

CHANGE IN LEAF ANATOMY, TOTAL CHLOROPHYLL CONTENT, AND CANOPY REFLECTANCE OF FIELD GROWN RICE AS A FUNCTION OF NITROGEN STATUS

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KEY WORDS: Leaf internal structure, Leaf total chlorophyll, Canopy reflectance, Rice, Nitrogen application rate

ABSTRACT: Experiments were conducted to examine leaf anatomical characteristics, total chlorophyll concentration (TCC), and canopy reflectance behavior as a function of nitrogen (N) status in field grown rice cultivar Tainung 67. Results showed that applying different rates of N fertilizer, from 0 to 180 kg N ha⁻¹ with 60 kg N ha⁻¹ intervals, changed plant N status and leaf TCC measured in the panicle initiation/formation stage. Reflectance of waveband regions in visible light negatively corresponded to leaf TCC. A diversity of anatomical features was also observed in leaves from tagged plants of varied N status grown in both first and second crops. Leaf thickness increased progressively with increasing aboveground N concentration (ANC). Leaf rolling extent was relieved by the increasing ANC so that a higher value of leaf rolling index (LRI) was computed, i.e., LRI changed positively with the ANC in a linear fashion. Changes in ratio of bulliform/mesophyll to ANC were best fitted to a quadratic function, implying stronger leaf movement strength occurred in plants with higher N. In the measured range of ANC, leaf water content (LWC) also changed in a curvilinear trend and was low in plants of lower level N. Of the same ANC, plants grown in first crop had higher LWC than those plants grown in second crop. Starch granules were shown loosely distributed and packed in the parenchyma cells of mesophyll in plants of higher ANC relative to those of lower ANC ones. Different application rates of N fertilizer also affected canopy reflectance behavior. The mean reflectance of blue, green, and red regions decreased while near-infrared region increased with the increasing ANC and LRI.

1. INTRODUCTION

To increase revenues while reducing costs of farming with the current economic depression in agriculture, farmers must seek whatever possible to cut down the expenditures of production and increase yield quality and quantity. Precision agriculture, a farming system based on spatially site-specific information and tactics to resolve spatial and temporal field variability, is one way presently being pursued and explored vigorously. Techniques such as remote sensing, global positioning system, geographical information system and variable rate technology are adopted dealing with various specific purposes. Remote sensors can play a valuable role in providing time-specific and time-critical information for precision agriculture, due to their capabilities in measuring biophysical indicators and detecting spatial variability. The current advancement in

utilizing remote sensing data shows promise in improving the efficiency and efficacy of management practices, particularly in reducing over-application of N fertilizer.

In addition to the most commonly use of remote sensing in yield prediction, the remotely-sensed data can also be used for on-site monitoring as well as real-time scouting of crop growth and variables related to plant physiology and biochemistry. However, the rationale and mechanisms behind the scenario should be identified and clarified and the biophysical parameters of interest must link with spectral characteristics of crop canopy before a practical application.

The success to the using of remote sensing data in crop management is the ability and capability to extract needed information from images at key growth stages during the growing season of the crop. In particular diagnosis of growth and nutrient status of the crop is critical for yield prediction, quality evaluation and N topdressing prescription. For rice, yield is closely related to growth (Chen and Yang, 2005) and N status prior to heading stage (Cui and Lee, 2002), and thus N topdressing at panicle initiation stage is of most crucial for rice yield and quality. With the appropriate indicator(s) related to rice growth and N condition, farmers are able to improve both yield and quality with a midseason N application to the right place at right time, and to protect environment from contamination as well. However, the basic to the changes in leaf internal structure and optical properties in response to the adjustment of N rates and in relation to plant N status should be delineated.

The objectives of this study were to investigate the effects of plant N status on leaf internal structure and canopy reflectance of rice plants in the stage of panicle initiation. Two field experiments were conducted to create various levels of plant N by applying different rates of N fertilizer to the paddy in 2001 and 2003. Changes in morphological, anatomical and reflectance characteristics of rice plants were determined to correlate with plant N content and the importance of these differential plasticity in response to changes in N status was discussed.

2. MATERIALS AND METHODS

2.1 Cultivation and Field Management

In the first experiment, field studies were carried out in the experimental farm of Taiwan Agricultural Research Institute (TARI) (23°30'N, 120°42'E) to measure ground-based remotely sensed high-resolution canopy reflectance spectra (330-1100 nm) during both first and second crops of 2001. Seedlings were machine-transplanted to 0.3 m wide north-south rows with plant distance of 0.18 m. Three field plots (replicates) were located on a loam soil (Fluvaquentic Dystrochrept) of a pH from 4.53 to 5.08. Each plot was 0.5-ha in size and was divided into four equal-area blocks laid out side-by-side. Four different application rates of N fertilizer in the form of ammonium sulfate were assigned individually to these four blocks in the sequence of 0, 50, 100, and 150% of local recommendation quantity (120 kg N ha⁻¹). For all N treatments, 70 kg ha⁻¹ of both P₂O₅ and K₂O were applied to the soil. In the second experiment, seedlings were transplanted north-south bound in Shiko experimental farm (23°34'N, 120°24'E) of TARI on a loam soil. Similar cultivation practices were applied as in the previous experiment. The soil pH was 6.01-6.51. Nitrogen was applied in four rates, 0, 45, 90 and 180 kg N ha⁻¹. Each block size was 50 m² (5 m wide × 10 m long) and plants (hills) were spaced 0.3 m × 0.18 m apart.

2.2 Measurements of Tissue Characteristics

For Experiment I, samplings of rice plants from targeted regions were made simultaneously with spectral measurements when rice plants were in the stage of panicle initiation. After canopy reflectance measurements, six hills were sacrificed each time from targeted spots of each of treated blocks to obtain fresh and dry weights of aboveground plant parts of the aboveground (leaf blades and culms). Dry weights were measured after oven-dried before milling. The powdered samples were for determination of aboveground total N content. Another three hills of rice plants were sampled in the morning at the same targeted regions as for spectral measurements to determine leaf anatomical characteristics to correlate against ANC and canopy reflectance. The photographs were taken under a light microscope operated at magnifications of 150X, 300X, and 600 X using a digital camera. Micrographs were analyzed with the image analysis software SimplePCI. Leaf thickness was measured from the traversed distance across the mesophyll of leaf blade. Leaf rolling index (LRI) was determined by dividing the distance of cross axis perpendicular to the axis of midrib of rolling leaves to the width of leaf section, where $0 < \text{LRI} \leq 1$ (1= flat leaf), and was used as an indicator of the magnitude of leaf rolling. The ratios of bulliform cells per mesophyll were computed for plants treated with different N rates. The LWC was calculated as: $\text{LWC (\%)} = [(\text{fresh weight of leaf blade} - \text{dry weight of leaf blade}) / \text{dry weight of leaf blade}] \times 100$ by using leaf blades excised from the sampled plants. For Experiment II, four hills of rice at panicle initiation stage were sampled from each of three targeted regions of each treatment to determine TCC of leaf blades on the days of spectral measurements.

2.3 Measurements of Canopy Reflectance Spectra

A 2-nm wide field-portable spectroradiometer (model LI-1800, LI-COR Inc., USA) connected to a quartz fiber-optic probe (LI-1800-10) and a remote cosine receptor with a field-of-view of 23 degrees (LI-1800-11) was used to take canopy spectral irradiance data near ground. The receptor was held downward by hand in a nadir-viewing 1.5 m above canopy surface, adjusted to plant height, to scan the upward reflected radiation. A notebook computer was connected to the unit automatically recorded the readings from all channels. Reflectance spectra were transformed from irradiance spectra with an attempt to correct for temporal variation in solar radiation and to reduce sunlight interference. The reflectance (R) of individual wavebands was calculated by dividing the vegetation irradiance measurements with the corresponding incident solar radiation measurements: $R = (\text{irradiance of vegetation at a specific waveband}) / (\text{irradiance of solar radiation at the same waveband}) \times 100\%$. Measurements were made close to solar noon on days with no overhead cloud cover to avoid the effects of solar angle and contributions from diffuse light. The hyperspectral canopy reflectance data were further aggregated into 10-nm interval wavebands in 350-1100 nm and used for comparisons of the effects of N applications. The mean reflectance of rice canopy in waveband regions of blue (425-490 nm, BLUE), green (490-560 nm, GREEN), red (640-740 nm, RED) and near-infrared (740-1100 nm, NIR) was calculated as four broader bandpasses for analyzing reflectance characteristics in relation to aboveground N content, LRI, and leaf total chlorophyll content. Differences between data from two cropping seasons were also compared.

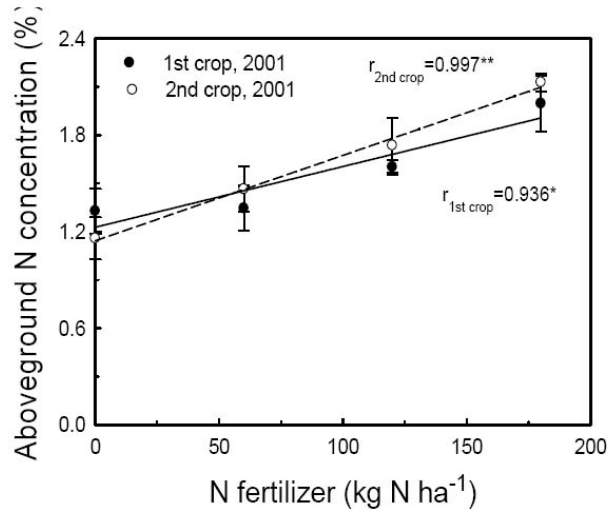


Figure 1. Changes in aboveground nitrogen concentration to the application rates of nitrogen fertilizer for rice plants (*Oryza sativa* L cv. Tainung 67) grown in both first and second crops of 2001. *: significant at the 0.05 probability level.

3. RESULTS AND DISCUSSION

Changes in plant N status were examined upon applications of different rates of N fertilizer. Results showed that application of different rates of N fertilizer to paddy field during growing periods changed ANC of rice plants measured in the panicle initiation/formation stage (Fig. 1), a linear trend with the increasing N application rates in both first ($r=0.936^*$) and second ($r=0.997^{**}$) crops of 2001. Alterations of plant N status by varied N application rates changed not only plant architecture but also leaf internal structure. As revealed in Fig. 2 (magnified from $\times 150$ to $\times 600$), mesophyll layer of leaf blades from the upper-most fully expanded leaves is mostly made up of fold parenchyma cells, which were densely packed with chloroplasts giving the dark green appearance when stained with 1% safranin-fast green. Vascular bundles were arranged parallel to each other throughout the leaf, and xylem, phloem, and bundle sheath of the vascular system were recognized. Bulliform cells, also called motor cells, were located in-between vascular bundles and bottom of the furrows of upper epidermal surface.

Leaf internal structure as a whole was found modulated by application of different rates of N fertilizer, including leaf thickness, leaf width and leaf rolling, cell size, starch granule packing and tissue arrangement (data not shown). Starch granules were shown loosely distributed and packed in the parenchyma cells in plants of higher ANC relative to those of lower ones (data not shown). In comparing growth performance between plants treated with different N rates, it may be considered as evidence indicating a more exhaustion of stored reserves in starch granules of leaves with higher N status. The carbon sources released from the depletion of stored reserves plus N compounds supplied from external N fertilizers are required in the processes of growth to support an active plant growth.

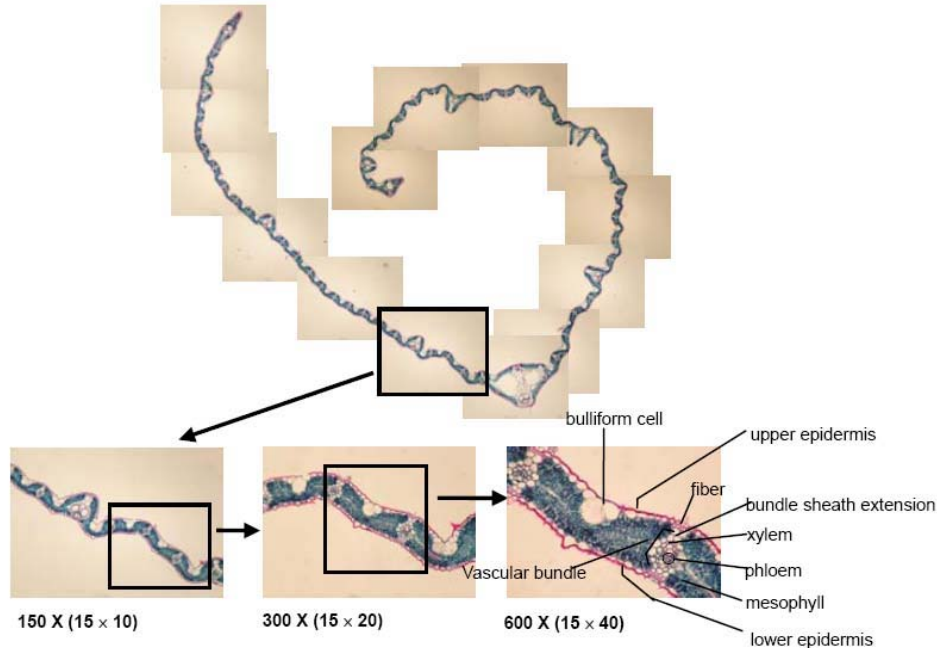


Figure 2. The anatomical cross-sections of leaf prepared from rice plants (*Oryza sativa* L. cv. TNG 67) at the panicle initiation/formation stage. A number of leaf internal structural characteristics were observed.

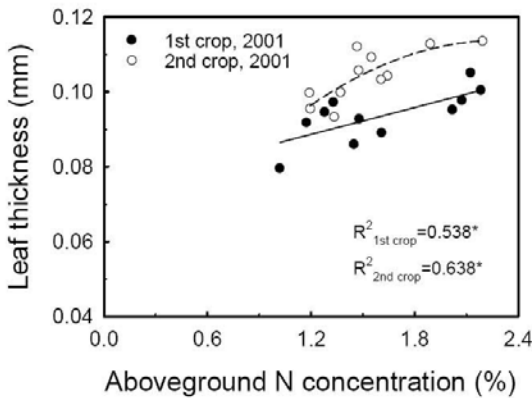


Figure 3. Changes in thickness of leaf blades to aboveground N concentration for rice plants (*Oryza sativa* L cv. Tainung 67) grown in both first and second crops of 2001.

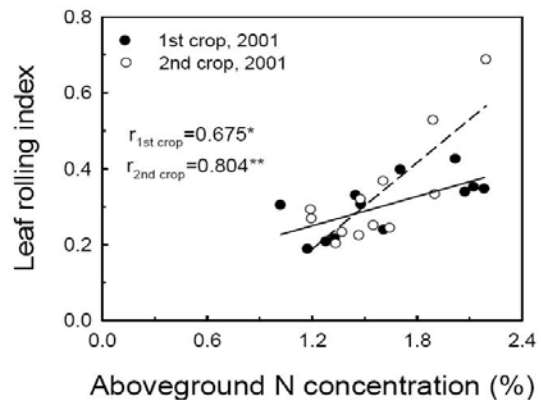


Figure 4. Changes in leaf rolling index (LRI) to aboveground N concentration for rice plants (*Oryza sativa* L cv. Tainung 67) grown in both first and second crops of 2001.

Mean leaf thickness increased progressively with increasing ANC (Fig. 3), while a positive linear relationship existed between LRI and ANC in both crops (Fig. 4). A lesser extent of leaf rolling was observed in leaves of higher ANC, i.e., a more flattened leaves in appearance with higher ANC. Changes in bulliform/mesophyll ratio to ANC were best fitted to a quadratic function in both crops (Fig. 5). When reached the plateau, further increase of N concentration resulted in a decrease of the ratio. In the measured range of ANC, LWC of the upper-most fully

expanded leaves also changed in a curvilinear trend (Fig. 6). Generally LWC was low in plants of lower N concentration, and leaves of plants grown in first crop had higher LWC level than those plants grown in second crop under the same N level. Since plant growth is known to be promoted by the application of higher rate of N and both length and width of leaf growth zone were found improved by the increased N status, the observed increase of leaf thickness and enhancement of LWC are reasonable under sufficient N condition. The phenomena also explain a positive relationship between LRI and ANC and an increase of bulliform/mesophyll ratio to the increasing N level. These facts imply that plants with higher N level may attain a superior leaf expanding vigor and thus impede leaf rolling. An enlargement of bulliform cells within mesophyll layer in response to a higher N is logical in that function of bulliform cells is to control leaf movements and more turgid leaves with larger leaf area were observed in plants with higher N level.

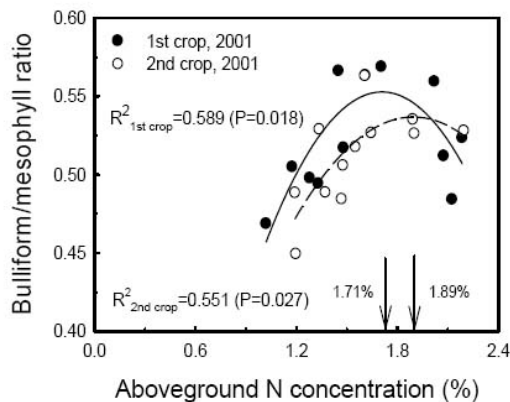


Figure 5. Changes in bulliform/mesophyll ratio to aboveground N concentration for rice plants (*Oryza sativa* L cv. Tainung 67) grown in both first and second crops of 2001.

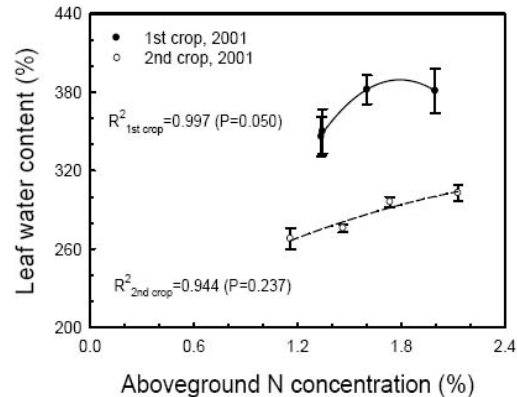


Figure 6. Changes in leaf water content (LWC) to aboveground N concentration for rice plants (*Oryza sativa* L cv. Tainung 67) grown in both first and second crops of 2001.

Application of varied rates of N fertilizer also affected canopy reflectance behavior of different waveband regions (Fig. 7). The mean reflectance of waveband regions at BLUE, GREEN, and RED decreased while NIR reflectance increased with increasing ANC. When plotted against LRI, it was again a negative correlation for reflectance at BLUE, GREEN, and RED and a positive correlation for reflectance at NIR (Fig. 8). It appears that vigorous vegetation growth promoted by higher rates of N fertilizer stimulates the absorption of visible light while reflects a great amount of NIR. Although not significant statistically due to limited sample size, higher ANC contributed to the increase of leaf TCC measured at panicle initiation/formation stage for plants grown in both crops, and this increase was more markedly in plants of second crop under the same N level (data not shown). When plotting reflectance of waveband regions in visible light to TCC, the relationships were negatively correlated in a linear trend (Fig. 9). Reflectance of wavebands in visible light was decreasing with an increase of leaf TCC, as that indicated in the relations to plant N concentration (Fig. 7).

The increases of reflectance at NIR were related to increases of crop biomass, leaf area index and canopy water content, and were affected by internal leaf structure and canopy architecture. The reduction in reflectance at RED region resulted from the increase of leaf TCC and N concentration. Nitrogen shortage was reported to reduce leaf chlorophyll content and hence, increase leaf transmittance at visible wavebands (Blackmer et al., 1996). The relationships

between reflectance of BLUE, GREEN, and RED and TCC obtained from this study supported all these facts. Rice plants with higher leaf TCC resulted from abundant N supply stimulated the absorption of solar energy in visible light. Results further showed that decrease in reflectance of BLUE, GREEN, and RED regions was influenced by the increments of LRI in plants with higher N status. Accordingly plants with more turgid and flatten leaves and higher TCC induced by higher N application rates tend to absorb more energy in visible regime to give a decreasing reflectance, but reflect greater extent of the near-infrared light. As a result, this study suggests that there exists differential morphological, anatomical, and photosynthetic plasticity in rice plants in response to changes in N status as consequences of application of different rates of N Aboveground N concentration (%) fertilizer during plant growth. Changes in leaf anatomy, leaf chlorophyll, and canopy reflectance behavior of rice plants showed reasonable correlation with the variation of N status and were closely correlated. But there is still more need to be uncovered about the ecophysiological significance and the importance these changes may have.

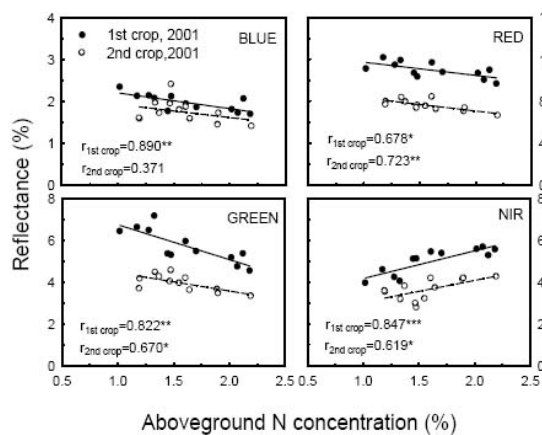


Figure 7. Changes of canopy reflectance in spectral regions of blue (425-490 nm, BLUE), green (490-560 nm, GREEN), red (640-740 nm, RED), and near-infrared (740-1100 nm, NIR) to aboveground N concentration for rice plants (*Oryza sativa* L cv. Tainung 67) grown in both first and second crops of 2001.

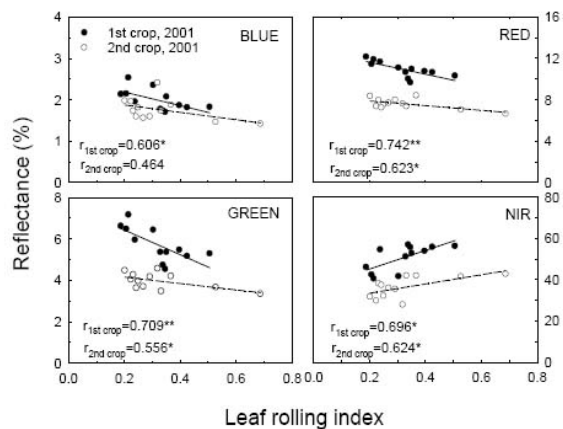


Figure 8. Changes of canopy reflectance in spectral regions of blue (425-490 nm, BLUE), green (490-560 nm, GREEN), red (640-740 nm, RED), and near-infrared (740-1100 nm, NIR) to leaf rolling index (LRI) for rice plants (*Oryza sativa* L cv. Tainung 67) grown in both first and second crops of 2001.

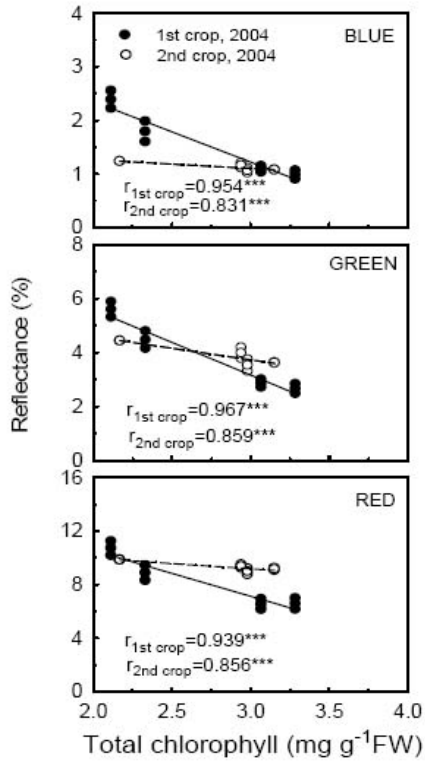


Figure 9. Changes of canopy reflectance in spectral regions of blue (425-490 nm, BLUE), green (490-560 nm, GREEN), and red (640-740 nm, RED) to leaf total chlorophyll content for rice plants (*Oryza sativa* L cv. Tainung 67) grown in both first and second crops of 2003.

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