Daytime and Nighttime Field Spectral Imagery of Ripening Paddy Rice for Determining Leaf Greenness and 1000-Grain Weight

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Abstract: A spectral-image observation technique to measure the reflectance information of rice plant organs (instead of the overall canopy) under either daylight or artificial light at night was developed, and examined for its usefulness as a method for the in-situ assessment of leaf greenness and grain quality during the ripening period. Generally, conditions are less significant for nighttime measurement since cloudiness does not affect the nighttime observations, and the evening calm helps extend the practical observation hours. A digital imaging system corresponding to the wavelength range of 450 nm to 720 nm, at intervals of 10 nm, collected spectral reflectance images of ripening rice-paddy plots on three clear days in daylight and during the night, respectively. The imaging system observed the rice canopies illuminated by the sun during the daytime and with two 100-W xenon lamps at night on each day. The reflectance values observed at several points on the illuminated leaves and panicles were normalized using the mean and the standard deviation for each spectrum. The normalized reflectance (NR) spectra obtained in the day and night agreed well with each other for the same target organs. Exemplary estimation trials for leaf greenness (SPAD value) using the NR spectra, and correlation analyses between 1000-grain weight of harvested rice grains and the NR spectra indicated that nighttime measurement could substitute for daytime measurement.

Key words: Artificial light source, Digital image, Reflectance, Rice, SPAD, 1000-grain weight.

In rice production in the warm and temperate climate regions of Japan, the recent rise of air temperature during the period from anthesis through harvest tends to worsen grain quality (Oh-e et al., 2007; Wakamatsu et al., 2007). Intensive research and precise assessment for rice grain development and growth in situ are necessary in order to improve crop growth diagnosis as well as to propose better farm management and practices over an area (Hasegawa et al., 2008). Under such circumstances, non-destructive remote sensing may be promising due to the potential for the intensive collection of field data using relatively less effort. Meanwhile, remote sensing techniques using handheld or low-altitude observations of spectral reflectance information can provide a great deal of information for the diagnosis, assessment and prediction of crop nutrient conditions, biomass and yields. So far, most ground observations of reflected light from crops have been made using wide-view spectroradiometers that have several to hundreds of wavelength channels at various spectral resolutions and wavelength ranges (Shibayama and Akiyama, 1986; Takebe et al., 1990; Zhu et al., 2007). As imaging techniques, video cameras or digital cameras have been tested mainly for the purpose of estimating leaf chlorophyll or nitrogen content (Nakatani and Kawashima, 1994; Adamsen et al., 1999; Matsuda et al., 2003; Ku et al., 2004; Omine, 2007; Okada and Ikeba, 2008). However, those instruments had only a few channels in broad spectral resolutions. Recently, in addition to high-spectral resolution (2 nm–10 nm) capability, imaging spectrometry has been introduced for the measurement of outdoor targets, including field-growing crops, as well as for plants in controlled environments such as greenhouses (Omasa et al., 2007). As a cost-effective system, Escobar et al. (1998) proposed a twelve-band airborne digital-video imaging system, and Oguma et al. (2000) tested a lightweight spectral video system for aerial observation. Regarding more complicated but high-spectral and spatial resolution instruments, so-called imaging spectrometers using AOTF (acousto-optic tunable filter), or LCTF (liquid crystal tunable filter) and CCD cameras have been developed (Tamura et al., 1998; Kurosaki et al., 2001a, 2001b) and applied for assessing plant’s biochemical properties such as the content of chlorophyll and nitrogen in rice seedlings (Inoue and Peñuelas, 2001). Endo et al. (2001) estimated and displayed the chlorophyll a concentration distribution on a detached tree leaf using an AOTF high-spectral resolution imager in the
laboratory environment. However, because such high-grade instruments are still experimental and costly, few comprehensive field observations have been done with respect to practical objectives. For relatively simple, cost-effective imaging spectroscopic observations, Minekawa et al. (2004) developed a crane-mounted high-spectral resolution imaging system using a line sensor that scanned mechanically above the target. They measured paddy fields in order to show the spectral responses from the panicles and leaves separately. Additionally, it has been found that disease and weather hazard damages on crops were detectable with these spectral images (Kosaka et al., 2007; Minekawa et al., 2007). Imaging spectrometry makes it possible to collect spectral information not only from the overall canopy but also from a part of an organ such as a panicle or even a spikelet. In other words the quick, non-destructive assessment of variations in the crop plant parameters within the concerned canopy or field will be feasible. Generally, crop plant canopies are not sufficiently homogeneous to draw precise information from restricted amounts of samples, and collecting plant samples often disturbs the targeted canopies themselves. Imaging spectrometry is supposed to work efficiently in various research scenarios if the plant parameters can be detectable using spectral responses.

In remote sensing using optical sensors in the field, normal data are difficult to acquire in the summer cropping season in East Asia due to thick clouds in the early summer to midsummer rainy season, in addition to typhoons in the autumn season (Akiyama and Kawamura, 2003). Therefore, we devised a concept of night observations using artificial light sources. The instability of illumination due to clouds or haze, which may have caused the measurement inaccuracy, as is often experienced during daytime measurements, would be resolved if measurements were made at night. Moving targeted plant organs should also be avoided. The likelihood of having calm evening conditions after sunset is high even if it has been windy throughout the afternoon.

Previous projects concentrating on the prediction of leaf greenness using visible wavelength range have recommended cloudy or overcast weather conditions, probably because the diffused light prevented the emergence of a strong contrast between the directly illuminated and the shadowed parts of targets, as well as “hot spots” or specular reflection effects (Takebe et al., 1990; Nakatani and Kawashima, 1994). However, in cloudy or overcast conditions it is inevitable that the results obtained will be more or less affected by the changing light intensity. Nighttime observations using an artificial light source do not need to be concerned about cloud cover and solar position, which may extend the opportunity for measurements. However, few studies have been conducted on field-reflectance imagery observations using artificial light sources for crop monitoring.

Shingu et al. (2002), at the Japan Aerospace Exploration Agency (JAXA), developed an imaging spectropolarimeter using LCTF to collect multiband imagery of reflected sunlight from landscapes containing vegetation. The system is easy-to-use, and was originally designed for the observation of spectropolarimetric images from an airplane, but it could also be used to obtain reliable measure spectral properties on the ground (Homma et al. 2004). We used this system together with electric lamps as an artificial light source to take spectral imagery of ripening rice plants in paddy fields.

The objectives of this study were to show the performance of reflectance image observations made separately for rice leaves and panicles during the day and night, and to investigate the possibility of substituting daytime observations with nighttime ones by instantiating estimations for leaf greenness (SPAD value) and grain quality (e.g., 1000-grain weight) during the ripening period.

Materials and Methods

1. Instruments

A visible-light LCTF (liquid crystal tunable filter) imaging spectropolarimeter (VLIS) developed by JAXA (Fig. 1) was mounted on a four-wheel observation truck that was operated by hand. The VLIS is able to measure spectrometric and polarimetric 656 pixel×480 line images of reflected light with a 13° field of view at selected wavelengths from 400 nm to 720 nm at intervals of 10 nm. This study concerned only spectrometric observations and consequently did not use the polarimetric function of the VLIS. The VLIS was attached on a tilting platform that looked down upon the targets from a height of 1.8 m above the ground. For the nighttime measurements the platform had side-overhang arms to which were attached a pair of 100-W xenon lamps (SOLAX-XC-100B, Seric Ltd., Tokyo, Japan). They were mounted beside the VLIS in order to illuminate the observed area with false solar light (color temperature: 5500 k; light intensity: 3000 cd; wavelength range: 300–780 nm; 80% of radiant heat being cut) (Fig. 2). For the daytime measurements, adjusting the diaphragm of VLIS kept proper exposure levels in addition to employing a neutral density filter (KENKO PRO ND8, transparency: 16%, Kenko Co., Ltd., Tokyo, Japan) according to the intensity of reflected light.

2. Experimental paddy field

The experimental site was located on the campus of the National Institute for Agro-Environmental Sciences (36° 01’ 28” N and 140° 06’ 29” E, 25 m a. s. l.). Eight plots of equal area (10 m×10 m) for two rice varieties (Oryza sativa L. ssp. japonica, cv.
Koshihikari and Nipponbare) were established in a 20 m × 40 m concrete-framed paddy field. The seedlings were transplanted on 4 June 2007 in north-south rows with a row width of 30 cm and an interhill space of 15 cm. The experimental design included a single basal fertilization level of 2 g N m⁻² applied with compound fertilizers (N-P₂O₅-K₂O = 8-8-8). Top-dressing was applied with compound fertilizers (N-P₂O₅-K₂O = 17-0-17) at rates of 0, 1, 2 and 3 g N m⁻² for the Koshihikari plots and 0, 2, 3 and 4 g N m⁻² for the Nipponbare plots on 13 July, and at rates of 0, 0, 0, 1 g N m⁻² for the Koshihikari plots on 21 August and 0, 0, 2, 2 g N m⁻² for the Nipponbare plots on 27 August. Approximately half the panicles in each hill of Koshihikari and Nipponbare began to emerge on 14 and 21 August, respectively (heading). We measured the leaf greenness (used as an indicator of leaf chlorophyll content per unit leaf area) using a hand-held optical sensor (SPAD-502, Konica Minolta, Inc., Tokyo, Japan) within a day from the corresponding VLIS observations. The respective means of the SPAD values taken from the uppermost leaves in five consecutive hills were averaged for three places per plot. The yield survey conducted on 10 October investigated twenty hills harvested in each plot to estimate the grain and brown rice yields, husking ratios and 1000-grain weights, as well as the total above-ground fresh and air-dried weights and the number of panicles. Actually, we weighed three sets of a hundred grains for each plot, then averaged and converted them into 1000-grain weight estimates.

3. Radiometric calibration

The VLIS was calibrated in two steps. The initial step was to correct the spatial sensitivity characteristics within the view images. A sunlit white reference board (SRT-99-120, W300 × H300 mm, Labsphere, Inc., New Hampshire, USA) was placed horizontally on the ground and then observed vertically from above with the VLIS at several diaphragm F-stops (1.4, 4, 5.6, 8, 11 and 16) with or without the ND8 filter. In each wavelength-channel image the mean of the VLIS output values of the central 10 × 10 pixels divided the entire image pixel output and was smoothed using a 21 × 21 pixel low-pass filter to obtain the correction parameters. The second step was made in the field at each instance of measurement. The procedure included corrections for changes in the illuminated light intensity and linearization for the output values against the observed light intensities. The output voltage from the VLIS is not necessarily linear to the incident light intensity over the entire dynamic range, given the characteristics of the imaging device employed (in this case a monochrome CCD camera). We therefore calibrated the VLIS in the field each time, just before the rice plant measurement, using a handmade eight-grade grayscale panel instead of a
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single white reference board. Eight gray-colored sections of cardboard (size: 257 mm × 364 mm × 1 mm thick) of different brightness (M-27, 28, 29 and 30, double-faced (trade name “Mermaid Board,” Muse Inc., Tokyo, Japan) were used as the reference panels. The reflectance spectra of the cardboard sections were measured beforehand using a spectrometer (USB4000, Ocean Optics, Florida, USA) under sunlight with the SRT-99-120 as the reference. The actual reflectance values were 0.05, 0.18, 0.28, 0.3, 0.32, 0.33, 0.43 and 0.58 at 550 nm. The eight gray-colored cardboard sections were cut into eight triangular pieces to make a reference panel for on-site use (Fig. 3).

4. Measurements for the rice plots
The spectral images of the rice plots were acquired from 1130–1400 on 20 August, and from 1945–2120 on 21 August. The second measurements were made on 3 September, from 1300–1520 and from 1835–2000. The final observations were conducted on 5 October, from 1120–1420 and from 1810–2020. The weather conditions were clear or partly cloudy for daytime measurements. The nighttime measurements were all made in calm conditions after sunset. There was no artificial light source within 100 m of the test site.

For each plant plot the reference panel for on-site use was held roughly perpendicular to the light axis of the VLIS in order to collect the reference images. At this occasion the proper F-stop and the use or non-use of the ND8 filter were determined by checking the images observed on the CRT monitor. Subsequently, after removing the panel we made one to six observations consecutively for the plot without changing the view position. This procedure was repeated once for each plot. The number of repetitions at each plot was not constant but depended on the stability of incident sunlight and the wind conditions that might move the plant organs during the measurement. It took approximately 30 s to make a single scan of wavelengths from 400 nm to 720 nm at intervals of 10 nm. The VLIS’s viewing angle of depression was approximately 35˚, and the average distance between the VLIS and the center of the target area was approximately 3 m, where the apparent view area had a size of W70 cm × H50 cm. The images included the upper half of approximately six hills containing mature rice plants. The soil surface was located approximately 50 cm below the paved levee upon which the observation truck of the VLIS moved along in a north-south direction. The azimuthal view directions were restricted to the west-east direction for the Koshihikari plots and the east-west direction for the Nipponbare plots because there was no wide levee in which to operate the observation truck between the areas of the two varieties.

5. Reflectance calculation procedure
First, the reference panel for on-site use and the subsequent plant observation images were spatially corrected in each wavelength channel using the correction parameter tables provided by the prescribed calibration. Then, for the images of the reference panel for on-site use, output values of three 10 × 10 pixel areas each were manually picked up from images of the eight gray triangular pieces and averaged for each wavelength channel. The previously measured reflectance values of the cardboard sections were regressed by a cubic polynomial expression of VLIS output value means. The polynomial equation thus provided the reflectance estimates for all the pixels in the following target images. Due to the small size of the viewing area (W70 cm × H50 cm), we ignored the effects caused by the non-uniformity of illumination in the nighttime measurements.

6. Data analysis
From the images that had been converted into reflectance values, we sampled five reflectance values...
averaged from 5 × 5 pixel areas located on the directly illuminated surfaces of leaves and panicles (Fig. 4). The reflectance median values were extracted from the number of images that were consecutively observed in field observations. The 5 × 5 pixel area in the observed image was no larger than the width of a completely expanded leaf or an emerging panicle. The five values of reflectance from leaves and/or panicles in each plot were averaged and used for subsequent analysis. To normalize the reflectance spectra, we applied the following formula:

\[ NR_x = \frac{R_x - R_m}{R_{std}} \]  

where \( NR_x \) is the normalized reflectance at \( x \)-nm wavelength channel, \( R_x \) is the original reflectance, \( R_m \) and \( R_{std} \) denote the mean reflectance value, and the standard deviation calculated from the concerned reflectance values from 450 nm to 720 nm wavelength channels, respectively. Reflectance values from 400 nm to 440 nm channels were omitted because the signal-to-noise ratio was insufficient to estimate reliable reflectance values in the wavelength range.

To evaluate the performance of measurements, we calculated correlation coefficients for the \( NR \) values at each channel and the SPAD values, grain yields, husking ratios and 1000-grain weights. In addition, we employed linear regression models consisting of the \( NR \) values as the predictor variables in order to estimate the SPAD values.

**Results**

1. **Reflectance signatures of illuminated leaves and panicles in daytime and nighttime**

   *Nipponbare* is a medium-maturing variety, and *Koshihikari* grows relatively earlier in the test site location. On the first measurement dates (20 and 21 August) there were few observable panicles in the *Nipponbare* plots. On the second measurement day (3 September) all eight plots of both varieties provided reflectance spectra from the leaves and panicles. One of the *Koshihikari* plots had lodged prior to the last measurement day (5 October) due to heavy fertilization and the damage from a typhoon. Thus we were able to collect 46 observations out of the total possible number (48 = 2 varieties × 4 plots × 3 dates × 2 times) for leaves, and 37 observations for panicles. The calculated reflectance values showed a rise at approximately 550 nm (green) and a dip at 660 nm (red), as well as a steep increase toward 720 nm (far-red) for the leaves and young panicles (Fig. 5), which is the generally observed spectral reflectance signature pattern of green plant organs. As they lost chlorophyll the leaves and panicle reflected more in the red wavelength range on the later measurement days. Because the daytime reflectance peak appearing at 440 nm is unusual in the typical spectral signatures of green plant organs (Fig. 5), the sensitivity of the light detectors in the wavelength range of 440 nm and shorter was assumed to be insufficient to secure the reliability of measured data. Accordingly, we omitted the data at wavelengths less than 450 nm from the following analyses. The obtained reflectance values were mostly larger in the daytime measurements than those obtained in the nighttime, even for the same kind of target in the same plot. The ratios of the nighttime reflectance to the daytime reflectance calculated at each wavelength channel were
approximately between 0.5 and 1.0 with no apparent wavelength-dependent characteristics (data not shown). This indicates that the original reflectance values of daytime and nighttime measurements required some conversion in order for use to make quantitative comparisons between them. One cause of this phenomenon may be the difference in the illumination conditions between day and night. Under sunlight the targeted plant organs are illuminated equally, but the light intensity from the lamps in nighttime greatly depends on the distance between the lamps and the target. We have not taken into consideration the distance variation in the image observed at night, which might decrease the averaged reflectance values taken at night in comparison with the reflectance measured under daylight.

2. Normalized reflectance (NR) of leaves and panicles

For equalizing the differences in daytime and nighttime reflectance levels, we introduced normalization using Eq. (1). Naturally, the normalized reflectance (NR) spectra lost information concerning the absolute amplitude levels but retained the characteristics of the spectral signatures (Fig. 6). For each observation day, the NR values for the leaves and panicles taken at the nighttime and the corresponding daytime NR values, both observed on the same date, showed a good match (Fig. 7). Although some discrepancies were still observed at the channels in the far-red range (close to 720 nm), the normalization generally seemed to succeed, so as to combine the daytime and nighttime spectra for further analysis.

3. Leaf greenness (SPAD) and yield survey data of targeted rice plots

Table 1 summarizes the measured leaf greenness (SPAD values) at the three radiometric observations, as well as the yield survey data such as the grain yields, total weight, 1000-grain weight of the harvests and so on. The SPAD values tended to decrease during the maturing period, but they decreased earlier in the less fertilized plots than in the more fertilized ones. The unexpected high air temperature at around the anthesis stage (a maximum air temperature of 38.6°C was recorded on 16 August) damaged the pollination process in the Koshihikari plots, which reduced the yields and 1000-grain weights of the variety (Hasegawa et al., 2008). Koshihikari in the most heavily fertilized plot lodged due to the strong wind and rain brought by a typhoon in September. Aside from that, the rice plants grew normally and the highly fertilized plots produced the average yield in the local district.

4. Leaf greenness (SPAD value) estimation using NRs

Simple correlation coefficients between the SPAD
and the normalized reflectance (NR) values were calculated at each wavelength channel (Fig. 8). The daytime and nighttime spectra showed similar wavelength responses, whereby the green band had positive and the red band had negative correlations with SPAD value. Some wavelength channels showed significantly high correlations; the probability level was 0.001 or less. However, a single-channel NR may not be sufficiently accurate to predict the SPAD values. Due to the normalization procedure, the NRs included certain negative values. Therefore, ratio-based band-to-band calculations such as the normalized difference vegetation index (NDVI = (Rx1 − Rx2) / (Rx1 + Rx2)) or the ratio vegetation index (RVI = Rx1 / Rx2) would often produce extraordinary calculation results. Therefore, to improve the prediction accuracy we employed linear regressions in order to use multiple wavelength channels of normalized reflectance (NR) values selected from all 28 of the wavelength channels from 450 nm to 720 nm. The observation number was 46 (23 observations each in the daytime and nighttime). The stepwise forward selection of variables with a threshold probability level of 0.25 selected the most efficient wavelength channels in the linear regression analyses. The variable selection steps were summarized in Table 2, indicating that the appropriate number of explanatory variables (wavelength channels) should be three or four, except for the intercept term, according to the Mallow's Cp indicator (Mallow, 1973). The wavelengths of 500 nm, 550 nm and 660 nm were finally selected based on a coefficient of determination ($R^2$) of 0.75. The partial regression coefficients for the three-channel NRs were very significant in the following equation:

$$SPAD = 26.37 + 8.79 \times NR500 + 3.72 \times NR550 - 12.64 \times NR660$$  

(2)

![Fig. 8. Simple correlation coefficients between the means of SPAD values measured for the uppermost leaves in the rice canopies and normalized reflectance (NR) in the wavelength channels (on the X-axis). An amalgamation of data from two varieties and three dates was used for the calculations. The horizontal broken lines indicate the 0.001 level of probability.](image-url)
The performance of Eq. (2) is presented as a scatter diagram of the estimated versus measured SPAD values in Fig. 9.

Using the three wavelength-channels above, we prepared four data subsets for validation tests: subset (I), Koshihikari measured in the daytime; subset (II), Nipponbare in the daytime; subset (III), Koshihikari in the nighttime; and subset (IV), Nipponbare in the nighttime. The numbers of observations for these subsets of (I)–(IV) were 11, 12, 11 and 12, respectively. The linear regression models obtained for the subsets were as follows:

Model (I): SPAD = 17.06 + 0.59 × NR500 − 0.72 × NR550 − 18.53 × NR660, $R^2 = 0.83$ (3),

Model (II): SPAD = 35.77 + 23.69 × NR500 + 9.09 × NR550 − 14.51 × NR660, $R^2 = 0.84$ (4),

Model (III): SPAD = 27.61 + 9.22 × NR500 + 3.94 × NR550 − 12.45 × NR660, $R^2 = 0.73$ (5),

and

Model (IV): SPAD = 29.81 + 13.84 × NR500 + 4.82 × NR550 − 15.32 × NR660, $R^2 = 0.90$ (6).

The daytime measurements for Koshihikari (subset (I)) gave relatively smaller partial regression coefficients for NR500 and NR550 than in the other subsets. We applied the model equations (3), (4), (5) and (6) to the data sets that excluded the subset used for model building. The number of observations was 34 or 35. Table 3 summarizes the coefficients of determination ($R^2$) and root mean square errors (RMSE) obtained. The $R^2$-value of 0.59 of the model derived from subset (I) was inferior among the other remaining models’ results, in which the $R^2$-values varied between 0.71 and 0.76. The smallest RMSE of 2.8 of the model Eq. (5) from subset (III) gave the best $R^2$-value of 0.76 among the four models. This subset was the data taken during the nighttime measurements for Koshihikari. The estimated SPAD values were plotted against the measured SPAD values in Fig. 10. The validations indicated that models derived by using a single variety and either of the measurement time (day or night) could well predict the SPAD values of both varieties.

Table 2. Forward selection of variables in stepwise linear regression analysis for estimating SPAD values of rice leaves using the normalized reflectance in the wavelength channels. Cp is Mallow’s Cp index, and P is the number of explanatory variables (including the intercept term).

| Step | Wavelength (nm) | Prob. > |t| | $R^2$ | $R^2*$ | Cp | P |
|------|-----------------|---------|---------|--------|--------|-----|---|
| 1    | 660             | <0.0001 | 0.65    | 0.64   | 18.6   | 2   |
| 2    | 500             | 0.006   | 0.71    | 0.69   | 10.6   | 3   |
|      | 660             | <0.0001 |         |        |        |     |
| 3    | 500             | 0.0005  | 0.75    | 0.73   | 5.1    | 4   |
|      | 550             | 0.01    |         |        |        |     |
|      | 660             | <0.0001 |         |        |        |     |
| 4    | 500             | 0.0007  | 0.77    | 0.74   | 4.3    | 5   |
|      | 510             | 0.098   |         |        |        |     |
|      | 550             | 0.004   |         |        |        |     |
|      | 660             | <0.0001 |         |        |        |     |

Prob. > |t|, probability of observing a sample value of partial regression coefficient as extreme as the value actually observed, assuming the null hypothesis $H_0$ is true; $R^*$, multiple correlation coefficient adjusted by degrees of freedom.

Fig. 9. Performance of the multiple linear regression model for estimating SPAD values of rice leaves using normalized reflectance at 500 nm, 550 nm and 660 nm.
5. 1000-grain weight and NR values

Because Koshihikari ripened faster than Nipponbare, the NR spectra from panicles were chosen from the data collected on 3 September for Koshihikari and 5 October for Nipponbare, respectively. However, the stages of the two varieties were not exactly the same. The duration after heading was approximately three weeks for Koshihikari but was more than six wk for Nipponbare. Due to the weather conditions in September and certain problems with the measuring system, there were no VLIS observations between 3 September and 5 October. In the preliminary experiment, however, we used the NR spectra of panicles on 3 September for Koshihikari, and 5 October for Nipponbare. The number of observations was 16 (= 2 varieties × 4 plots × 2 daytime and nighttimes). Simple correlation coefficients between the NRs and the grain yield, husking ratio and 1000-grain weight were plotted against the wavelength (Fig. 11). The NR spectra were closely correlated with the 1000-grain weight and the husking ratio, but poorly correlated with the grain yield. Because the NRs of panicles were observed on a small part of each panicle, probably from one or two spikelets, the grain yield per unit land area might not be detectable. It is interesting that NRs showed relatively high correlations with the 1000-grain weight as well as the husking ratio, both of which should be related to the degrees of grain filling. Negative correlations in the wavelength range of 530 nm to 560 nm (green) and positive correlations in 640 nm to 700 nm (red – far-red) were significant at the 0.1% level for both daytime and nighttime measurements. Fig. 12 presents a scatter gram of the NR690 against the 1000-grain weight. Mingling the varieties and daytime and nighttime observations, the following model was obtained:

\[ \text{NR690} = -4.59 + 0.200 \times (1000\text{-grain weight}), \]

\[ R^2 = 0.79 \]  

(7)

The NR690 values of both varieties in day- and nighttime observations appear to line up on or around the common line of the regression model.

Discussion

The reflectance of the leaves and panicles was assumed to be similar in the daytime and nighttime. The condition of being damp with dew and/or guttation is something to be concerned about here, but we observed no dewdrops on the leaves and panicles during the measurements. This might be explained by the fact that our nighttime measurements were performed within two hours after sunset, and the field calibration procedure by which a person in the target plant plot held the reference panel for on-site use (Fig. 3) probably shook the dew from the plant organs. Dewdrops on the plant leaves and panicles may affect the measured reflectance when observations are carried out from midnight to early morning. Another
possibility (other than dew) may be the changes in canopy structure brought about by the nyctinastic movement of leaves, which is often observed in leguminous crops (Isoda et al., 1993). The difference in the reflectance of the same surfaces but in various angular orientations against the incident light and view directions is also an important and intriguing issue for future studies.

Naturally, daytime measurements are useful and convenient as long as the weather conditions are favorable. The validation test for SPAD value estimation using a common model for daytime and nighttime observations showed that a model calibrated at night could also be effective for daytime observations, and that the converse would also be true. For practical application, it is essential to calibrate the models for each location, variety and growth stage, but it takes time to do so. Therefore, the above-mentioned fact may actually reduce the risk of deficit in calibration and practical measurements and substantially increase the opportunity for observation.

A substantial problem with nighttime measurement was that the illumination would not be uniform in the targeted area because the light intensity is in inverse proportion to the square of distance between the light source and the target. It is necessary to calibrate the light intensity at each point in the observed area in order to determine the absolute values of reflectance. However, this is technically complicated in field conditions. To solve this problem, we employed a normalization technique that applies mathematical calculation on the reflectance observed in more than one wavelength channel, which is widely used in field spectrometry and remote sensing. For example, NDVI is one of the most versatile normalization indices using two-channel reflectance or radiance.

The effects of normalization for multi-channel spectral data have been investigated by, for example, Ono and Fujiwara (2002). They proposed a method dividing each channel reflectance by the sum of the entire channel reflectance values, which was reported to be suitable for multichannel satellite sensors. We
tentedly employed and tested normalization using the mean and the standard deviation using the entire wavelength channels in this study. Although this study did not directly evaluate the variation of normalized reflectance (NR) from similar targets located at different positions in the area observed, it revealed the daytime and nighttime spectra comparable with each other. This may be partly attributed to the fact that the observed targets were restricted to green leaves and panicles, which were also fully illuminated. The procedure of selecting illuminated spots and parts of plant organs in the observed images could solve the problem of mixed pixels caused by shadows and background pixels, which has not been resolved in field radiometry using wide-view spectroradiometers.

Few studies have been conducted for the purpose of directly observing rice panicles and/or spikelets in order to assess grain quality in the field before harvest. Our study demonstrated that field imaging spectrometry during the daytime and nighttime could detect spectral characteristics (i.e., changes in color) of panicles in situ. At agricultural experiment stations the changes in spikelet color, as well as the water content, have been periodically (every two or three d) examined in ripening period so as to determine the best time for harvest (Mr. K. Morita at the Toyama Prefectural Agricultural, Forestry and Fisheries Research Center, Toyama, Japan, in personal communication). Collecting panicle samples from average hills, threshing by hand, selecting kernels larger than a specific size, and manually classifying the kernels into color classes in a short time in order to avoid rapid discoloration require skill and considerable labor. The developed spectral imagery method is completely nondestructive, so it may be a satisfactory observation tool for engineers and researchers studying the development of spikelets and panicles of cereal crops from physiological and agro-meteorological viewpoints during the ripening period.

The correlation between 1000-grain weight, husking ratio and NR spectra may be a unique finding in field spectrometry. The correlations at some wavelength bands may be high regardless of the varietal differences. Those relations were clearly detected in both daytime and nighttime observations. The rate of color change of ripening spikelets on panicles may relate to the function of reflectance for estimating grain quality, but at this stage of study, it is only a matter of speculation. The observations were restricted to a single occasion for each variety. The difference in the average 1000-grain weight levels of the varieties might apparently improve the correlation coefficients (Fig. 12). The spikelet sterility for Koshihikari caused by a high air temperature (Hasegawa et al., 2008) might have varied the 1000-grain weight more than would be expected under normal weather conditions. This may have been an advantage in our correlation calculation. The general applicability of Eq. (7) itself might therefore be limited. Further study is necessary to develop a concrete method using multiple wavelength channels, and reveal the appropriate conditions and stages for a reliable prediction. The results of this study indicate that imaging spectroscopy may have potential for assessing 1000-grain weight before harvest under either daylight or artificial light at nighttime.

Nighttime observations with imaging spectral measurement systems may not only extend the opportunity of field observations by ensuring the performance of nondestructive sensing but could also enable 24 hr field monitoring of the physiological conditions of crop organs, for example, on a single spikelet on a panicle. It may greatly support current and future activities in field research to determine the effects of fluctuating environmental parameters (such as the ambient air temperature) on grain yield and quality.

Conclusions

This study showed the possibility of nighttime field spectral image measurement targeting very narrow spots on plant organs illuminated by artificial light. There is less cloud cover and solar position improves the prospects of refining field radiometric methodologies, warranting further investigation. The method may be useful for studying the mechanisms of grain development and the environmental influence on grain quality.

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