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FUNGAL DISEASE

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Ratio of rice reflectance for estimating leaf blast severity with a multispectral radiometer

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Abstract Rice reflectance was measured to determine the spectral regions most sensitive to leaf blast infection with a multispectral radiometer. As disease severity increased, reflectance also increased in the 400–500nm (blue), 570–700nm (red), and 900–2000nm regions but decreased in the 500–570nm and 700–900nm regions. The increased reflectance in the blue and red regions may be attributed to decreased chlorophyll and carotenoid contents in response to the blast infection. The maximum and minimum reflectance differences occurred at 680nm and 760nm for the nondiseased and diseased rice, respectively. The spectral location of maximum sensitivity was 675nm regardless of disease severity. Rice reflectance ratios were evaluated as indicators of leaf blast severity. Two ratios, R550/R675 (reflectance at 550nm divided by reflectance at 675nm), and R570/R675 quantified the significant disease severity. These wavelengths were selected based on the sensitivity minima and maxima. The ratios of nondiseased rice plants varied depending on growth stage. The variation in ratios must be considered when they are used to estimate leaf blast severity.

Key words *Magnaporthe grisea* · Multispectral radiometer · Remote sensing · Rice leaf blast · Spectral reflectance

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Introduction

Rice blast disease caused by the ascomycete *Magnaporthe grisea* is the principal fungal disease in rice because of its wide distribution and its destructiveness under favorable conditions. The disease results in severe damage under cool summer conditions in northern Japan. Blast fungus affects the leaves, on which it causes diamond-shaped white to gray lesions with dark green to brown borders surrounded by a yellowish halo. The lesions sometimes enlarge, coalesce, and kill entire leaves, leading to complete drying out of infected leaves. The pathogens also infect the nodes of stems. After heading, the blast fungus attacks the spikelets, nodes at the base of the panicle, and branches of the panicle. Because spores that form on the leaf blast lesions are inoculum leading to panicle blast, it is more important to control leaf blast. In Japan, a preventive fungicide for leaf blast is applied to nursery boxes at transplanting time or to the water in the field before epidemics start in most rice fields. When leaf blast occurs despite the preventive application, a curative fungicide is also applied to the rice foliar. Fungicide is commonly applied for panicle blast at the booting and fully heading stages.

The ability to detect diseases early and quantify severity accurately is fundamental for plant disease assessment and management. Nutter et al. (1993) reported significant variation among raters when visually assessing the dollar spot severity in creeping bentgrass. It has been suggested that differences in human vision occur because individuals differ in their ability to perceive various wavelengths of light, which can lead to differences in their estimates of disease severity (Nilsson 1995b). Multispectral radiometer provides more accuracy and precision than visual estimates of disease severity (Nilsson 1995a). It is impossible to estimate accurately the diseased areas and severity over a wide range because formal investigations by public pest management staff also have limits related to labor in Japan. Remote sensing techniques using multispectral scanners may provide an easily available permanent record of disease severity for large areas.

Some researchers (Jackson 1986; Komada 1989; Nilsson 1995a; Ogawa 1991) have reviewed the use of radiometric remote sensing in plant pathology and crop protection. Corn leaves have greater reflectance after inoculation with *Helminthosporium maydis* than do healthy leaves in the chlorophyll (500–700 nm) and water (1450–1950 nm) absorption regions (Safir et al. 1972). Radiometry has been used to assess the severity of early blight of tomato (Lathrop and Pennypacker 1980), rust, and late leaf spot of peanut (Nutter 1989) and dollar spot of bentgrass (Nutter et al. 1993). Leaf reflectance changes in response to eight stress agents including pathogens have been documented for six vascular plant species (Carter 1993). There have also been reports of field experiments using ground-based radiometers to assess the severity of watermelon disease (Blazquez and Edwards 1986), various leaf and root diseases (Nilsson 1991), anther smut disease in *Silene dioica* (Nilsson and Carlsson 1994), barley stripe disease (Nilsson and Johnsson 1996) and Rhizoctonia blight in creeping bentgrass (Raikes and Burpee 1998). As the incidence of panicle blast increased at the dough stage, R470/R570 (reflectance at 470 nm divided by reflectance at 570 nm), R520/R675, and R570/R675 decreased significantly (Kobayashi et al. 2001). At the yellow-ripe stage, R550/R970 and R725/R900 were useful for estimating the panicle blast severity as measured in terms of the percentage of diseased spikelets.

Yamamoto et al. (1995) found that an infection of rice blast fungus increased leaf temperatures by 1.1°C (determined with an infrared radiative thermometer) under upland nursery conditions. However, there have been no reports on the response of rice spectral reflectance to leaf blast infection within the visible, near-infrared, and mid-infrared regions, which include most of the incident solar spectrum. The objectives of this study were to (1) identify the spectral region in which rice reflectance was affected by leaf blast infection; and (2) select wavebands for ratio computation and determine if the selected ratio could detect and quantify the severity of the disease.

Materials and methods

Response of leaf blade and whole plant reflectance to leaf blast infection

Rice seedlings (*Oryza sativa* L., cv. Hatsuboshi) were transplanted (to a 30-a field) in Fukushima Prefecture on May 13, 1995. The planting density was 28 seedlings/m^2. A basal application of fertilizer was performed at a rate of 50/70/60 kg/ha ($N/P_2O_5/K_2O$). Rice plants at the young panicle formation stage were uprooted on July 16 from the field where leaf blast occurred naturally. The leaf blast severity for each rice plant was assessed using an ordinary scale reported by Asaga (1981) (Table 1). This scale uses 11 classes that are assigned to plant samples having corresponding degrees of leaf blast infection. Leaf blades collected from the same plant were cut and placed immediately on wet filter paper in a petri dish to minimize water loss.

Table 1. Evaluation of leaf blast severity

Score	Degree of leaf blast severity	Diseased leaf area (%)
0	No susceptible lesions observed	0
1	A few susceptible lesions	0.1
3	Several susceptible lesions	0.5
5	Many susceptible lesions	2.0
7	Many susceptible lesions with a few dead leaves and stunt symptom[a]	11.0
9	Many dead leaves and severe stunt symptom	55.0
10	All plants dead[b]	(120)

Leaf blast severity was assessed using an ordinary scale reported by Asaga (1981)

[a] The lesions enlarged, coalesced, and killed entire leaves. In severe cases plants became stunted and died

[b] All leaves and stems were dead

Leaf reflectance in the petri dish was measured in the laboratory.

Seedlings (cv. Akitakomachi) were transplanted to a 3-a field at the National Agricultural Research Center for the Tohoku Region on May 13, 1996. Planting density was 20 seedlings/m^2. A basal application of fertilizer was performed at a rate of 70/175/70 kg/ha ($N/P_2O_5/K_2O$) with an additional application at 20/20 kg/ha (N/K_2O) on July 12. Diseased rice plants were transplanted to the center of the field as the inoculum of leaf blast on June 27. Rice plants at the young panicle formation stage were uprooted to 1/5000-a Wagner pots Wagner pots on July 25, and the reflectance of potted rice plants was measured the next day in the field.

Measurement of nondiseased rice reflectance at various growth stages

Rice seedlings (cv. Akitakomachi) were transplanted to a 1-ha field of the Andosols located at the National Agricultural Research Center for the Tohoku Region on May 14, 1997. The planting density was 20 seedlings/m^2. A basal application of fertilizer was performed at 70/175/70 kg/ha ($N/P_2O_5/K_2O$) with an additional application at 20/20 kg/ha (N/K_2O) on July 15. Leaf blast did not occur in the field. Rice canopy reflectance was measured on May 12 (flooded paddy field before transplanting), June 19 (initial tillering stage), June 30 (active tillering stage), July 21 (young panicle formation stage), and August 3 (initial heading stage) in the field.

Spectroradiometric data

Reflectance was measured with a multispectral radiometer (MSR-7000; Opto, Tokyo, Japan) as described earlier (Kobayashi et al. 2001). Reflectances were measured for leaf blades in petri dishes in the laboratory (Fig. 1A–C), three pots in the field (Fig. 1D–F), and nondiseased rice canopies in the field (Fig. 2). Each of the leaf blades in the petri dish and three rice plants had the same disease inci-

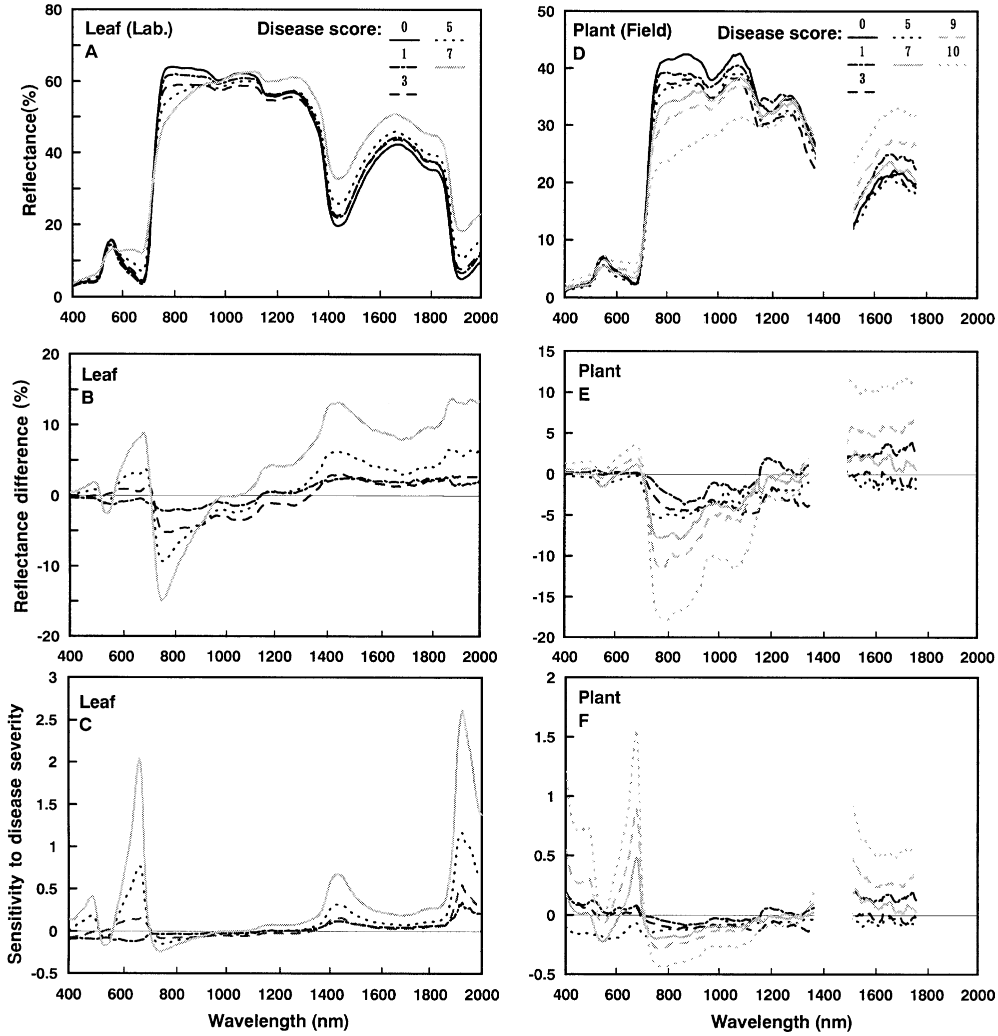

Fig. 1. Reflectance spectra, reflectance differences, and reflectance sensitivity to leaf blast infection. **A, D** Spectral reflectance on infection with blast fungus (*Magnaporthe grisea*). **B, E** reflectance differences for diseased versus nondiseased plants. **C, F** spectral sensitivity to leaf blast infection. **A–C** Spectral reflectance of leaf blades (cv. Hatsuboshi) in a petri dish was measured in the laboratory. **D–F** Spectral reflectance of whole rice plants (cv. Akitakomachi) was measured in the field. Diseased rice plants in the field were transplanted to pots for the measurement. The reflectance difference was computed by subtracting the mean reflectance of the nondiseased rice from that of the diseased rice. The spectral sensitivity was computed by dividing the reflectance difference by the reflectance of nondiseased plants

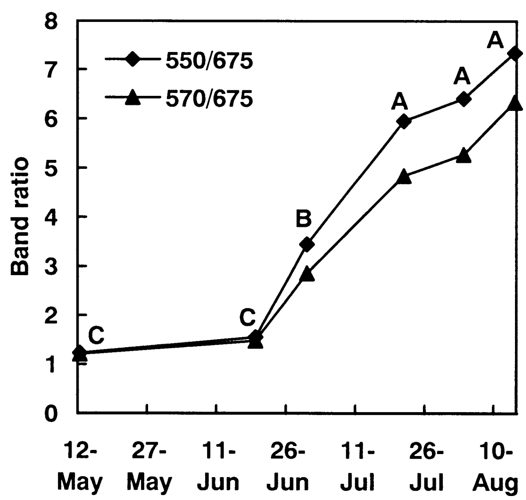

Fig. 2. Reflectance ratios of nondiseased rice canopies (cv. Akitakomachi) in the field. *12 May*, flooded paddy field before transplanting; 19 June, initial tilling stage; 30 June, active tillering stage; 21 July, panicle formation stage; 3 August, initial heading stage. Different capital-case letters indicate that the ratio differed significantly

dence. Three scans were made for each sample, changing the field of view for each scan. A reflectance standard ($BaSO_4$; Labsphere, North Sutton, NH, USA) was measured just before and after the spectral measurement of rice plants at the same position in both the field and laboratory experiments.

Data analysis

Reflectance spectra, relative to the $BaSO_4$ standard, were calculated by dividing the rice radiance by the reference radiance from a $BaSO_4$ standard for each wavelength. Reflectance sensitivity at a given wavelength was computed by dividing the reflectance difference (obtained by subtracting the reflectance of nondiseased rice from that of diseased rice at each spectroradiometer wavelength channel) by the reflectance of nondiseased rice at each channel (Carter 1991). The reflectance, reflectance differences, and reflectance sensitivity for each severity represent means of three replicates.

Reflectance difference and sensitivity maxima and minima determined the wavelengths from which numerator and denominator reflectances should be selected to calculate ratios (Carter 1994; Kobayashi et al. 2001). The wavelength, based on past research on plant stress, the peaks and troughs of spectral reflectance, and a first derivative curve infected with blast fungus were also used for the analysis; the methods were described previously (Kobayashi et al. 2001). Band ratios of each sample represent means for three replicates. To determine the extent to which ratios were affected by blast fungus infection, the values for diseased rice were compared with those for nondiseased rice using ANOVA and Dunnett's mean test. Products of the ANOVA procedure (SAS Institute, Cary, NC, USA) included the probability of obtaining a higher value for the F statistic ($Pr > F$) and the coefficient of multiple determination (r^2). $Pr > F$ indicates the significance of F: the probability that random variation could have explained the differences in reflectance between the ratios of diseased versus nondiseased rice or different disease severities.

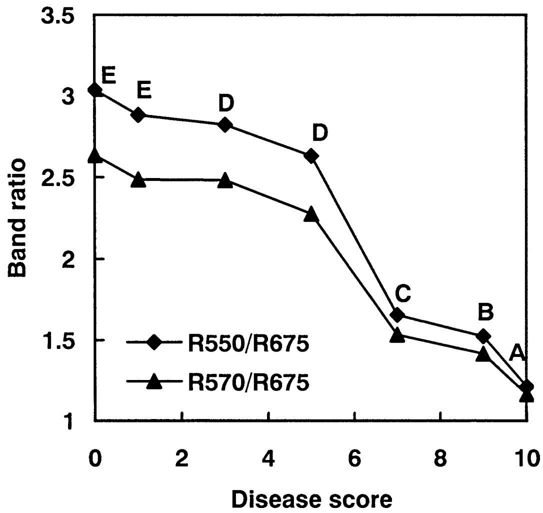

Fig. 3. Relation between reflectance ratios and disease severity of whole rice plants infected by *Magnaporthe grisea*. R550/R675 and R570/R675 were calculated from the reflectance data of Fig. 1D. Different capital-case letters indicate that the ratio differed significantly

Results

Reflectance spectrum of diseased leaf blades

As disease severity increased, leaf reflectance also increased in the 400–500, 570–700, and 900–2000 nm regions and decreased in the 500–570 and 700–900 nm regions (Fig. 1A). The maximum differences in the reflectance in response to leaf blast infection occurred in the 495–500 and 680–705 nm ranges at three or more disease scores and the 1440–1445 and 1880–1885 nm regions at all scores (Fig. 1B). Minima occurred in the 545 and 760–770 nm regions at three or more disease scores. Negative differences were observed in the 715–1065 nm region for different scores of disease severity. The sensitivity of the reflectance for the uninfected leaves was high at visible wavelengths, with maxima centered at 490 and 675 nm for three or more scores (Fig. 1C). Reflectance sensitivity in the near-infrared reflectance (NIR: 700–1300 nm) region was near zero regardless of disease severity. The wavebands of high sensitivity were in the mid-infrared region (MIR: 1300–2000 nm), located at 1440 and 1925 nm. The magnitude of sensitivity varied with the severity of leaf blast infection.

Reflectance spectrum of diseased rice plants

Leaf reflectance measured in the laboratory was generally higher than the reflectance of whole potted plants measured in the field (Fig. 1A,D). The reflectance response of diseased rice plants in the field was similar to that of diseased leaf blades in the laboratory. Maxima and minima occurred at 680 and 760 nm, respectively, at five or more disease scores (Fig. 1E). The sensitivity maxima occurred at 675 nm regardless not only of disease severity but of plant parts (Fig. 1C,F). Variability in the magnitude of disease sensitivities was small in the NIR region. In the MIR region, it was impossible to measure reflectance in the 1320–1500 nm and 1760–1980 nm regions in the field. General reflectance responses were similar between cvs. Hatsuboshi and Akitakomachi (data not shown).

Rice plant reflectance ratios

The wavelengths selected for the ratios were based on the results noted above and past research (Fig. 1D–F). R550/R675 (reflectance at 550 nm divided by reflectance at 675 nm) and R570/R675 decreased as the severity of leaf blast increased. These bands, based on the wavelengths of sensitivity minima and maxima, showed significant differences in disease scores of 0–1, 3–5, 7, 9, and 10 (Fig. 3). The R550/R675 and R570/R675 ratios for nondiseased rice were about 1.0 from May 12 to June 19 and rapidly increased from June 19 to July 21. After July 21 the ratios exhibited a slight increase (Fig. 2). These ratios differed significantly ($P < 0.05$) between the stages of initial tillering, active tillering, and panicle formation.

Discussion

The absorbance curve for living rice leaves has two distinct peaks at 437 and 678 nm due to chlorophyll *a* and at 473 and 639 nm due to chlorophyll *b* (Inada 1980). Carotenoids are absorbed in the 455–483 nm region. Thus, the increased reflectance in the blue and red regions consistent with the disease severity may be attributed to decreased chlorophyll and carotenoid contents in response to the blast infection (Fig. 1A) (Riedell and Blackmer 1999). When the data are presented as reflectance versus wavelength (as in Fig. 1A), the effects on the reflectance are often difficult to evaluate quantitatively (Carter 1993; Riedell and Blackmer 1999). This is particularly true where the slope of the reflectance curve is large, as in the red–infrared transition region (e.g., at 715 nm in Fig. 1A). Hence, to represent more clearly the reflectance response to leaf blast severity, reflectance difference and sensitivity are useful for identifying specific wavelengths in which the reflectance is most strongly affected by leaf blast infection (Carter 1993; Riedell and Blackmer 1999). Carter (1993) summarized leaf reflectance responses for a variety of stress agents in several species. Differences in reflectance are reported to occur in the green and red wavelengths, and major sensitivity peaks are found in the 590–610 nm (orange) and red spectra (Carter 1993). In the case of leaf blast, however, major reflectance differences and sensitivity peaks occurred in the blue and red regions. This might be explained by the difference in plant species and stress inducing agents.

Several reports have assessed the internal structure of a leaf using NIR reflectance and have related the linear relation of the total dry mass to the simple difference between the reflectance at 1100 and 1200 nm (Shibayama and Akiyama 1989). A similar reduction in NIR reflectance caused by a pathogenic infection has been reported in other pathosystems (Nielsson and Johnsson 1996; Nielsson and Carlsson 1994; Natter 1989; Nutter et al. 1993; Raikes and Burpee 1998). These cases might be explained by a decrease in dry mass and change in the internal structure caused by leaf blast infection (Fig. 1A,D). However, the sensitivity of reflectance to leaf blast infection in the NIR region was near zero (Fig. 1C,F). It may be difficult to assess disease severity in the NIR region. When water is lost from a leaf, absorption decreases, and reflectance tends to increase in the MIR range. In the laboratory, the prominent sensitivity maxima in the water absorption bands at 1440 and 1925 nm that occurred with fungal infection might be characteristic of a decrease in the leaf's water content (Fig. 1C,F). Infections of powdery mildew fungus in golden euonymus (*Euonymus japonica*) yielded sensitivity maxima near 1450 nm, 1900–1950 nm, and 2500 nm (Carter 1993). Thus, these locations of sensitive maxima may appear in response to fungal infection as a result of dehydration. MIR reflectance responds consistently only when leaf blast has developed sufficiently to cause severe leaf dehydration.

Remote sensing is used to detect leaf blast in the field, not in the laboratory. The leaf reflectance response in the laboratory was compared with rice reflectance in the field. Water vapor in the atmosphere strongly absorbs solar radiation in the field. As regions of absorption in the 1320–1500 nm and 1760–1980 nm ranges are especially strong, it was difficult to compare rice reflectance in the laboratory with that in the field (Fig. 1D) (Kobayashi et al. 2001). The reflectance response in the laboratory was similar to that in the field, especially in the range of 400–1300 nm. This suggests that data obtained in the laboratory are applicable to field observations. The relation between leaf reflectance and rice plant reflectance in the 400–2000 nm range was similar. It seems that the results of leaf reflectance are applicable to rice plants in the field.

Although it may be sufficient to analyze only the relative change in reflectance, better information can be obtained by combining data from various spectral ranges. Many formulas and vegetation indices (e.g., spectral ratios, normalized difference indices, perpendicular vegetative indices) have been developed to reduce multispectral data to a single number for assessing physiological characteristics such as leaf area, biomass, nitrogen status, water content, and stress (Carter 1994; Inada 1985; Peñuelas et al. 1993; Shibayama and Akiyama 1989; Takebe et al. 1990). Dividing the leaf reflectance measured within a stress-sensitive waveband by the reflectance measured within a relatively stress-insensitive waveband may largely correct variations in irradiance, leaf orientation, irradiance angles, and shading (Carter 1994). The reflectance near 695–700 nm divided by the reflectance near 670–675 nm indicated the precise chlorophyll *a* content of soybean leaves (Chappelle et al. 1992). The ratios reported to indicate plant stress are R420/R695, R605/R760, and R695/R760, which were based on sensitivity minima and maxima (Carter 1994). At the yellow-ripe stage, R550/R970 and R725/R900 differed significantly with the disease severity of panicle blast (Kobayashi et al. 2001). In this study, these ratios were not affected by leaf blast infection. R550/R675 and R570/R675, based on sensitivity minima and maxima, effectively assessed disease severity. R570/R675 was used to estimate panicle blast severity, as measured in terms of the percentage of diseased spikelets at the dough stage (Kobayashi et al. 2001) Hence, this ratio is useful as an indicator of both leaf and panicle blast disease. The absorptivity of photosynthesis pigments is relatively low, around 550 nm (Inada 1980). With regard to ratios that incorporated reflectance at 550 nm, similar results were obtained when reflectance at 610 nm was used (data not shown). This result and the low sensitivity of reflectance in the 550–610 nm region to infection (Fig. 1F) indicated that reflectance in any wavebands throughout the 550–610 nm range could be divided into the reflectance at 675 nm to produce a stress-sensitive ratio. Thus, disease severity may be quantified by measuring the reflectance percentages of these bands. As plant growth progressed, R550/R675 and R570/R675 of nondiseased rice canopy tended to increase from the transplanting stage to the panicle formation stage. If these ratios are applied as an indicator of leaf blast before the panicle formation stage, a change in the ratio depending on the difference in growth stage must be considered. Early detection of leaf blast might be useful when deploying control procedures in a timely fashion. More studies to assess the potential of airborne or satellite sensors as a tool to detect the occurrence of leaf blast are necessary to estimate disease severity over large production areas.

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