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Modeling grain protein formation in relation to nitrogen uptake and remobilization in rice plant

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Abstract Protein concentration of grain is an important quality index of rice, and formation of grain protein largely depends on pre-anthesis nitrogen assimilation and post-anthesis nitrogen remobilization in the rice plant. The primary objective of this study was to develop a simplified process model for simulating nitrogen accumulation and remobilization in plant and protein formation in rice grains on the basis of an established rice growth model. Six field experiments, involving different years, eco-sites, varieties, nitrogen rates, and irrigation regimes, were conducted to obtain the necessary data for model building, genotypic parameter determination, and model validation. Using physiological development time (*PDT*) as general time scale of development progress and cultivar-specific grain protein concentration as genotypic parameter, the dynamic relationships of plant nitrogen accumulation and translocation to environmental and genetic factors were quantified and synthesized in the present model. The pre-anthesis nitrogen uptake rate by plant changed with the *PDT* in a negative exponential pattern, and post-anthesis nitrogen uptake rate changed with leaf area index (*LAI*) in an exponential equation. Post-anthesis nitrogen translocation rate depended on the plant nitrogen concentration and dry weight at anthesis as well as residue nitrogen concentration of plant at maturity. The nitrogen for protein synthesis in grains came from two sources: the nitrogen pre-stored in leaves, stem and sheath before anthesis and then remobilized after anthesis, and the nitrogen absorbed directly by plant after anthesis. Finally, the model was tested by using the data sets of different years, eco-sites, varieties, and N fertilization and irrigation conditions with the root mean square errors (*RMSE*) 0.22%–0.26%, indicating the general and reliable features of the model. It is hoped that by properly integrating with the

existing rice growth models, the present model can be used for predicting grain protein concentration and grain protein yield of rice under various environments and genotypes.

Keywords rice, grain protein formation, nitrogen uptake, simulation model

1 Introduction

Rice is one of the most important food crops in the world. During the past 20 years, substantial progress has been made in the area of rice growth modeling (Lin et al., 2003), and several simulation models have been developed and tested under wide conditions, such as CERES-Rice (Ritchie et al., 1987), ORYZA2000 (Bouman et al., 2001), SIMRIW (Horie et al. 1995), RCSODS (Gao et al. 1992) and Rice Grow (Meng, 2002). These simulation models comprehensively quantify the processes of growth and development in rice and their relationships with environmental factors and culturing practices. They can predict eco-physiological processes and yield formation in rice under the influences of soil nutrition and water status. Applications of these growth models have generated significant benefits in yield prediction and management decision support in rice production (Lin et al., 2003). However, none of these models quantify the dynamic processes of grain quality formation in rice, which restricts their predictability in grain quality. Since superior grain quality of rice has become a key target in current rice production (Cheng and Zhu, 1998; Huang et al., 1998; Li et al., 2005), it is of urgent importance to develop a grain quality model in rice so as to realize the dynamic prediction and management regulation in both grain quality and grain yield of rice.

Protein concentration of rice normally accounts for 5%–9% of grain dry weight, and is a key index to reflect grain quality property, especially in nutritional quality, of paddy rice. Formation of protein in rice grains is closely related to plant nitrogen status and affected by variety traits (Jiang et al., 2003; Yang et al., 2002). Leaf nitrogen status affects leaf area development and leaf photosynthesis, thus regulating grain productivity (Grindlay 1997; Hasegawa and Horie, 1996;

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Sheehy et al., 2004; Yin et al., 2000). Accumulation of nitrogen in the vegetative organs and its subsequent allocation to reproductive organs in rice are the important processes for grain protein formation (Norman et al., 1992; Sheehy et al., 2004). From the seedling stage, the rice plant starts to take up nitrogen from the soil to meet the needs of structural growth and subsequent grain protein accumulation after anthesis, and the uptake rates vary with different genotypes and environmental conditions (Dingkuhn 1996; Jiang et al., 2003; Ntanos and Koutroubas, 2002; Ying et al., 1998). From jointing to booting, a large amount of nitrogenous compounds such as amino acids and proteins are stored in the tissues of stem, sheath and leaves (Jiang et al., 2003; Ntanos and Koutroubas, 2002). Then, during the grain filling period, a large amount of nitrogen is required, and the amount of nitrogen absorbed by the plant during this period is much smaller than the demand for nitrogen accumulation in grains, so a large part of nitrogen is translocated from the vegetative organs, especially from leaf blades, in the form of amino acids to the grains for protein synthesis (Mae, 1997). These processes construct a nitrogen-flow involving absorption, storage, translocation, and synthesis of nitrogen compounds in rice plant (Dingkuhn, 1996; Huang et al., 1998). In general, a greater plant nitrogen concentration at anthesis would result in a greater nitrogen translocation to grain, thus a higher grain protein concentration in rice (Ntanos and Koutroubas, 2002).

So far, most researches have focused on the characteristics of nitrogen uptake and utilization in relation to grain yield formation and protein accumulation in rice, along with the regulating effects of nitrogen fertilization and genotypes (Huang et al., 1998; Jiang et al., 2003; Ntanos and Koutroubas, 2002; Sheehy et al., 2004; Ying et al., 1998). Yet few studies have comprehensively investigated the dynamic processes, the quantitative patterns of nitrogen accumulation, and the protein formation in rice grains. In the existing popular rice growth models such as CERES-Rice (Ritchie et al., 1987) and ORYZA2000 (Bouman et al., 2001), the nitrogen concentration and protein formation in grains was normally determined by calculating the fraction of nitrogen partitioned into grains in the final amount of nitrogen accumulated in the plant with the concept of nitrogen harvest index (*NHI*). This approach might give a reasonable prediction of grain protein concentration based on accumulation and distribution of dry matter and nitrogen in grains, but hardly explain the time-course processes of nitrogen flow inside the plant, and neglect the interaction among pre-anthesis nitrogen accumulation,

post-anthesis nitrogen uptake, and nitrogen remobilization in relation to grain nitrogen accumulation. Therefore, further study is necessary to develop a process model to simulate nitrogen flow in plants and to predict protein formation in rice grains under diverse conditions.

The objectives of this study are to quantify the dynamic relationships of plant nitrogen accumulation and remobilization to environmental and genetic factors in rice, and to develop a simplified nitrogen flow process-based model for predicting grain protein formation in rice, by using the data obtained from six field experiments. This work would provide a quantitative tool to support dynamic prediction and then cultural regulation of grain protein index in rice production.

2 Materials and methods

2.1 Experiment description

In total, there were six different field experiments for this study, involving different eco-sites, rice varieties, nitrogen rates, and irrigation regimes, in three years from 2001 to 2003, and the various treatments are briefly summarized in Table 1. For all the experiments, the daily climatic data including maximum and minimum temperatures, precipitation, and radiation were obtained from the weather stations installed in the experiment sites.

2.1.1 Experiments No. 1 and No. 2

Experiments No. 1 and No. 2 were two duplicated experiments conducted in 2001 and 2002, consisting of different varieties and eco-sites in three countries. In 2001 there were four eco-sites, including Iwate (39°91'N), Shimane (35°51'N) and Kyoto (35°N) of Japan, and Nanjing (32°03'N) of China, and in 2002 there were five eco-sites, including Iwate (39°91'N) and Shimane (35°51'N) of Japan, Nanjing (32°03'N) and Lijiang (26°N) of China, and Changmai (18°45'N) of Thailand. In both years, nine varieties were provided by Kyoto University of Japan, including five Indica types: Takanari (TAK), IR72 (IR72), Sankeiso (SK), Ch86 (Ch86) and IR65564-44-2-2 (IR65), and four Japonica types: Nipponbare (NIP), Takenari (TEN), Banten (BA), and WAB450-1-B-P-38-HB (WA).

Table 1 Basic conditions in six different field experiments

Experiment	Year	Site	Treatment	Number of levels	Number of cultivars
1	2001	China, Japan, Thailand	Varieties and sites	9 × 6	9
2	2002	China, Japan, Thailand	Varieties and sites	9 × 6	9
3	2002	Jiangsu of China	Varieties and sites	10 × 8	10
4	2002	Nanjing of China	Nitrogen rates and irrigation regimes	5 × 2	1
5	2003	Nanjing of China	Nitrogen rates and irrigation regimes	5 × 2	1
6	2003	Nanjing of China	Nitrogen rates and varieties	4 × 4	4

Both experiments were in a two-factorial randomized complete block design with three replications and plot size of 15 m². The sowing and transplanting dates were April 12 and May 15 at Iwate, April 17 and May 16 at Shimane, April 30 and May 22 at Kyoto, May 11 and June 15 at Nanjing, April 7 and May 5 at Lijiang, and June 24 and July 22 at Changmai, respectively. The application rates of N, P₂O₅, K₂O were all 120 kg/hm², row and hill spacing was 30 cm × 15 cm, with two plants per hill. Other routine management followed the local standard practices. After harvest, the grain protein concentrations of all plots were measured according to the NY 147–88 rice quality protocol established by the Ministry of Agriculture of China (1988), which was determined as the product of grain nitrogen concentration and conversion coefficient of 5.95.

2.1.2 Experiment No. 3

Experiment No. 3 was carried out in 2002 with different varieties and locations within Jiangsu province of China. There were eight eco-sites including Kunshan (31°31'N), Lishui (31°57'N), Jingtian (31°73'N), Danyang (31°94'N), Rudong (32°36'N), Baoying (33°22'N), Sihong (33°42'N), and Huaiyin (33°66'N), and 10 varieties including five Indica types: Changyou 1, Sidao 10, Yangdao 6, Fengyouxiangzhan, and Shanyou 63, and five Japonica types: Wuyujing 7, Wuyujing 3, Lianjing 3, Guanglinxiangjing, and Zaofeng 9. Each site was in a randomized complete block design with three replications and plot size of 20 m². The sowing and transplanting dates were May 10 and June 12, respectively, and row and hill spacing was 30 cm × 15 cm, with two plants per hill. The application rates of N, P₂O₅, K₂O were all 150 kg/hm², and other routine management followed the local standard practices. After harvest, the grain protein concentrations of all plots were measured with the protocol as mentioned in Experiment No. 1.

2.1.3 Experiments No. 4 and No. 5

Experiments No. 4 and No. 5 were two similar experiments conducted at the experiment station of Nanjing Agricultural University in 2002 and 2003. The treatments included combinations of different nitrogen application rates and irrigation regimes, using the variety of Wuxiangjing 9 (Japonica). There were five nitrogen rates of 0, 75, 150, 225 and 300 kg/hm² and two irrigation regimes as shallow water irrigation and intermittent irrigation. The experiments were in a randomized complete block design with three replications and plot size of 20 m². The sowing and transplanting dates were May 10 and June 12, respectively, and row and hill spacing was 30 cm × 15 cm, with two plants per hill. The application rates of P₂O₅ and K₂O were all 150 kg/hm², and other routine management followed the local standard practices. After harvest, the grain protein concentrations of all plots were measured with the protocol as mentioned in Experiment No. 1.

2.1.4 Experiment No. 6

Experiment No. 6 was conducted at the experiment station of Nanjing Agricultural University in 2003, with nitrogen rates and varieties as experiment factors. There were four Japonica varieties including Wuxiangjing 9, Nipponbare, Yangdao 6, and Takanari and four nitrogen rates of 0, 90, 180, and 270 kg/hm². The experiment was in a randomized complete block design with three replications and a plot size of 20 m². The sowing and transplanting dates were May 11 and June 12, respectively, and row and hill spacing was 30 cm × 15 cm, with two plants per hill. The application rates of P₂O₅ and K₂O were all 150 kg/hm², and other routine management followed the local standard practices.

During the experimental period, the plants were sampled at the stages of tiller, panicle initiation, heading, filling, and maturity, respectively, and panicles were sampled every six days after anthesis. Green leaf blade area was measured with the CI-203 (CID, Vancouver, WA) area/meter. Above-ground population dry matter was weighed after oven-dried at 70°C to constant weight. Total nitrogen concentrations of plants were determined with semi-micro Kjeldahl method. The grain protein concentrations were measured with the protocol as mentioned in Experiment No. 1.

3 Data analysis and utilization

By analyzing the detailed data from Experiment No. 6 as well as the recent literatures on plant nitrogen flow and grain protein formation in rice (Huang et al., 1998; Jiang et al., 2003; Ntanos and Koutroubas, 2002; Piao et al., 2003; Ying et al., 1998), the dynamic processes of nitrogen uptake and translocation in rice plant were comprehensively characterized to construct the conceptual model and logical relationships. On the basis of the rice growth model (RiceGrow) established in the authors' laboratory (Meng, 2002; Meng et al., 2003; Meng et al., 2004), and using physiological development time (PDT) as general time scale of development progress (Cao and Moss, 1997; Meng et al., 2003), the fundamental functions and algorithms were formulated for quantitative description of the processes involved in plant nitrogen dynamics and grain protein accumulation in rice.

Then, cultivar-specific genotypic parameter as grain protein concentration was estimated for the different cultivars used in the experiments, by using the data from different eco-sites and varieties in Experiment No. 1 and from four eco-sites of Lishui, Rudong, Baoying, and Huaiying in Experiment No. 3.

Finally, the data from Experiments No. 2, No. 4, and No. 5, and from the other four eco-sites of Kunshan, Danyang, Jingtian, and Sihong in Experiment No. 3 were used for testing the model performance under different conditions. The root mean square errors (RMSEs) between the observed and predicted values were calculated to indicate the accuracy of model simulation under the tested circumstances (Bouman

et al., 2001; Cao and Luo, 2003; 1997), along with 1:1 plotting of the observation against the prediction for displaying the goodness of fit.

4 Model description

The nitrogen required for protein synthesis in rice grains is largely from remobilization of nitrogen accumulated in the vegetative organs before anthesis and uptake of nitrogen from the soil by plant after anthesis. Therefore, simulation on the dynamic of grain protein formation in rice must be based on a comprehensive analysis and quantitative description of the processes involved in nitrogen uptake, accumulation, remobilization, and utilization in plant.

5 Pre-anthesis nitrogen uptake

The nitrogen for growth of new plant organs in rice is primarily from both nitrogen uptake by the roots and nitrogen remobilization from the aging organs (Huang et al., 1998; Peng and Gassman, 1998; Su et al., 2001). Yet all nitrogen accumulated in the rice plant is originally from the soil. In the present study, from germination to anthesis, the rate of nitrogen uptake or accumulation in the rice plant changed in a Logistic curve and genotypic differences were becoming more obvious with the progress of development (Fig. 1). The PDT was a useful parameter to represent development progress (Cao and Luo, 2003; Cao and Moss 1997; Meng et al., 2003), so the general time-course pattern of nitrogen uptake rate before anthesis in rice was described in a negative exponential model, as described in Equation (1).

$$PNURPB_{(PDT)} = MNURP \left(1 - \exp \left(\frac{-INURP \times PDT}{MNURP} \right) \right) \quad (1)$$

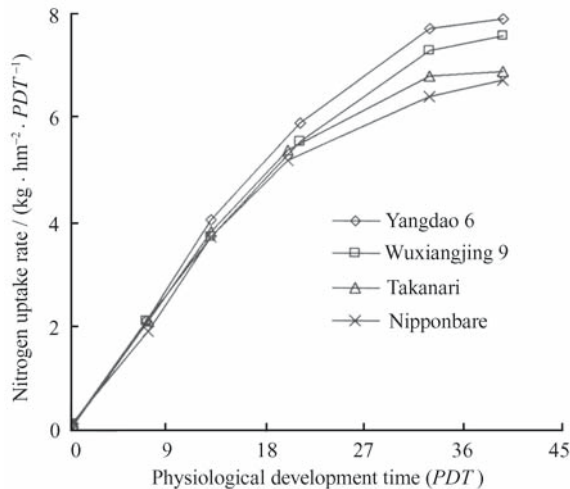


Fig. 1 Relationship between nitrogen uptake rate before anthesis and PDT in different rice genotypes

In Equation 1, PDT is physiological development time, shortly defined as development time under the optimum conditions (Cao and Luo, 2003; Cao and Moss 1997), obtained from the phasic development module of the RiceGrow model as detailed by Meng (2002) and Meng et al., (2003); $PNURPB_{(PDT)}$ is potential nitrogen uptake rate/unit PDT at PDT time ($\text{kg} \cdot \text{hm}^{-2} \cdot \text{PDT}^{-1}$) before anthesis; $INURP$ is the initial nitrogen uptake rate or accumulation rate/unit PDT ($\text{kg} \cdot \text{hm}^{-2} \cdot \text{PDT}^{-1}$), defined as the product of grain nitrogen concentration and sowing rate (Eq. (2)); $MNURP$ is the maximum nitrogen uptake rate/unit PDT ($\text{kg} \cdot \text{hm}^{-2} \cdot \text{PDT}^{-1}$) calculated from Equation (3),

$$INURP = PC \times SR / 5.95 \quad (2)$$

$$MNURP = NCM \times DWAP \quad (3)$$

in which PC (%) is the cultivar-specific grain protein concentration, i.e., genotypic parameter, normally ranging 5%–9%; 5.95 is the conversion coefficient from nitrogen concentration to protein concentration in grains; SR (kg/hm^2) is sowing rate, provided by users; NCM (%) is the maximum nitrogen concentration in plant, with a range of 3%–4% (Jiang et al., 2003; Piao et al., 2003; Sheehy et al., 1998; Wang, 1994), and is set as the average of 3.5% in this model; $DWAP$ ($\text{kg} \cdot \text{hm}^{-2} \cdot \text{PDT}^{-1}$) is dry matter increment/unit PDT under the maximum leaf area index (LAI), with the value from the biomass production module in the RiceGrow model (Meng, 2002; Meng et al., 2004).

Then potential nitrogen uptake rate/day before anthesis ($PNURDB_{(i)}$, $\text{kg} \cdot \text{hm}^{-2} \cdot \text{day}^{-1}$) can be calculated from Eq. (4), in which $\Delta PDT_{(i)}$ is increment of physiological development time on day i , obtained from the development module of the RiceGrow model (Meng, 2002; Meng et al., 2003); $PDT_{(i)}$ is the accumulated physiological development time from sowing date till day i ; and $PNURPB_{(PDT_{(i)})}$ is potential nitrogen uptake rate per unit PDT at $PDT_{(i)}$ before anthesis.

$$PNURDB_{(i)} = PNURPB_{(PDT_{(i)})} \times \Delta PDT_{(i)} \quad (4)$$

Under the actual growing conditions, the nitrogen uptake by plant before anthesis is affected by the nitrogen status and water supply in the soil, and thus the actual nitrogen uptake rate can be calculated from Equations (5) and (6),

$$ANURDB_{(i)} = PNURDB_{(i)} \times \min(FN_{(i)}, FW_{(i)}) \quad (5)$$

$$ANAB_{(i)} = \sum_{i=0}^n ANURDB_{(i)} \quad (6)$$

in which $ANAB_{(i)}$ and $ANURDB_{(i)}$ are actual amounts of total nitrogen accumulation and daily nitrogen uptake, respectively, on day i (kg/hm^2) before anthesis; $FN_{(i)}$ and $FW_{(i)}$ are nitrogen and water deficit factors on day i , calculated from the nutrient balance module (Zhuang, 2001; Zhuang et al., 2004) and water balance module in soil-crop system (Hu, 2002; Hu et al., 2004), respectively.

6 Post-anthesis nitrogen uptake

After anthesis, rice plant continues to absorb a certain amount of soil nitrogen for protein synthesis in grains. Analysis on the present experiment data indicated that the nitrogen uptake rate/unit PDT from the soil by plant after anthesis was exponentially related to leaf area index during this period (Fig. 2), and positively related to capacity of plant nitrogen accumulation, which could be expressed in Equation (7).

$$ANURPA_{(PDT)} = \Delta DW_{if} \times (NC_{if} - NCT) \times (0.02 \times \exp(0.8482 \times LAI_{(PDT)})) \quad (7)$$

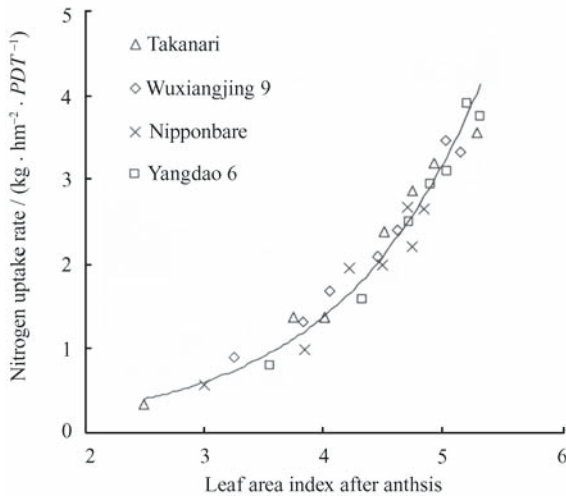


Fig. 2 Relationship between nitrogen uptake rate and leaf area index after anthesis in rice

In the equation, $ANURPA_{(PDT)}$ ($\text{kg} \cdot \text{hm}^{-2} \cdot \text{PDT}^{-1}$) is actual nitrogen uptake rate/unit PDT at PDT time after anthesis; ΔDW_{if} ($\text{kg} \cdot \text{hm}^{-2} \cdot \text{PDT}^{-1}$) is increment/unit PDT of above-ground population dry matter at the initial filling, obtained from the biomass production module in the RiceGrow model (Meng, 2002; Meng et al., 2004); NC_{if} (%) is the plant nitrogen concentration at the initial filling, obtained from Eq. (8); NCT (%) is minimum nitrogen concentration that could be translocated in plants, and is set at about $0.55 NC_{if}$ (Jiang et al., 2003; Sheehy et al., 1998; Wang, 1994); $LAI_{(PDT)}$ is the leaf area index at PDT time during grain filling, obtained from the biomass production module in the RiceGrow model (Meng, 2002; Meng et al., 2004).

$$NC_{if} = ANAB_{if} / DW_{if} \quad (8)$$

$$NCT = 0.55 NC_{if} \quad (9)$$

In Eq. (8), $ANAB_{if}$ (kg/hm^2) is the amount of plant nitrogen accumulation (Eq. (6)) at initial filling; DW_{if} (kg/hm^2) is above-ground dry weight at initial filling, obtained from the biomass production module in the RiceGrow model (Meng, 2002; Meng et al., 2004).

Then actual nitrogen uptake rate/day on day i after anthesis ($ANURDA_{(i)}$, $\text{kg} \cdot \text{hm}^{-2} \cdot \text{day}^{-1}$) can be calculated from Equation (10),

$$ANURDA_{(i)} = ANURPA_{(PDT(i))} \times \Delta PDT_{(i)} \quad (10)$$

in which $\Delta PDT_{(i)}$ is the increment of physiological development time on day i , obtained from the development module of the RiceGrow model (Meng, 2002; Meng et al., 2003); $PDT_{(i)}$ is the accumulated physiological development time from sowing date till day i ; and $ANURPA_{(PDT(i))}$ is the actual nitrogen uptake rate/unit PDT at $PDT_{(i)}$ after anthesis (Equation (7)).

7 Post-anthesis nitrogen remobilization

After anthesis, grain growth starts and requires large amount of nitrogen for protein synthesis. During this period, almost all nitrogen absorbed by plants is translocated into grains, and meanwhile the nitrogenous compounds including amino acids stored in plant organs before anthesis are also remobilized to grains when the amount of N absorbed by the plants is smaller than the demand for N accumulation in grains (Dingkuhn, 1996; Huang et al., 1998; Mae, 1997; Ntanos and Koutroubas, 2002). For the purpose of simplification, the nitrogenous compounds stored in plant organs before anthesis are all considered as the transferable nitrogen, and move to grains in a simple process of remobilization.

The nitrogen translocation rate from vegetative organs to grains is used to represent the daily remobilization intensity of the transferable nitrogen stored in plant before anthesis. An integrated analysis on the present experiment data and literature (Ntanos and Koutroubas, 2002; Peng and Cassman, 1998; Ying et al., 1998) indicated that the nitrogen translocation rate to grain after anthesis exhibited a dynamic changing pattern of slow-fast-slow with the progress of grain filling (Fig. 3), and the value largely depended on plant nitrogen

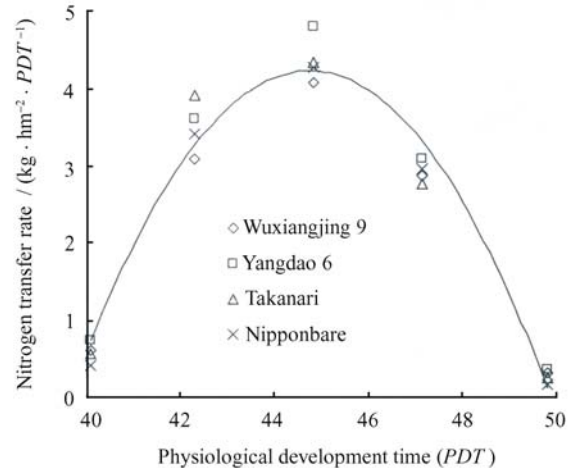


Fig. 3 Relationship between nitrogen translocation rate after anthesis and PDT in rice

accumulation at anthesis as well as amount of residue nitrogen in plant at maturity, as formulated in Equation (11).

$$ANTRP_{(PDT)} = (ANAB_{if} - NCT \times DW_m) \times (-0.0015 \times PDT^2 + 0.138 \times PDT - 3.06) \quad (11)$$

In Eq. (11), $ANTRP_{(PDT)}$ ($\text{kg} \cdot \text{hm}^{-2} \cdot \text{PDT}^{-1}$) is the actual nitrogen translocation rate/unit PDT at PDT time; $ANAB_{if}$ (kg/hm^2) is amount of plant nitrogen accumulation (Equation 6) at initial filling; NCT (%) is the minimum transferable nitrogen concentration in plant (Eq. (9)); DW_m (kg/hm^2) is above-ground dry weight (kg/hm^2) at maturity, obtained from the biomass production module in the RiceGrow model (Meng, 2002; Meng et al., 2004); PDT is physiological development time, obtained from the development module of the RiceGrow model (Meng, 2002; Meng et al., 2003).

Then actual nitrogen translocation rate/day on day i ($ANTRD_{(i)}$, $\text{kg} \cdot \text{hm}^{-2} \cdot \text{day}^{-1}$) can be calculated from Equation (12),

$$ANTRD_{(i)} = ANTRP_{(PDT_{(i)})} \times \Delta PDT_{(i)} \quad (12)$$

where $\Delta PDT_{(i)}$ is the increment of physiological development time on day i , obtained from the development module of the RiceGrow model (Meng, 2002; Meng et al., 2003); $PDT_{(i)}$ is the accumulated physiological development time from sowing date till day i ; and $ANTRP_{(PDT_{(i)})}$ is the actual nitrogen translocation rate/unit PDT at $PDT_{(i)}$ (Equation (11)).

8 Grain protein accumulation

On the base of the data from the present experiments as well as literatures (Huang et al., 1998; Jiang et al., 2003; Ntanos and Koutroubas, 2002; Wei et al., 2002), during grain development in rice, the pattern of grain protein accumulation is essentially consistent among different cultivars. In general, the protein synthesis in grain starts on the fifth day after anthesis (40 PDT) till physiological maturity (57 PDT). During this period, the nitrogen substrate for protein synthesis in rice grains is from two sources. One source is from the remobilization of the nitrogenous compounds stored in the leaf, sheath and stem before anthesis, which occurs from initial filling (40 PDT) to late filling (50 PDT), accounting for about 70% of the total nitrogen in grains. The other source is from nitrogen uptake by plant roots from initial filling (40 PDT) to physiological maturity (57 PDT), accounting for 30% of total nitrogen in grains. In addition, the process of protein formation is influenced by air temperature (T).

Taking all considerations, the following algorithm can be formulated for simulating the dynamic process of protein formation in rice grains.

$$GPA_{(i)} = 5.95 \times NAG_{(i)} \quad (13)$$

$$NAG_{(i)} = \begin{cases} \sum_{t=0}^k ((ANURDA_{(t)} + ANTRD_{(t)}) \times FT_{(t)}) & 40 < PDT_{(i)} \leq 50 \\ \sum_{t=k}^n (ANURDA_{(t)} \times FT_{(t)}) & 50 < PDT_{(i)} \leq 57 \end{cases} \quad (14)$$

In Eq. (13) and (14), $GPA_{(i)}$ (kg/hm^2) is amount of protein accumulation in grains on day i ; $NAG_{(i)}$ (kg/hm^2) is amount of nitrogen accumulation in grain on day i ; 5.95 is conversion coefficient from nitrogen to protein; $ANURDA_{(i)}$ ($\text{kg} \cdot \text{hm}^{-2} \cdot \text{day}^{-1}$) is nitrogen uptake rate/day on day i after anthesis, calculated in Equations 7–10; $ANTRD_{(i)}$ ($\text{kg} \cdot \text{hm}^{-2} \cdot \text{day}^{-1}$) is actual nitrogen translocation rate/day on day i as described in Equation 12; $FT_{(i)}$ is temperature impact factor on day i as obtained in the following Equation (15).

The optimum temperatures for grain filling and protein formation in rice are 24–27°C, with minimum limit of 16–18°C and maximum limit of 37–40°C (Gao et al., 1992; Huang et al., 1998; Zhou et al., 1997). According to the dynamic relationship between the temperature and protein accumulation in grains, the following sine functions were derived to obtain the impact factor of temperatures ($FT_{(i)}$),

$$FT_{(i)} = \begin{cases} \sin[(T_{(i)} - T_b)/(T_{ol} - T_b) \times \pi/2] & T_b \leq T_{(i)} < T_{ol} \\ 1 & T_{ol} \leq T_{(i)} \leq T_{oh} \\ \sin[(T_m - T_{(i)})/(T_m - T_{oh}) \times \pi/2] & T_{oh} < T_{(i)} \leq T_m \\ 0 & T_m < T_{(i)}, \text{ or } T_{(i)} < T_b \end{cases} \quad (15)$$

where $T_{(i)}$ (°C) is daily mean air temperature on day i ; T_m (°C) and T_b (°C) are maximum and base temperatures for protein synthesis in grains, and are set at 40°C and 16°C, respectively; T_{ou} (°C) and T_{ol} (°C) are upper and lower limits of optimum temperature ranges for protein synthesis, and are determined to be at 25°C and 27°C, respectively, in Indica rice, and 24°C and 26°C, respectively, in Japonica rice (Bi, 1980; Zhou et al., 1997).

9 Grain protein concentration

The time-course protein concentration in rice grains can be obtained as ratio of grain protein accumulation to grain yield from day five after anthesis (40 PDT) to physiological maturity (57 PDT) as shown in Equation (16),

$$GPC_{(i)} = GPA_{(i)} / GY_{(i)} \times 100 \quad (16)$$

where $GPC_{(i)}$ is grain protein concentration on day i (%); $GPA_{(i)}$ is amount of grain protein accumulation on day i (kg/hm^2); $GY_{(i)}$ is grain yield on day i (kg/hm^2), extracted from the biomass partitioning module of the RiceGrow model (Meng, 2002; Meng et al., 2004).

10 Model test

The present model, programmed with VC++ language into a standard software component, can be incorporated into the RiceGrow simulation model as established and validated by Meng (2002). Operation of the present model requires one cultivar-specific parameter, grain protein concentration, while the other input variables can be initiated by taking the data or output items from the growth model RiceGrow. With the grain protein data obtained from the cultivar and eco-site experiment in 2001, the characteristic grain protein concentrations of the varieties TAK, IR72, SK, Ch86, IR65, NIP, TEN, BA, and WA, used in Experiments No.1 and No.2, were determined to be 6.81%, 6.14%, 6.99%, 6.34%, 7.96%, 6.82%, 6.97%, 7.45%, and 8.04%, respectively. With the experiment data from Lishui, Rudong, Baoying and Huaiying in Experiment No. 3, the characteristic grain protein concentrations for the different cultivars used in the study were estimated at 6.46%, 6.28%, 7.04%, 7.45%, and 7.92%, respectively, for Indica cultivars of Changyou 1, Sidao 10, Yangdao 6, Fengyouxiangzhan and Shanyou 63, and 6.43%, 6.5%, 7.05%, 6.65%, and 6.45%, respectively, for Japonica cultivars of Wuyujing 7, Wuyujing 3, Lianjing 3, Guanglinxiangjing, and Zaofeng 9. According to the data from Experiment No. 6, the grain protein concentration of cultivar Wuxiangjing was set at 6.51%.

With the characteristic grain protein concentrations of different cultivars, weather data and management data during the periods of the experiments, and initial soil conditions before the experiments, the present model was used to predict the grain protein concentrations of nine rice cultivars grown under five ecological environments of Experiment No. 2 in 2002. The results indicated a high goodness of fit between the simulated and observed values under the different conditions, with R^2 of 0.8763 and $RMSE$ of 0.26%. Figure 4A displays the 1:1 relationship between the simulated and observed grain protein concentrations of different cultivars under the eco-sites of Japan, Thailand, and China. In addition, the data from four eco-sites of Kunshan, Jingtian, Danyang, and Sihong in Experiment No. 3 were used to test the model performance under different conditions in Jiangsu region. Figure 4B shows the 1:1 relationship between the simulated and observed grain protein concentrations of five Indica varieties and five Japonica varieties at different experiment locations, with R^2 of 0.8417 and $RMSE$ of 0.22%. It appears that the present model can give reliable prediction of the grain protein concentrations of different rice cultivars under varied ecological environments.

Since the grain protein formation in rice is significantly affected by soil nitrogen supply and irrigation regime, the two-year data from the combined nitrogen and water treatments in Experiments No. 4 and No. 5 were used for further validation of the model. The simulation results on the grain protein concentrations indicated that the predicted values were well close to the observed values (Fig. 5), with

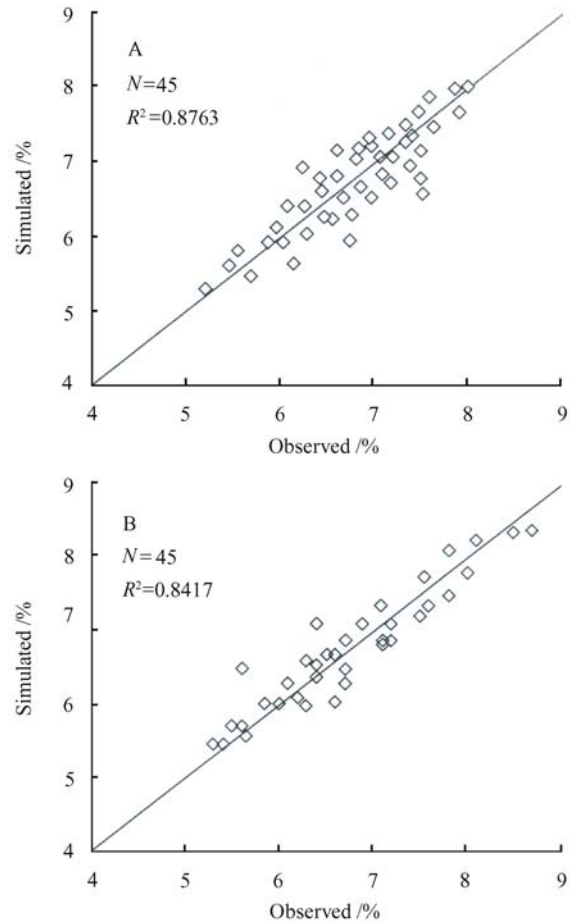


Fig. 4 Comparison of the simulated with measured grain protein concentrations of rice under different eco-environments of three countries (A) and of local areas (B), respectively

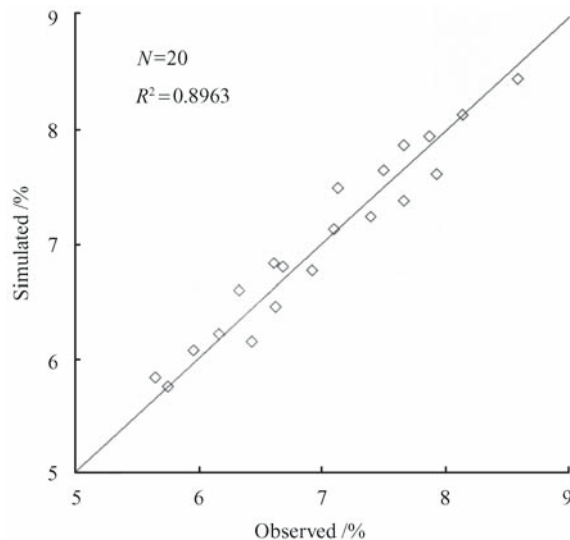


Fig. 5 Comparison of the simulated with measured grain protein concentrations of rice under different nitrogen rates and irrigation levels

R^2 of 0.8963 and $RMSE$ of 0.22%. These results on model testing indicated that the present model was accurate and applicable for prediction of grain protein concentrations under varied conditions of nitrogen fertilization and irrigation management in rice production.

11 Discussion

The nitrogen absorbed by rice plant is mainly used for structural growth and non-structural storage in plant before anthesis and grain protein accumulation after anthesis. A large amount of nitrogenous compounds in the form of amino acid or protein is stored in the stem, sheath and leaves before anthesis, and then remobilized in the form of amino acid to grain for protein synthesis after anthesis, forming a dynamic nitrogen flow involving the processes of uptake, storage, remobilization and synthesis of nitrogen compounds (Dingkuhn, 1996; Huang et al., 1998; Wei et al., 2002). So far, most of the existing studies are limited to characterizing the properties of nitrogen absorption, translocation and utilization in plant (Ntanos and Koutroubas, 2002; Peng and Cassman, 1998; Ying et al., 1998; Sheehy et al., 2004), and protein accumulation and concentration in grains of rice (Jiang et al., 2003; Su et al., 2001). Yet, there is a lack of information about quantitative representation of nitrogen flow dynamic in plant and protein formation in grains of rice. Based on simplified processes of nitrogen accumulation and translocation in rice plant and following the main line of the *PDT*, this research established a general simulation model of protein formation in rice grains. Although incorporated some empirical relationships, the model exhibits reasonable explanation and practical predictability, and thus lays a foundation for quantitatively predicting the dynamic changes of the grain protein concentration and protein yield of different rice cultivars under various conditions of ecological environments and production managements. This work can be considered as a further expansion and refinement of the existing growth and yield simulation models of rice (Bouman et al., 2001; Gao et al., 1992; Horie et al., 1995; Meng, 2002; Ritchie et al., 1987), and is of useful values for protein yield prediction, quality eco-zoning, and management decision support in rice production.

The present model of grain protein formation in rice includes different parameters and variables in its algorithms. Among them, the grain protein concentration of a specific cultivar is a required input as genotypic parameter, whereas other variables are initiated from the output values of or shared with an established rice growth model (RiceGrow) in the authors' laboratory (Hu, 2002; Meng, 2002; Zhuang, 2001), including the physiological development time from development module, above-ground dry weight and leaf area index from organ growth module, and nitrogen and water deficit factors from environment module. This implies that the present model of grain protein formation is based on the operation of growth simulation model, although it constructs the following processes of nitrogen flow dynamic in plants.

Thus, by linking with available rice growth simulation systems such as the RiceGrow (Meng, 2002; Meng et al., 2004) or other proper simulation systems, it is possible to realize simultaneous prediction of plant growth dynamic, grain yield formation, and grain protein accumulation in rice. Overall, the present model itself is of unique features such as low parameter requirement, high integration, and strong applicability.

The model was well tested with different data sets of field experiments involving the factors of eco-sites, cultivars, fertilization and irrigation. The validation results on grain protein concentrations indicated that the overall performance of the model was reliable and accurate under different conditions. Yet, in the experiments of cultivars and locations, there was no relatively large deviation of prediction among the cultivars at few sites (Fig. 4). Besides the experimental errors, the results suggest that these cultivars are relatively sensitive to the specific environmental conditions of the experiment regions, which needs further quantitative representation. Overall, the present model is only a preliminary effort on predicting grain protein formation in rice, and thus needs a wide testing under diverse circumstances and constant improvement in the future research. In addition, with better understanding and data accumulation in quality physiology and ecology in rice, it is necessary to elucidate the dynamic patterns and inter-relationships on formation of different protein components in rice grains including albumin, globulin, gliadin, and glutenin. This will lead to building a more comprehensive simulation model on formation dynamics of protein components in rice grains by expanding the present model of total protein accumulation.

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