The measurements were made from 1000 h to 1300 h under natural sunlit and light-saturated conditions (PPF $>1200\ \mu mol\ m^{-2}\ s^{-1}$) for ten genotypes on clear days during the period from PI to maturity (MT) in both years. Leaf temperature was $31 \pm 1{\cdot}1$ °C and the CO_2 concentration at the leaf surface averaged $346 \pm 6{\cdot}2\ \mu mol\ mol^{-1}$ over measurements. The values measured at a relative humidity (RH) <70 % were discarded. P_n and g_s were averaged for four developmental stages; from PI

to 2 weeks before heading (2 WBH), from 2 WBH to full heading (FH), from FH to 2 weeks after full heading (2 WAH) and from 2 WAH to MT. At least 12 leaves were used to determine P_n and g_s for each developmental stage for each genotype.

Measurement of leaf nitrogen concentration

In the pot experiment, the nitrogen concentration of the leaf used for gas exchange measurements was measured, and in the field experiment the nitrogen concentration of the whole canopy leaves of two plants harvested at PI, 2 WBH, FH, 2 WAH and MT of each plot was measured. After measurement of leaf area with a meter (LI-3000, LI-COR), they were oven-dried at 80 °C for at least 72 h, weighed and then the N concentration measured by the Kjeldahl method for both the experiments. Nitrogen concentrations in leaves at the four developmental stages in the field experiment are averaged values of the concentrations at the beginning and the end of each stage.

The model

Based on Fick's law, P_n was given by the product of g_s and the difference between CO_2 concentrations on the leaf surface (C_a) and in intercellular airspaces (C_i). Similarly, P_n can also be expressed by the product of g_w and the difference between CO_2 concentrations in intercellular airspaces and chloroplast stroma (C_c). Namely,

$$P_n = g_s(C_a - C_i) \quad (1)$$

$$P_n = g_w(C_i - C_c) \quad (2)$$

Photosynthetic response to change in CO_2 concentration is generally presented as a Michaelis–Menten equation. However, the response from zero to atmospheric CO_2 concentration can also be regarded as linear apparently, with the initial slope representing carboxylation capacity (Farquhar and Sharkey, 1982). Since Makino *et al.* (1987) showed that there is little variation in the amount of Rubisco per unit of leaf-soluble protein or its kinetics among diverse rice genotypes and their wild relatives, carboxylation capacity is, in this study, assumed proportional to N with an empirical proportionality constant k_1:

$$P_n = k_1(N - N_0)(C_c - \Gamma^*) - R_L \quad (3)$$

where R_L is the mitochondrial dark respiration rate in the light, N_0 is N when the amounts of Rubisco reached zero, as reviewed by Evans (1989), and Γ^* is the CO_2 compensation point when P_n is zero in the absence of R_L. Γ^* was fixed considering the consistency of the Rubisco kinetic parameters among *Oryza* species (Makino *et al.*, 1987) and set to be 43·8 μmol mol^{-1} for rice leaves at 30 °C, referring to Horie (1981).

The dark respiration rate in the dark (R_d) was about 5 % of P_n over all cultivars and developmental stages in this study (data not shown). High irradiance inhibited mitochondrial dark respiration (Brooks and Farquhar, 1985) and R_L was much smaller than R_d at 30 °C (Atkin *et al.*, 2000). On the basis of these results, it was assumed that R_L has little effect on the absolute value of P_n and could be neglected.

Then, combining eqns (1)–(3) and assuming $R_L = 0$, the following equation was obtained:

$$P_n = \frac{k_1 g_s (N - N_0)}{g_s + k_1(1 + g_s/g_w)(N - N_0)}(C_a - \Gamma^*) \quad (4)$$

Since the internal conductance, g_w, was not measured in this study, eqn (4) includes three unknown values: k_1, N_0 and g_w. These were estimated by applying the measured data set of P_n, N, g_s and C_a, and the constant, Γ^*, to eqn (4). Thus, these estimates represent the average of the individual leaves. Two different assumptions on the nature of g_w in the calculation were also made. The first was based on von Caemmerer and Evans (1991) and Evans *et al.* (1994) that g_w was correlated with S_c, which in turn was proportional to N. In this case, g_w was represented by

$$g_w = k_2 N \quad (5a)$$

where k_2 was an empirical parameter. The second was based on Loreto *et al.* (1992) and Lauteri *et al.* (1997), and g_w was proportional to g_s with little variation in the similar proportionality constant among plant species. In this case,

$$g_w = k_3 g_s \quad (5b)$$

where k_3 is another empirical constant. Using eqns (4) and (5a) or (5b), P_n of the ten genotypes at different developmental stages were regressed against their N, g_s and C_a to obtain best estimates for the values of parameters k_1, N_0 and k_2, or k_3 on the assumption that these parameter values are independent of genotypes and developmental stages. A least-squares method for non-linear functions was applied for this regression to minimize the sum of squared errors between measured and estimated P_n values. The regression was determined separately for the data set of the pot experiment and that of the field experiment, because of the different sampling methods for N measurements. P_n measurements on 240 leaves (ten genotypes × eight leaves × three developmental stages) were analysed for the pot experiment and 40 measurements (ten genotypes × four developmental stages) for the field experiment in 2001. The 40-data set from the 2002 field experiment was used for validation of the model developed from the 2001 field experiment.

RESULTS

Pot-grown rice

P_n and N of pot-grown rice drastically declined by 3 WAH, but g_s declined only slightly (Table 1). P_n differed significantly among the genotypes and ranged from 12·9 to

TABLE 1. *Genotypic differences in photosynthetic rate, stomatal conductance for CO_2 and leaf nitrogen content per unit area for the ten rice genotypes grown in pots at panicle initiation (PI), heading and 3 weeks after heading (3 WAH)*

	Photosynthetic rate (μmol m^{-2} s^{-1})			Leaf nitrogen content per unit area (g m^{-2})			Stomatal conductance for CO_2 (mol m^{-2} s^{-1})		
Cultivar	PI*	Heading	3 WAH	PI	Heading	3 WAH	PI	Heading	3 WAH
'Takanari'	$18{\cdot}7^{ab}$	$23{\cdot}4^{a}$	$12{\cdot}5^{a}$	$1{\cdot}39^{ab}$	$1{\cdot}57^{a}$	$0{\cdot}95^{ab}$	$0{\cdot}39^{a}$	$0{\cdot}32^{a}$	$0{\cdot}36^{a}$
'IR72'	$18{\cdot}7^{ab}$	$21{\cdot}6^{abc}$	$13{\cdot}9^{a}$	$1{\cdot}43^{ab}$	$1{\cdot}43^{abc}$	$0{\cdot}95^{ab}$	$0{\cdot}28^{abc}$	$0{\cdot}34^{a}$	$0{\cdot}36^{a}$
'Shanguichao'	$15{\cdot}3^{bcd}$	$22{\cdot}6^{ab}$	$10{\cdot}4^{ab}$	$1{\cdot}24^{bc}$	$1{\cdot}38^{abc}$	$0{\cdot}76^{bc}$	$0{\cdot}20^{bc}$	$0{\cdot}32^{a}$	$0{\cdot}25^{ab}$
'Ch86'	$12{\cdot}9^{d}$	$14{\cdot}4^{d}$	$5{\cdot}8^{c}$	$1{\cdot}05^{c}$	$1{\cdot}08^{d}$	$0{\cdot}67^{c}$	$0{\cdot}19^{c}$	$0{\cdot}19^{b}$	$0{\cdot}11^{c}$
'Nipponbare'	$15{\cdot}3^{bcd}$	$18{\cdot}0^{c}$	$7{\cdot}8^{bc}$	$1{\cdot}24^{bc}$	$1{\cdot}49^{ab}$	$0{\cdot}92^{abc}$	$0{\cdot}19^{c}$	$0{\cdot}18^{b}$	$0{\cdot}10^{c}$
'Takenari'	18^{abc}	$17{\cdot}5^{cd}$	$6{\cdot}8^{bc}$	$1{\cdot}42^{ab}$	$1{\cdot}42^{abc}$	$0{\cdot}91^{abc}$	$0{\cdot}20^{bc}$	$0{\cdot}20^{b}$	$0{\cdot}10^{c}$
'Koshihikari'	$16{\cdot}9^{abc}$	$20{\cdot}4^{abc}$	$10{\cdot}1^{abc}$	$1{\cdot}38^{ab}$	$1{\cdot}22^{cd}$	$1{\cdot}05^{a}$	$0{\cdot}28^{abc}$	$0{\cdot}19^{b}$	$0{\cdot}20^{bc}$
'Banten'	$14{\cdot}3^{cd}$	$18{\cdot}8^{c}$	$10{\cdot}4^{ab}$	$1{\cdot}24^{bc}$	$1{\cdot}30^{bcd}$	$0{\cdot}91^{abc}$	$0{\cdot}23^{bc}$	$0{\cdot}24^{ab}$	$0{\cdot}19^{bc}$
'NPT'†	$15{\cdot}3^{bcd}$	$19{\cdot}6^{bc}$	$10{\cdot}3^{ab}$	$1{\cdot}56^{a}$	$1{\cdot}43^{abc}$	$0{\cdot}97^{ab}$	$0{\cdot}18^{c}$	$0{\cdot}24^{ab}$	$0{\cdot}17^{bc}$
'WAB'†	$19{\cdot}9^{a}$	$18{\cdot}2^{c}$	$12{\cdot}3^{a}$	$1{\cdot}37^{ab}$	$1{\cdot}38^{abc}$	$1{\cdot}15^{a}$	$0{\cdot}34^{ab}$	$0{\cdot}19^{b}$	$0{\cdot}23^{b}$

*Values are shown as mean of eight leaves.
†'NPT' and 'WAB' indicate IR65564-44-2-2 and 'WAB'450-I-B-P-38-HB, respectively.
Figures followed by a different letter are significantly different at the 5 % level among genotypes with the Tukey test.

19·9, 14·4 to 23·4 and 5·8 to 13·9 μmol m^{-2} s^{-1} at PI, heading and 3 WAH, respectively. P_n was significantly higher in 'Takanari', 'IR72' and 'WAB' than in 'Ch86' at all developmental stages. There were also significant genotypic differences in N and g_s at each developmental stage. 'Ch86' consistently had the lowest N throughout development, while 'Takanari', 'IR72' and 'NPT' had the highest N. 'Takanari' maintained significantly higher g_s than 'Ch86', 'Nipponbare' and 'Takenari'. Genotypes with higher P_n tended to have higher N and/or g_s.

P_n values of the ten rice genotypes were plotted against N (Fig. 1A) and against g_s (Fig. 1B) for each developmental stage. The relationship between P_n and N in the ten genotypes was not significant at a given developmental stage, but was significant with a correlation coefficient, r, of 0·85 ($P < 0{\cdot}001$) when all the data at different stages were aggregated. In contrast, P_n of the ten genotypes was significantly correlated with g_s at each stage, with an r of 0·77, 0·81 and 0·91 at PI, heading and 3 WAH, respectively ($P < 0{\cdot}01$), but the regression lines differed with developmental stages.

Field-grown rice

Figure 2 shows P_n, N and g_s for the different developmental stages of the ten genotypes grown under field

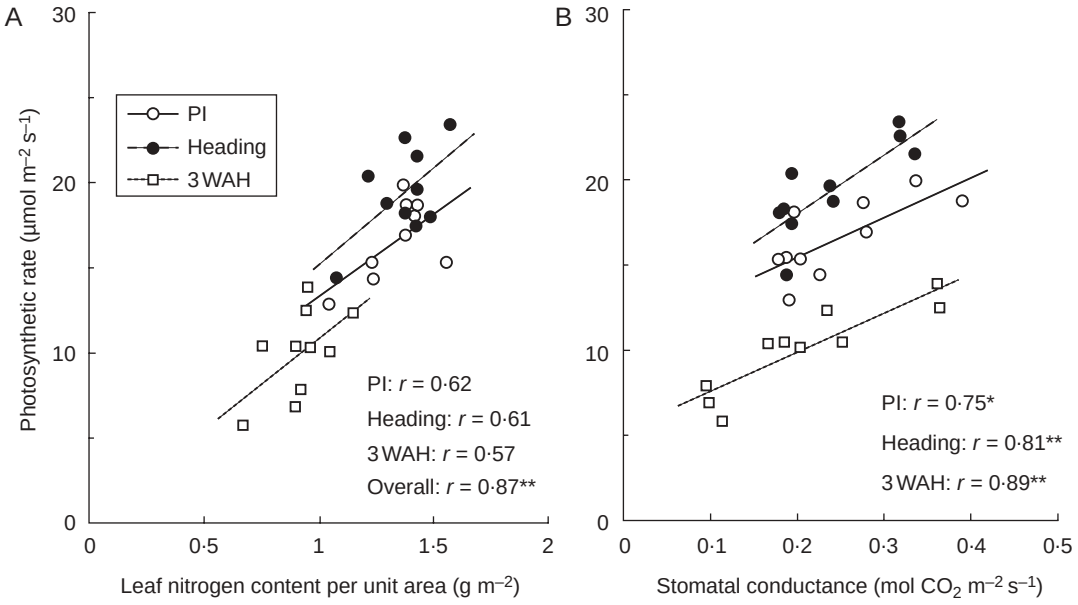

FIG. 1. Relationships between photosynthetic rate and nitrogen content per unit area of leaves (A), and stomatal conductance for CO_2 (B) of ten genotypes of rice grown in pots at panicle initiation (PI), heading and 3 weeks after heading (3 WAH). Each point means an average value of eight leaves for each genotype. * denotes significance at the 5 % level, and ** at the 1% level.

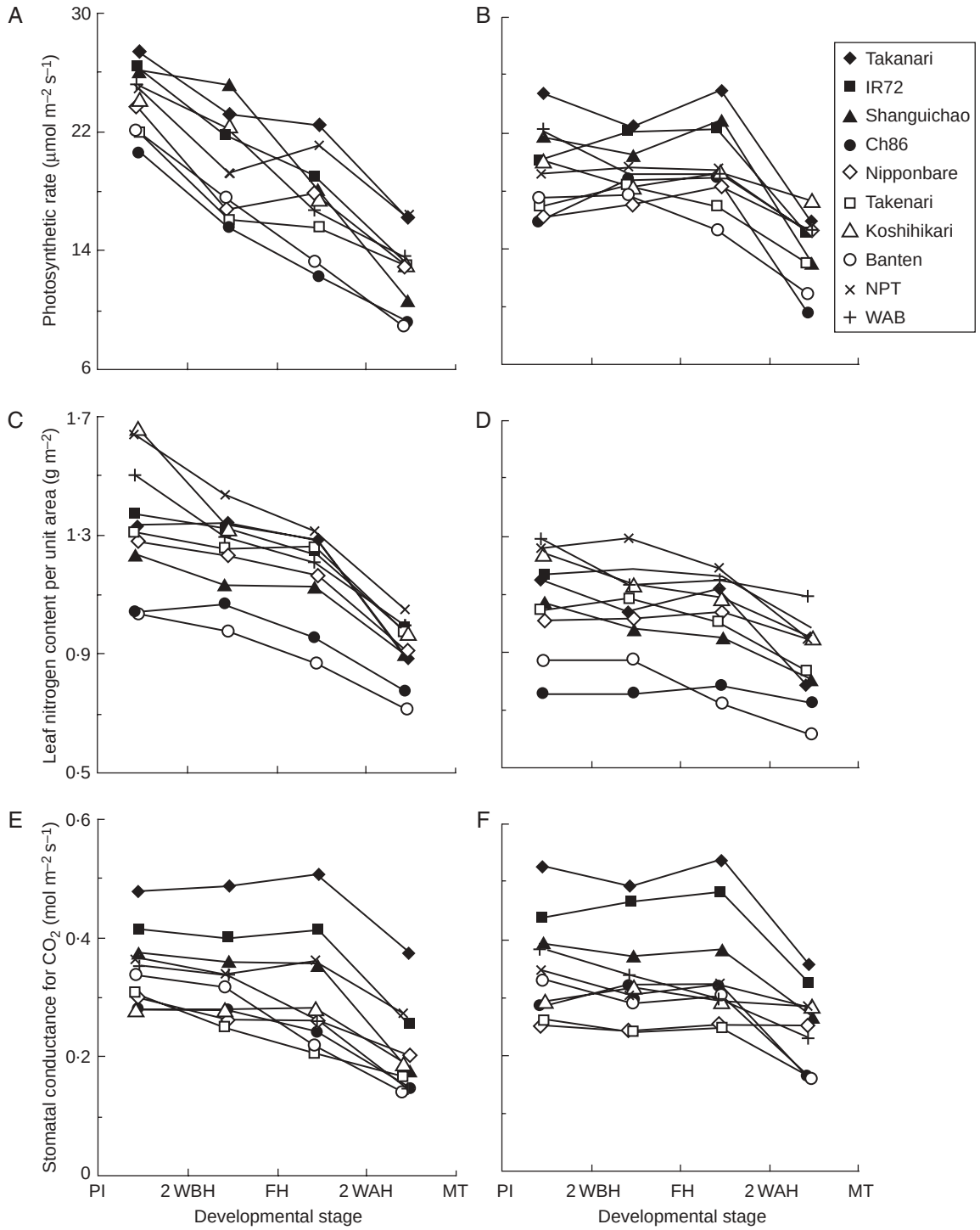

FIG. 2. Changes of photosynthetic rate (A, B), leaf nitrogen content per unit area (C, D) and stomatal conductance for CO_2 (E, F) with developmental stage of ten rice genotypes grown under field conditions in 2001 (A, C, E) and 2002 (B, D, F).

conditions in 2001 and 2002. P_n and N of all the genotypes changed markedly with stage of development. Plants grown in 2001 had greater P_n and N at the early reproductive stages than those in 2002. This might be related to the higher solar radiation before PI in 2001 than in 2002, which affects soil temperature and thus nitrogen mineralization. In contrast to P_n and N, the ontogenetic change of g_s was small in all ten genotypes in both years, except for the late grain-filling stages (2 WAH to MT).

Considerable genotypic differences were observed in P_n, N and g_s at each stage in both years. P_n and g_s of

TABLE 2. *Contributions (% of sum of squares) of genotype (G), developmental stage (D), year (Y) and their interactions (G × D, D × Y, G × Y) to total variance in photosynthetic rate, leaf nitrogen content per unit area and stomatal conductance for CO_2 for the ten rice genotypes during the developmental period from panicle initiation to 2 weeks after heading in the field experiment*

Factor	Photosynthetic rate	Leaf nitrogen content per unit area	Stomatal conductance for CO_2
Genotype (G)	44·9**	56·7**	80·7**
Developmental stage (D)	16·3**	6·4**	1·0*
Year (Y)	1·8**	23·3**	0·8*
G × D	4·6*	4·0**	3·5
G × Y	2·6*	1·1*	2·5*
D × Y	18·8**	1·3**	1·0*
Total	89·0	92·7	89·5

*Significant at $P = 0{\cdot}05$; **significant at $P = 0{\cdot}01$.

'Takanari', 'IR72' and 'Shanguichao' were greater than those of 'Ch86' and 'Banten' throughout development. While 'NPT' maintained high *N*, its P_n was intermediate among the cultivars. There were similarities in these genotypic differences in P_n, g_s and *N* in the field experiment in the two years and also in the pot experiment. Genotypic differences in P_n, *N* and g_s were $>5{\cdot}3$ μmol m^{-2} s^{-1}, 0·48 g m^{-2} and 0·20 mol CO_2 m^{-2} s^{-1}, respectively, at each developmental stage in the two years.

Three-way analysis of variance (ANOVA) was carried out to evaluate the effects of genotype (G), developmental stage (D) and year (Y) and their interactions on total variance of P_n, g_s and *N* in the ten genotypes at three developmental stages up to 2 WAH in the two years (Table 2). G explained 44·9, 46·2 and 80·7 % of the total variance of P_n, *N* and g_s, respectively. D and D × Y gave significant effects not only on the variance of P_n but also on that of *N*, while their effects on g_s were very limited.

P_n of the ten genotypes grown in the field in 2001 and 2002 was plotted against *N* and g_s for each developmental stage (Fig. 3). As in the pot-grown rice, *N* poorly explained genotypic difference of P_n at a given stage except at the late grain-filling stage, but g_s explained the genotypic difference of P_n at all developmental stages in both years with the higher correlation coefficients. There was a significant linear relationship between P_n and *N* over all genotypes and developmental stages, with an *r* of 0·80 in 2001 and of 0·67 in 2002 ($P < 0{\cdot}001$). Since there was no association between g_s and *N* of the ten diverse cultivars in any of the pot and the two field experiments, the genotypic variation of g_s was thought to be independent of that of *N*.

Model application

Figure 4 shows the results of the application of P_n, g_s and *N* data obtained for the ten genotypes at three

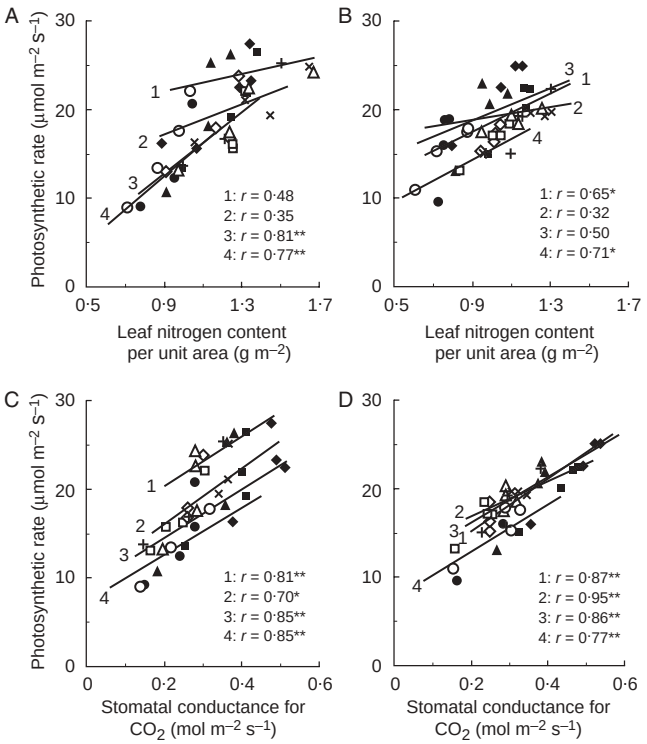

FIG. 3. Relationships between photosynthetic rate and nitrogen content per unit area of leaves (A, B), and stomatal conductance for CO_2 (C, D) of ten rice genotypes measured at four different developmental stages from field experiments in 2001 (A, C) and 2002 (B, D). The number on the lines show the developmental stage: 1, panicle initiation to 2 weeks before heading (2 WBH); 2, 2 WBH to heading; 3, heading to 2 weeks after heading (2 WAH); 4, 2 WAH to maturity. Symbols are the same as in Fig. 2. *denotes significance at the 5% level, and **at the 1% level.

developmental stages of pot-grown rice to the two models assuming that g_w is proportional to *N* (eqn 5a, called the *N*-model hereafter) and that g_w is proportional to g_s (eqn 5b, called the g_s-model hereafter). The g_s-model accounted for the measured P_n of the ten rice genotypes at all developmental stages better than the *N*-model did ($R^2 = 0{\cdot}85$ and 0·79 for the former and the latter, respectively.). The *N*-model underestimated P_n of 'IR72' and 'Banten', but overestimated that of 'Ch86', 'NPT' and 'Takenari'. On the other hand, the g_s-model simulated the measured genotypic and ontogenetic variation of P_n well, with smaller biases for all the cultivars.

Similarly, the g_s-model explained more of the variation in P_n of the ten genotypes grown under field conditions in 2001 than the *N*-model (Fig. 5, $R^2 = 0{\cdot}80$ and 0·53 for the former and the latter, respectively, $P < 0{\cdot}001$). For the model validation, the two models with the parameter values of k_1, N_0 and k_2, or k_3 estimated from the 2001 field experiment were applied to the measured data in 2002. The P_n measured in 2002 was also well explained by the g_s-model with $R^2 = 0{\cdot}66$ ($P < 0{\cdot}001$), whereas the *N*-model could not explain the measured P_n better than *N* alone.

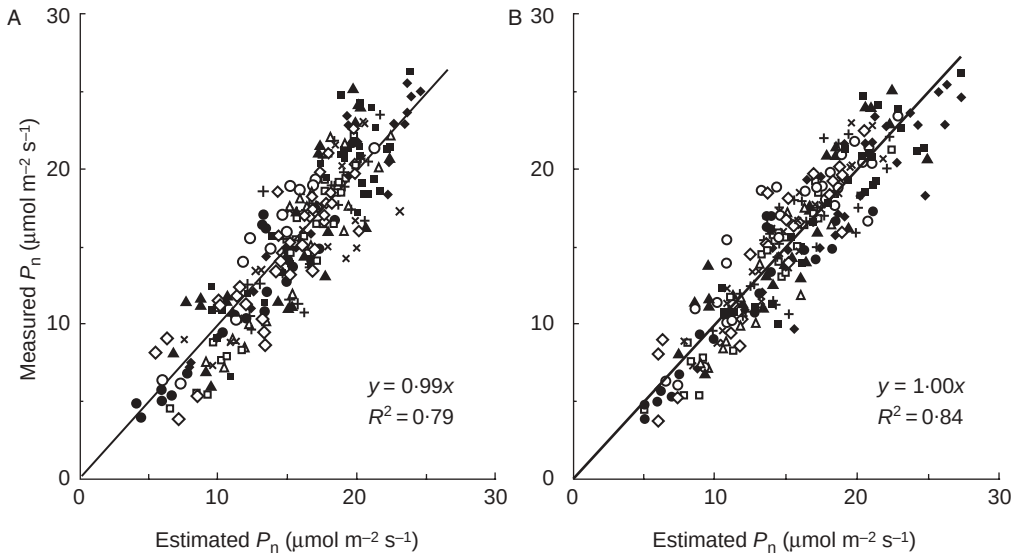

FIG. 4. Relationships between measured and estimated leaf photosynthetic rate (P_n; μmol m^{-2} s^{-1}) by the models (eqn 4) assuming that the internal conductance (g_w) is proportional to leaf nitrogen content per unit area (A), and assuming that g_w is proportional to stomatal conductance (B), using data from the pot experiment. Data points include those at panicle initiation, heading and 3 weeks after heading. Symbols are the same as in Fig. 2. Both the relationships are significant at the 1% level.

DISCUSSION

Considerable genotypic differences in P_n were evident for rice throughout development in both the pot and field experiments (Table 1, Fig. 2). Sasaki and Ishii (1992) reported that recent cultivars had higher P_n during grain filling as a result of the breeding of high-yielding *japonica* cultivars in Japan. In the present study, high-yielding *indica* genotypes such as 'Takanari', 'IR72' and 'Shanguichao' had higher P_n throughout development. The ten genotypes in the field had sufficient leaf area for full light interception with LAI >4·0 during the late reproductive stages in both years (data not shown). This suggests that the high biomass productivity of the three genotypes, as indicated earlier (Horie *et al.*, 2003), would be attributable to their high P_n.

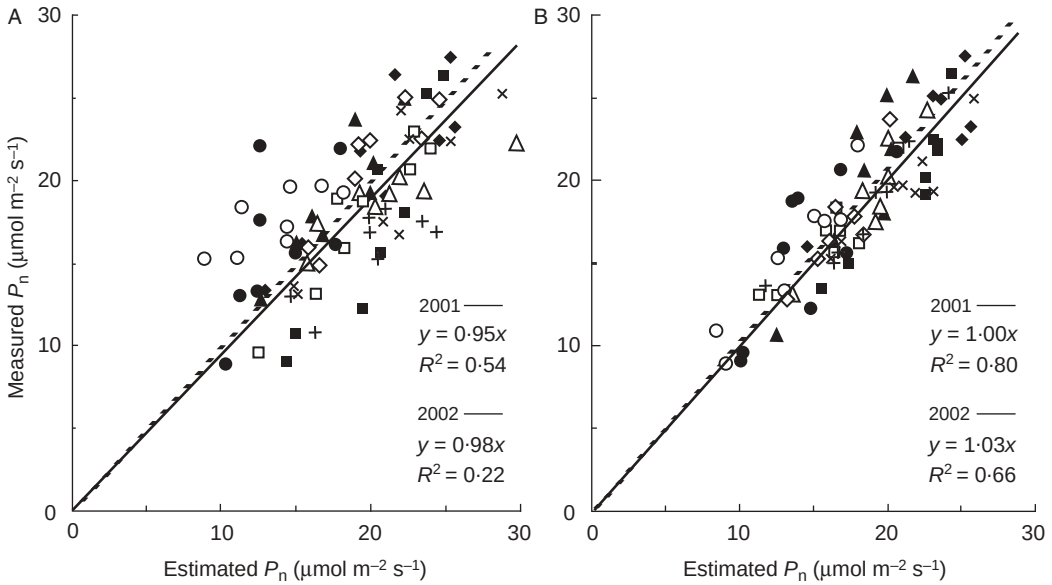

FIG. 5. Relationships between measured and estimated leaf photosynthetic rate (P_n; μmol m^{-2} s^{-1}) by the models (eqn 4) assuming that the internal conductance (g_w) is proportional to leaf nitrogen content per unit area (A), and that g_w is proportional to stomatal conductance (B), in 2001 (solid line) and 2002 (dashed line) field experiments. The parameter values estimated from the 2001 experiment were applied to the 2002 model analysis. Data points include those at four different developmental stages from panicle initiation to maturity. Symbols are the same as in Fig. 3. All the relationships are significant at the 1% level.

The present data show that g_s was a stable and mainly genotypic trait with small ontogenetic change except for the late grain-filling stage (Table 2). Although N varied partly with developmental stage and year, the ranking of genotypes was very conservative. The genotypic difference in P_n at each developmental stage was better explained by g_s than by N while the change of P_n with development was explained by that of N (Figs 1 and 3). These results confirmed that both g_s and N are the major factors determining the variation of rice P_n among genotypes and developmental stages. It is noticeable that the genotypic variation in N was unrelated to that in g_s among the rice genotypes used here.

There are many reports indicating strong correlations of P_n with N in rice, as reviewed by Sinclair and Horie (1989). A number of empirical models have been proposed to explain the variation of P_n solely by N for rice and other crops (Sinclair and Horie, 1989; Peng *et al.*, 1995; Boote *et al.*, 1998). However, N alone did not explain the genotypic difference of P_n of diverse *Oryza* species (Takano and Tsunoda, 1971; Cook and Evans, 1983). The model proposed here based on N and g_s explained P_n measured for the ten genotypes grown under both pot and field conditions at different developmental stages very well (Figs 4 and 5). Therefore, a significant improvement of the model based on N in explaining the genotypic difference of P_n by incorporating g_s was demonstrated.

Table 3 shows the parameter values estimated by the model for pot-grown and field-grown rice. The estimated N_0 values of 0·35 for pot-grown rice and 0·27 for field-grown rice were similar to the value of 0·3 reported previously on rice (Sinclair and Horie, 1989). There were noticeable differences between the estimated values in the pot- and field-grown rice; the former had larger N_0 and smaller k_1 values than the latter. These differences probably reflected the differences in N measurements. While N was measured for the P_n-measured leaf in the pot experiment, it was an average for the whole leaf canopy in the field experiment and lower than that of the P_n-measured leaf since leaf nitrogen usually decreases from the top to the bottom of the canopy. The reason for the difference in the estimate for k_3 between the pot and field experiments is not clear. Although boundary layer conductance is not involved in calculated g_s, the different boundary layer conductance in the different measurement apparatus between pot and field experiments might have affected g_s. Also, the different conditions for plant culture such as rhizosphere size might have affected g_s relative to g_w. However, the g_w/g_s ratios (k_3) of 1·21 estimated for pot-grown and 0·89 for field-grown rice agree with the experimental reports that g_w is comparable with g_s (Loreto *et al.*, 1992; Epron *et al.*, 1995). A modified model that assumes g_w to be infinitely large ($k_3 = +\infty$) results in substantially lower goodness of fit ($R^2 = 0{\cdot}79$ and 0·53 for the pot and the 2001 field experiments, respectively) than the model proposed here (not shown). The similarities of the parameter values N_0 and k_3 from the model analysis to those from previous studies led to the presumption that the values were estimated reasonably, from a data set with large variations in P_n, N and g_s and with differences between N and g_s among the genotypes and developmental stages.

P_n responses to N increase are curvilinear and can be simulated by applying the estimates from the pot-grown rice to Eqn 4 (Fig. 6). The model shows that P_n responses to N differ with g_s values. The P_n increase of genotypes with low g_s, such as *japonica*, is smaller than that of genotypes with high g_s. This suggests that effective breeding of rice genotypes with higher P_n might be achieved through parallel efforts on improving both N and g_s. Further, 'Takanari', which is regarded as one of the most high-yielding cultivars, exhibited the highest g_s of 0·39 mol m^{-2} s^{-1} at PI (Table 1). The P_n response curve with g_s of 0·4 mol m^{-2} s^{-1} corresponded closely with the function showing maximum P_n for the existing rice genotypes reported by Sinclair and Horie (1989) in a wide range of N. The model predicts that P_n would increase approx. 13 % at the N level of 1·5 g m^{-2} if g_s is further improved from 0·4 to 0·6 mol m^{-2} s^{-1}.

The model explained the variation of P_n better when it assumed a constant g_w/g_s ratio for both experiments. Co-ordinated variations in g_w and g_s have been reported for plant species with similar leaf morphology (Loreto

TABLE 3. *Parameter values estimated from the model application (eqns 4 and 5b) for the photosynthetic rates measured for the ten genotypes grown under pot (2002) and field (2001) conditions at different developmental stages, and their goodness of fit*

	k_1 (mol CO_2 g N^{-1} s^{-1})	k_3 (ratio)	N_0 (g N m^{-2})	Bias	R^2
Pot experiment	0·10	1·21	0·35	1·00	0·84
Field experiment	0·13	0·89	0·27	1·00	0·80

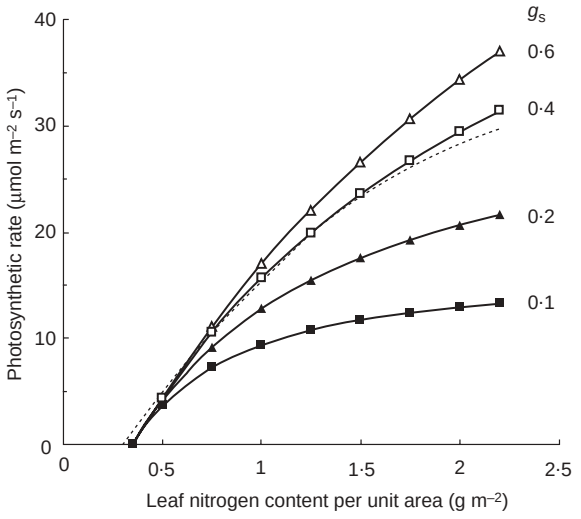

FIG. 6. Response curves of photosynthetic rate to increased leaf nitrogen content per unit area with different stomatal conductance (g_s). These curves are derived from the data for pot-grown rice (Table 3) applied to eqn (4). Symbols represent g_s of 0·1, 0·2, 0·4 and 0·6 mol CO_2 m^{-2} s^{-1} as indicated. The dashed line is the response curve reported by Sinclair and Horie (1989).

et al., 1992; Lauteri *et al.*, 1997; Hanba *et al.*, 2003). This co-ordination may explain the high correlation between g_s and P_n observed at a given developmental stage.

The strong correlations between P_n and g_s are partly due to the greater variability of g_s than N. In the pot experiment, the coefficient of variance in g_s was 29, 26 and 47 % at PI, heading and 3 WAH, respectively, much larger than those in N, i.e. 11, 10 and 15 % at the respective developmental stages. Similar differences were also observed in the field experiments. g_s is determined by complex traits such as stomatal density and stomatal size. Kawamitsu *et al.* (1987) reported a large genotypic difference in stomatal density between rice cultivars (600–1400 mm^{-2}), but Maruyama and Tajima (1990) revealed that the genotypic difference in g_s was mainly due to stomatal aperture. However, little is known about how g_s as well as g_w is quantitatively determined by morphological and physiological factors, and further information on their genetic variations would be needed for genetic improvement of P_n.

In conclusion, a model was constructed to explain genotypic and ontogenetic variation of P_n based on N and g_s, using the experimental data from pot and field experiments. Assuming that variation of g_w is proportional to that of g_s, the model adequately explained the variation between genotypes, grown under different conditions. The model showed different curvilinear responses of P_n to an increase in N depending on g_s, suggesting that simultaneous improvements of both N and g_s are essential for an effective breeding of genotypes with higher P_n. Further, the model proposed here would contribute to construct the rice growth and yield simulation model as a basal photosynthesis sub-model.

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