Ultraviolet-induced Fluorescence of Rice Leaf as Influenced by Nitrogen Application and Cultivars

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Abstract

New measurement system of ultraviolet-induced fluorescence was developed for detecting the secondary metabolic materials and chlorophyll fluorescence in order to evaluate the photosynthetic activity of crops. In this study, 4 rice cultivars; Koshihikari, IR72, Banten (traditional javanica variety), and CH86 (traditional indica variety), were grown in pots and nitrogen fertilizers were added in 5 different levels. Fluorescence of leaves was measured before and after the heading stage. Leaf blades were excited by light of 370 nm wavelength, which was derived by passing light from xenon lamp through a band-pass filter. The results showed that the fluorescence peaks were located in the region of 400-650 nm, 685 nm and 740 nm, and the spectrum patterns varied among cultivars and different nitrogen levels. Fluorescence intensity (400-650 nm wavelength) observed under high nitrogen condition were relatively low as compared with observed under low nitrogen conditions. These results indicated that the fluorescence pattern of the leaf blade was affected from plant growth and nitrogen level of soil. Our findings show that spectrum analysis of ultraviolet-induced fluorescence is useful for evaluation of rice plant nutrition.

Keywords: ultraviolet-induced fluorescence, nondestructive, rice

Introduction

Development of plant nutrition diagnosis systems is required for sustainable food production in order to conserve resources. Diagnosis of crop is able to provide good information about plant conditions, especially the nutrition status of a plant. Moderate amounts of fertilization make it possible to conserve resource, and cost and to half nutrient enrichment of the soil.

In rice cultivation, increase of yield was established by restricting nitrogen supply in the middle of the growth stage (Matsushima *et al*., 1964; Matsushima *et al*., 1966), as excess nitrogen caused lodging. Nitrogen restriction was determined with basis on leaf color (Matsushima *et al*., 1970; Matsuzaki *et al*., 1974). This cultivation method was termed the V-shaped rice cultivation. Using leaf color to enable diagnosis is delaminated with information only of the leaf surface. However, recent studies have attempted express to express the condition of a plant through analysis of internal leaf conditions; many methods have succeeded more easily with indirect estimation. Direct detection will be expected to estimate photosynthetic ability and function in the future of diagnosis.

A useful diagnosis method is light detection by chlorophyll fluorescence. Fluorescence detection has been used as a nondestructive, noncontact, and continuous detection method to evaluate photosynthetic ability (Zoran *et al*., 1999). Chlorophyll concentration and water stress are monitored by calculating the ratio of chlorophyll fluorescence (Saito, 2005).

The choice of an excitation light source is important in chlorophyll fluorescence because it determines the fluorescence information that can be obtained. Visible (blue) excitation for plants emits both red (RF, 630–700 nm) and far-red fluorescence (FRF, 700–800 nm). Ultraviolet (UV) excitation emits RF, FRF, and blue-green fluorescence (BGF, 400–630 nm). The BGF spectrum includes information on leaf materials and structure, and this information reveals plant cultivation conditions that are related to chlorophyll a information.

In this study, we developed a new UV-induced fluorescence measurement system to detect the fluorescence of secondary metabolic materials and of chlorophyll in order to evaluate the photosynthetic activity of crops. To achieve this purpose, we investigated whether our system was able to distinguish rice cultivar and nitrogen fertilizer levels.

Materials and Methods

Plant material

Four rice cultivars, Koshihikari (Japonica), IR72, Banten (traditional javanica variety), and CH86 (traditional indica variety), were grown in pots and nitrogen fertilizers were added in 5 different amounts (Table 1). The rice was grown in the Shinshu University greenhouse in Nagano Prefecture (35°51′N, 137°56′E, 740 m above sea level). Measurement of leaf fluorescence was carried out twice, before and after the heading stage, in 2010. The fluorescence of the cut leaves was measured after dark adaption for 30 min at 25°C. Room temperature was maintained at 25°C with air conditioning.

Table 1. Plant material

Cultivar	Ecotype	Plant type	Notes
Koshihikari	Japonica	Standard	Japanese commercial var.
IR72	Indica	Multi tillering	
BANTEN	Javanica	Large panicle	
CH86	Indica	Grassy	Yellow leaf

Fluorescence measurements

Figure. 1 illustrates the new fluorescence measurement system (FMS) for detection of the fluorescence spectrum. In this system, leaf blades were excited by a 370 nm-wavelength light derived by passing light from a xenon lamp (LAX101, Asahi Spectra Co., Ltd., Japan) through a band-pass filter at 370 nm (XBPA370, Asahi Spectra Co., Ltd., Japan). UV light was directed to the target leaf through a quartz fiber. The band-pass filter had a center wavelength of 370 nm with a 10 nm half-bandwidth. The detector unit was a Photonic Multichannel Analyzer (PMA-11, Hamamatsu Photonics, Japan). A long-pass filter (XUL0400, Asahi Spectra Co., Ltd. Japan) was positioned in front of the detection fiber and the reflection of light below a 400 nm wavelength was ensured. The detected fluorescence wavelength was 400–850 nm. The distance between the edge of the fiber and the leaf sample was adjusted to approximately 5 mm. The PMA was operated in 50 msec durations for each leaf area (point) and this was repeated 1000 times in the same area to determine fluorescence excitation. Each leaf sample consisted of 5 areas (point). The acquisition spectra were determined as the highest peak of the spectrum that had a maximum intensity at 680 nm and 740 nm in the 1000 runs. The acquisition spectra were identical to the mean spectra of peak timing in the Kautsky curve (Govindjee, 1995).

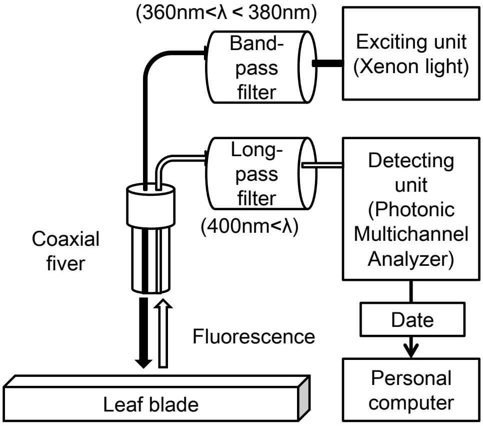
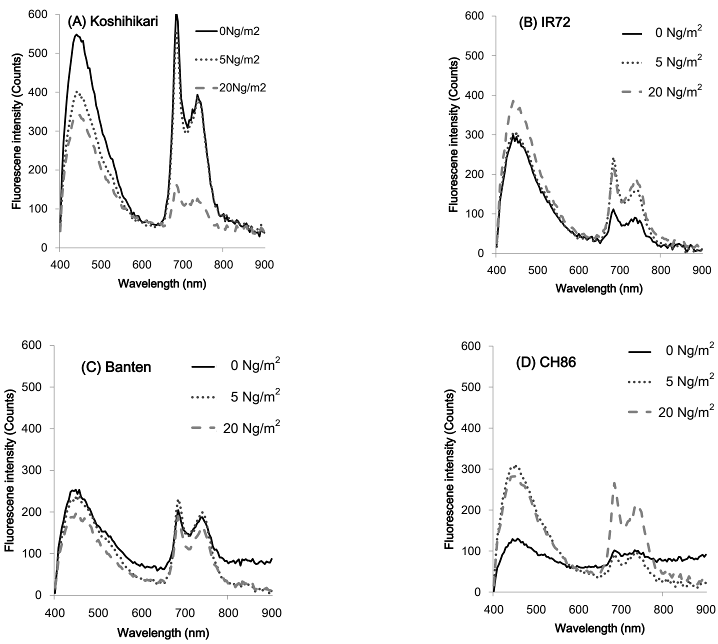


Figure 1. Schematic diagram of fluorescence measurement system. Detected bandwidth of spectrum had wavelengths of 400–850 nm.

Results and Discussion

Figure 2 indicates the fluorescence patterns of leaf blades at various nitrogen levels. The fluorescence peaks were in the region of 400–650 nm, 685 nm, and 740 nm, and the spectrum patterns varied with the different nitrogen levels. The intensity of BGF observed under high-nitrogen conditions was low compared with BGF intensity observed under low-nitrogen conditions.



(A) Koshihikari, (B) IR72, (C) BANTEN, (D) CH86.

Figure 2. Fluorescence spectra of rice leaf. The spectra by excitation at 370 nm were measured at the leaf apex prior to the heading stage.

Figure 3 illustrates the fluorescence spectrum of each cultivar. Fluorescence intensity varied with each cultivar, and high BGF intensity was obtained from the Koshihikari spectrum. These results might describe the fluorescence information containing the condition of plant metabolic material affected by nitrogen levels. The effect of nitrogen treatment was related to BGF intensity. BGF is caused by excitation of ferulic acid derivatives, other phenylpropanoids, and NAD(P)H; RF and FRF are caused by excitation of chlorophyll a (Cerovic *et al*., 1999). BGF enables monitoring of the influence of nitrogen on the degree of growth. We believe that this system can function as a nitrogen level monitor at the same growth stages in rice.

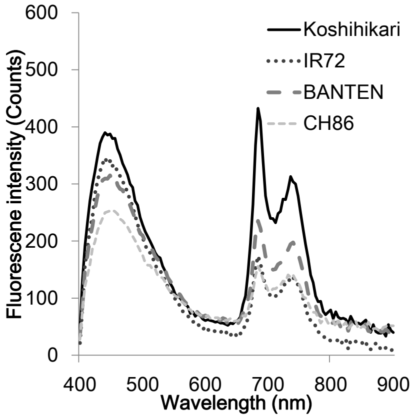


Figure 3. Fluorescence spectra for the cultivars. The spectra by excitation at 370 nm were measured at the leaf apex prior to the heading stage. All cultivars were grown at 5 Ng/ m^2.

The fluorescence spectrum of each cultivar demonstrated different patterns. However, we also need to consider leaf structure, rate of development, and the response to nutrients. In fluorescence detection, it has been suggested that a balance of FRF and BGF enables estimation of wheat lamina growth (Meyer, 2003). In this study, the balance of FRF and BGF may have enabled monitoring of the degree of growth for the cultivars. This result suggests that the detection for cultivars requires further testing and analysis in combination with their growth rates and conditions.

In conclusion, the differences in these results indicate that soil nitrogen levels and different cultivars influence the fluorescence patterns of leaf blades. Our findings show that spectrum analysis of UV-induced fluorescence is useful for evaluation of rice plant nutrition. In the future, UV laser-induced fluorescence methods will be developed if a new diagnosis system becomes necessary.

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