Spatial Distribution of Leaf Area Index and Leaf N Content in Relation to Grain Yield and Nitrogen Uptake in Rice

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Abstract: Exploring approaches to optimizing spatial distributions of leaf area index (LAI) and leaf nitrogen content (LNC) should be useful for increasing grain yield in rice (Oryza sativa). The primary objective of this study was to characterize the variation of LAI and LNC distributions within the canopy in relation to grain yield and N uptake in rice. Two experiments with different N fertilization rates under conventional and intermittent irrigation (CI and II, respectively) were conducted in 2002 and 2003, using Japonica rice cultivar Wuyujing9. The results showed that grain yield and N uptake were significantly different among application rates of N (N rates), but did not differ between CI and II. LAI distribution at full heading was affected significantly by N rate but hardly by the irrigation method. Individual LAI increased with the N rate. To achieve a high yield, the proper distribution of LAI in the canopy can be designed as the largest in the 2nd leaf from the top, followed by the 3rd and 4th leaves, and the smallest in the top leaf. LNC on the base of both area and dry matter at 15 days after full heading decreased from the top to lower leaves in the canopy, and significantly increased with the N rate. Grain yield was enhanced linearly with the increasing N content of the upper two leaves, but hindered by the high N content of lower leaves. These results indicate that the spatial distributions of both LNC and LAI could be optimized to achieve maximum canopy photosynthesis and grain yield in rice.

Key words: Grain yield, Leaf area index, Leaf N content, N uptake, Spatial distribution.

Rice yield must be improved by 43% in 30 years from 2000 to meet the demands of population growth in the world (Cassman, 1999). Increasing canopy photosynthesis facilitates the increase of grain yield in rice. Optimization of leaf N distribution associated with LAI distribution in rice canopy increased canopy photosynthesis by more than 20% during grain filling (Goudriaan, 1995; Shiratsuchi et al., 2006), when most of the grain dry matter is normally produced (Cock and Yoshida, 1972; Murata and Matsushima, 1975; Tsunoda, 1978; Yoshida, 1984). Thus improving distributions of leaf area index (LAI) and leaf nitrogen content (LNC) in the canopy is possibly a desirable way to increase yield in rice (Goudriaan, 1995; Yin et al., 2000; Shiratsuchi et al., 2006), instead of high nitrogen fertilizer input and much water consumption (Guerra et al., 1998; Bouman and Tuong, 2001), which led to the lower nitrogen use efficiency than the present rice production situation in China (Xing and Zhu, 2000; Zhu et al., 2000; Jaynes et al., 2001; Peng et al., 2002).

Recently, the site-specific nitrogen fertilizer management in rice was introduced to irrigated rice production, based on the leaf N status with leaf colour chart (LCC), and fertilizing according to LCC to increase N use efficiency as well as high yield (Wang et al., 2001; Alam et al., 2006). More convenient methods and portable spectral meters have been developed to monitor leaf colour. For example, the specific spectral bands were identified to monitor LNC and LAI (Peng et al., 1996; Xue et al., 2004). The hypothesis was that the LAI and LNC of individual leaves could be predicted by the portable spectral meters (Such as SPAD502, LCC, Sunscan system), and from which grain yield and N uptake were deduced. Proper function and accurate use of these portable meters rely on characterizing the distribution in the canopy of LAI and LNC. This would help to detect N status in rice plants and predict the end harvest by a non-destructive method on the basis of crop physiological processes. However, few investigators have explored the spatial variations of LAI and LNC within canopy in relation to the end yield and N uptake in rice under different N rates and water supplies.

Therefore, we did two field experiments in rice with five nitrogen rates under two water regimes, i.e. conventional irrigation (CI) and intermittent irrigation (II). The objectives of the study were: (1) to characterize the grain yield and N uptake, and spatial distributions of LAI and LNC of rice under various N fertilization and under CI and II; (2) to quantify the relationships of the yield and N uptake with the distributions of LAI and LNC within the rice canopy.
Materials and Methods

1. Location

The experiment was carried out at the experiment station of Nanjing Agricultural University (32°04′ N, 118°48′ E) China in 2002 and 2003. The soil type is a clay loam with 1.15 g kg⁻¹ total N, 23.9 mg kg⁻¹ available P, 95.7 mg kg⁻¹ available K, and 20.5 g kg⁻¹ organic matter.

2. Experimental design

The experiment was a two-factor split plot design in both years. The main factor of water management consisted of two treatments, i.e., CI and II. CI aimed at keeping the fields continuously submerged with a water level of 2 to 5 cm. Soil water content in II was maintained between about 85% and saturated conditions by drainage or irrigation, monitored by frequent soil sampling and water content measurements. Under both CI and II, nitrogen fertilizer (urea) was applied at five rates, i.e., 0, 75, 150, 225 and 300 kg N ha⁻¹, when 60% was applied before transplanting, and another 20% at 15 days after jointing, and the remaining 20% at 25 days after jointing. In addition to nitrogen, 135 kg P₂O₅ ha⁻¹ and 210 kg K₂O ha⁻¹ were applied as basal fertilizer to all plots.

Plot size was 10 × 3 m² with three replications for each treatment. Each plot was separated by plastic sheet up to 30 cm below and above the soil surface to reduce water and nitrogen flows between adjacent plots as much as possible. The soil was plowed and puddled before transplanting.

Japonica rice cultivar Wuyujing9 was used in the study. In 2002, rice was sown on May 11 and on June 15 six-leaf seedlings were transplanted, one seeding per hill, with 25 and 15 cm spacing between hills and rows, respectively. In 2003, seeds were sown on May 15 and transplanted on June 15. In both years, fields were kept free from weeds, pests and diseases by applying herbicides and pesticides when needed following the local standard procedures.

3. Measurements

(1) Dry matter

Eight hills of plants were sampled at full heading, 15 days after full heading, and at maturity. Four hills of plants were separated into leaves, culms and leaf sheaths, dead and yellow leaves, and panicles. The leaves were separated according to each nodal position. The remaining samples were used to measure whole plant dry weight. All samples were oven-killed at 105°C for 1 hr, and then dried at 80°C till constant
weight. At maturity, plants from 3 m² of each plot were harvested manually to determine the yield on the basis of 14% moisture content. The ripening percentage was determined from the number of filled grains per spikelet, in a NaCl solution with a specific gravity of 1.03.

(2) Leaf area and LAI
The area of green leaf at each nodal position was measured.
measured using a CI-203 Portable Laser Area Meter (CID Inc., Washington, USA) at full heading, and LAI was calculated for individual leaf.

(3) Nitrogen content
After drying, the samples were weighed and grounded. Nitrogen contents of plant were determined by the micro-Kjeldahl method (AOAC, 1984), following digestion in a $\text{H}_2\text{SO}_4 - \text{H}_2\text{O}_2$ solution. N uptake at maturity was shown as total N absorbed into shoot. Leaf nitrogen content on the basis of leaf area at 15 days after full heading was calculated from the N content on the basis of weight and leaf area.

(4) Weather data
Weather data including daily radiation, precipitation, maximum and minimum temperatures were monitored by the weather stations installed at the experimental site.

Results and Discussions

1. Weather
The weather conditions during rice growth periods in 2002 and 2003 were different although the temperature variation showed a similar pattern (Fig. 1). The total precipitation in 2003 was one and a half times greater than that in 2002 over the growing period of rice crop. The frequent and large rainfall in 2003 resulted in relatively less radiation in 2003 than in 2002, especially 20% lower after full heading.

2. Yield, yield component and N uptake
Grain yield and N uptake at maturity were consistently lower in 2003 than 2002 (Fig. 2), and this yearly difference could be caused by lower radiation during rice ripening in 2003, when most biomass of grain yield was produced (Cock and Yoshida, 1972; Murata and Matsushima, 1975). The interactions between years and irrigation method, and between years and the rate of N application (N rates) were basically not significant (Table 1), and thus the trends of yield and N uptake under CI and II and N rate were similar in the two years.

The difference in yield was significant under different N rates, but not between CI and II (Table 1), which is consistent with the previous studies (Bouman and Tuong, 2001; Belder et al., 2004). Grain yield initially increased with increasing N rate, and then decreased at a high N rate (Fig. 2). The number of spikelets and ripening percentage were both significantly affected by the N rate while no difference between CI and II (Table 1). The number of spikelets increased with increasing N rate, indicating that the sink enlarged by N fertilization, whereas ripening percentage decreased with increasing N rate, from 74 percent at 0 kg N ha$^{-1}$ to 61 percent at 300 kg N ha$^{-1}$.

![Fig. 4. Cumulative LAI from the top to 4th leaf at full heading positions in rice canopy. Open symbols are the data from 2002, and closed symbols from 2003. Triangle symbols under conventional irrigation (CI), and rectangle symbols are under intermittent irrigation (II). The equations are from the regressive linear relationships between cumulative LAI and leaf position, for the highest and lowest values.](image-url)
This inverse pattern between the number of spikelets and ripening percentage is consistent with the previous report by Horie et al. (1997). The general low ripening percentage suggested a source limitation for grain filling, which might have resulted from the insufficient assimilation supply during ripening since most biomass of grain yield was produced after flowering (Cock and Yoshida, 1972; Murata and Matsushima, 1975).

Similar to the yield, N uptake increased significantly in both years with N rates. Statistical analysis showed no difference in N uptake between CI and II (Table 1). Nevertheless, N uptake at a low N rate was higher under CI than II, but vice versa at a high N level (Fig. 2), indicating that the water regime affected N uptake through the differential microbe-mediated soil N-transformations (nitrification and denitrification) (Li et al., 2001). In our experiments under II the soil conditions converted from aerobic to anaerobic and vice versa, while under CI the fields were always under anaerobic conditions. The grain yield increased linearly from the initial N uptake, and then leveled off or even decreased at a high N uptake. This may be because rice yield was mainly driven by the amount of N uptake required (Witt et al., 1999), and levelled off or even decreased when N was not a limiting factor of rice growth (van Keulen, 1982).

3. LAI

LAI at full heading increased with increasing N rate (Fig. 3). LAI normally reached maximum value and thereafter decreased (Sharma and Singh, 1999). The vertical distribution of LAI along canopy fitted the polynomial curves, i.e., the leaf area was the smallest in the 1\textsuperscript{st} leaf from the top, the largest in the 2\textsuperscript{nd} leaf and gradually decreased in the lower leaves (Fig. 3). The spatial distribution of LAI was affected by N rate in this study. LAI of lower leaves in the canopy decreased dramatically at 0 and 300 kg N ha\textsuperscript{-1} while at 150 kg N ha\textsuperscript{-1} LAI remained more stable in the lower leaves.

Cumulative LAI from the 1\textsuperscript{st} to 4\textsuperscript{th} leaves from the top increased linearly irrespective of N rate or irrigation method, and all the lines regressed to the origin (Fig. 4). This suggested that cumulative LAI from the top to the 4\textsuperscript{th} leaf could be quantified by regression line. Accumulation of LAI increased linearly from the top to lower leaves and thus lower leaves were shaded. The optimum distribution of LAI for light interception is that all the green leaves in the canopy receive as much light as possible (Goudriaan, 1995), and shading by upper leaves should be minimized by proper reduction of the leaf angle and LAI of upper leaves. However, leaf angle is mainly determined genetically, while LAI distribution can be significantly affected by fertilizer management. For example, a better LAI distribution was obtained at fertilizer rate of 150 kg N ha\textsuperscript{-1} which gave a high yield in this study.

4. LNC

LNC on the basis of both area and dry matter at 15 days after full heading decreased in the canopy from the top to lower leaves (Fig. 5), which is consistent with the report of Shiratsuchi et al. (2006), and the previous studies with other crop species (Field, 1983; Hirose and Werger, 1987; Pons et al., 1989). Yin et al. (2000) described the LNC distribution from the top to basal leaves using an exponential equation, and indicated that the leaves at lower layers senesced when the LNC was lower than the minimum LNC for leaf photosynthesis. LNC increased significantly with increasing N rate. No significant difference in LNC was found between CI and II with 150 kg N ha\textsuperscript{-1} under conventional irrigation, respectively, and II, II150 and II300 show 0, 150 and 300 kg N ha\textsuperscript{-1} under intermittent irrigation, respectively.
unit leaf area at 15 days after full heading from the 2nd leaf from the top was significantly larger under CI than under II, which is consistent with the N uptake under the two irrigation systems (Table 1). This indicates that water management affected the distribution of LNC. Thus, N rates and irrigation method could regulate the spatial distribution of LNC within the canopy, and an optimum distribution of LNC may maximize canopy photosynthesis as proposed in the previous studies (Gimenez et al., 1994; Anten et al., 1995; Connor et al., 1995; Goudriaan, 1995; Shiratsuchi et al., 2006).

5. Relationship of Yield to LAI and LNC

Grain yield increased with increasing LAI of individual leaves within rice canopy until an optimal value of LAI, and the yield decreased with a further increase in LAI (Fig. 6). We fitted the relationships between yield and LAI to two linear regression lines with the highest coefficient (Fig. 6). The optimum LAI for the yield varied with the nodal position of the leaf. In order to produce the highest yield, the 2nd leaf from the top should be the largest, followed by the 3rd and 4th leaves, and the 1st leaf (flag leaf) had the smallest area. In this study, the optimum LAI distribution of the 1st, 2nd, 3rd and the 4th at full heading was 0.90, 1.16, 1.01, and 0.92, respectively. The distribution of LAI at full heading was affected by N rate, and at 150 kg N ha⁻¹ the distribution of LAI in the canopy was close to this optimum distribution (Fig. 3).

The yield increases linearly with increasing LNC because photosynthetic rate is positively correlated with LNC in many crop species (van Keulen and Seligman, 1987; Sinclair and Horie, 1989; Xu et al., 1995), but it levelled off or even decreased with LNC exceeding 1.26 and 1.12 g m⁻² for the 3rd and 4th leaf in this study (Fig. 7), where light intensity was low and maintenance and respiration rates were high (Goudriaan, 1995). Thus the relationships between yield and LNC depended on leaf positions due to the different availability of light. The difference in the relationships between yield and LNC depending on the position in the canopy suggested that the distribution of LNC in the canopy should be improved for high canopy photosynthesis by increasing LNC of upper leaves and reducing it in lower layers. This was in agreement with a recent study by Shiratsuchi et al. (2006), who optimized the LNC for maximum canopy photosynthesis by increasing LNC in the upper leaves and reducing LNC in the lower leaves.

6. Correlation of N uptake with LAI and LNC

Fig. 8 shows the relationship between N uptake and
LAI of individual leaves at full heading stage. LAI of individual leaves at full heading were smaller in 2003 than in 2002, which is consistent with the yield. N uptake at maturity increased linearly with increasing LAI of the individual leaves at full heading. A similar linear relationship between LAI and N uptake was reported in trees by Carlyle (1998). These linear relationships suggested that N uptake at maturity could be estimated through LAI which is easily measured by non-destructive methods, e.g., Sunscan system. Fig. 9 shows that the LNC on a dry matter basis at 15 days after full heading was lower in 2003 than in 2002. In addition, there was a significant linear relationship between N uptake and leaf N content on a dry matter basis in all the leaves in the canopy, which is consistent with the previous study (Bouman et al., 2001).

**Conclusions**

Grain yield and N uptake in rice were significantly affected by the N rate, but not by the irrigation method, CI and II. Grain yield increased linearly with increasing N uptake from 0 kg N ha\(^{-1}\), and then leveled off or even decreased at higher N uptake.

The spatial distribution of LAI varied significantly with the N rate. LAI increased with increasing N rate. LNC on the bases of both area and dry matter decreased from the top to lower leaves in the canopy, and LNC of individual leaves significantly increased with increasing N rate.

The grain yield increased linearly with increasing LAI of individual leaves till an optimal value, above which yield leveled off or decreased. In this study, the optimum LAI was identified as 0.90, 1.16, 1.01, and 0.92 for the 1st, 2nd, 3rd and 4th leaf from the top, respectively. Grain yield was enhanced by increasing LNC of the upper two leaves, but hindered by a large LNC of lower leaves.

The linear relationship between N uptake and LAI of individual leaves at full heading was quantified, along with the linear relationship between N uptake and LNC on the basis of dry weight.

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Fig. 8. N uptake at maturity as functions of LAI downward stems at full heading in rice canopy. Open symbols are the data from 2002, and closed symbols are from 2003.

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