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The Relationship between SPAD Values and Leaf Blade Chlorophyll Content throughout the Rice Development Cycle

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Abstract

The Soil and Plant Analyzer Development (SPAD) meter is a chlorophyll meter used worldwide due to its convenience. The present study was conducted to investigate the relationship between SPAD values and rice leaf blade chlorophyll content throughout the entire rice development cycle, and determine whether it is possible to apply a nonlinear model to the relationship. A field experiment was conducted in which the rice communities of two cultivars ('Nipponbare' in 2000 and 'Kinuhikari' in 2002) with wide-ranging chlorophyll content were generated by varying nitrogen fertilizer rates. Data sets comprising SPAD values for leaves sampled from the various rice communities and actual chlorophyll content of the same leaf measured spectroscopically were created. A linear model for each cultivar was applied to the relationship between the SPAD value and chlorophyll content of the same leaf at each developmental stage. Their relationship revealed a good fit. Subsequently, linear and nonlinear models were applied to the data through all rice development stages of each cultivar. A nonlinear model for each cultivar revealed a better fit to the relationship between SPAD values and rice blade chlorophyll content than the linear model. The following nonlinear models were then generated to fit the data:

$$Chl_{Ni} = 0.965 \exp(0.036\ Sp) - 1\ (r^2 = 0.93)$$

$$Chl_{Ki} = 0.925 \exp(0.038\ Sp) - 1\ (r^2 = 0.94)$$

where, Chl_{Ni} and Chl_{Ki} denote the leaf blade chlorophyll content (mg/g, flesh weight) for Nipponbare and Kinuhikari, respectively, and Sp is the SPAD value. The coefficients of the nonlinear models obtained for each cultivar were very similar. The possibility of applying a nonlinear model to the relationship between the SPAD value and rice leaf blade chlorophyll content was confirmed with high coefficients of determination. To establish a broadly applicable calibration equation based on a nonlinear model for evaluating the chlorophyll content in the rice leaf blade using a SPAD meter, more studies are needed using various cultivation data sets.

Discipline: Crop production
Additional key words: nonlinear model, nitrogen fertilizer, top dressing

Introduction

The chlorophyll or nitrogen content in crop leaves is important information for managing the nitrogen status and monitoring the physiological activity of crops. The Soil and Plant Analyzer Development (SPAD) meter is a simple, convenient, portable and nondestructive chlorophyll meter used worldwide to manage crop nitrogen status and monitor crop activity.

Many studies have used SPAD meters to diagnose crop nutritional status (Furuya 1987, Bullock and Anderson 1998, Lopez-Bellido et al. 2004, Wu et al. 2007, Asai et al. 2009) or photosynthetic activity (Inoue 1987). However, SPAD meters only provide researchers with a measure of relative leaf chlorophyll or nitrogen content. In order to determine the absolute leaf chlorophyll or nitrogen content based on SPAD values, the relationship between SPAD values and actual leaf chlorophyll or nitrogen content

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throughout a crop's entire development cycle must be investigated and a calibration equation developed.

Many researchers have investigated the relationship between SPAD values and chlorophyll or nitrogen content in rice leaf blades to assess the nitrogen status of rice for purposes of top dressing fertilization management (Takebe et al. 1990, Inada 1994a, Inada 1994b). Therefore, most studies on said relationship were conducted for the specific development stage of panicle formation when top dressing is typically applied (Takebe et al. 1990, Inada 1994a, Inada 1994b).

Inada (1994b) investigated the relationship between chlorophyll meter values (KI-1, Sanshin Kougyou, Japan) and the nitrogen content of rice leaf blades from the early to ripening stages, and proposed linear calibration equations for each rice development stage. In the same study, the relationship between SPAD values and the nitrogen content of rice leaf blades throughout the entire rice development cycle suggested that an exponential function could be applied to the relationship between both parameters. However, apart from this study, there are few reports concerning the relationship between SPAD value and chlorophyll content throughout the entire rice development cycle.

Therefore, this study was intended to (i) investigate the relationship between SPAD values and the chlorophyll content of rice leaf blades on a fresh weight basis using the data of all rice development stages, and (ii) confirm the possibility of applying a nonlinear model to the relationship between SPAD values and the chlorophyll content of rice leaf blades.

Materials and methods

Field experiments were conducted in 2000 and 2002 in a paddy field of the National Agriculture and Food Research Organization (NARO), Agricultural Research Center (Tsukubamirai, Ibaraki). Rice communities with wide-ranging chlorophyll content were generated by varying nitrogen fertilizer rates. Seven and four unreplicated treatment plots were established in 2000 and 2002, respectively, with plots receiving different rates of nitrogen fertilizer. Each plot was 50 m^2 (10 m × 5 m) in size. Rice cultivars of 'Nipponbare' and 'Kinuhikari' were used in 2000 and 2002, respectively.

A compound fertilizer containing N, K_2O, and P_2O_5 was applied at rates of 0, 4, 9 and 13 kg/10 a in 2000, and at rates of 0, 3, 6 and 9 kg/10 a in 2002 as a basal dressing. Top dressings were also applied to some of these plots. Tables 1 and 2 list the fertilization treatments for each plot.

Rice seeds were sown on April 27th (117th day of the year) in 2000 and on April 30th (day 120) in 2002. Seedlings were planted on May 25th (day 145) in 2000 and on May 22nd (day 142) in 2002, when the seedling leaf numbers had reached 3.5. Tables 3 and 4 list the cultivation practices used each year. The plant spacing was 0.3 m between rows and 0.15 m between hills.

The same SPAD 502 chlorophyll meter (Konica Minolta, Japan) was used to measure leaf color in 2000 and 2002. Two hills were sampled from each treatment plot and measurements were performed for the two to four uppermost expanded leaf blades on a single stem from each hill. Six points on each leaf were chosen for measurement with SPAD. These comprised three pairs of points on both sides of the midrib were sampled near the leaf tip, in the middle of the leaf, and near the leaf bottom. The mean of these six SPAD values was recorded.

After the measurement of leaf color with the SPAD meter, chlorophyll in each leaf blade was extracted as follows: First, the leaf blade was removed from the leaf sheath and cut with scissors into pieces 2-mm wide. These pieces were then soaked in a flask with 20 ml of 80% acetone for 48 hours at 5ºC (Takebe et al. 1990). In this process, chlorophyll in the leaf blades was extracted in acetone. The

Table 1. Fertilization regime utilized for Nipponbare in 2000.

Treatment	Basal dressing (kg /10 a)			Top dressing (kg /10 a)	
	N	P_2O_5	K_2O	N	K_2O
N 13-1	13.0	13.0	13.0	5.6	5.6
N 13-0	13.0	13.0	13.0	0.0	0.0
N 9-1	9.0	9.0	9.0	3.9	3.9
N 9-0	9.0	9.0	9.0	0.0	0.0
N 4-1	4.0	4.0	4.0	1.7	1.7
N 4-0	4.0	4.0	4.0	0.0	0.0
N 0-0	0.0	0.0	0.0	0.0	0.0

Table 2. Fertilization regime utilized for Kinuhikari in 2002.

Treatment	Basal dressing (kg /10 a)			Top dressing (kg /10 a)	
	N	P_2O_5	K_2O	N	K_2O
K 9	9.0	9.0	9.0	7.9*	3.9**
K 6	6.0	6.0	6.0	2.6	2.6
K 3	3.0	3.0	3.0	1.3	1.3
K 0	0.0	0.0	0.0	0.0	0.0

*Top dressing of nitrogen was applied three times: 2 kg/10 a on June 12, 2 kg/10 a on June 26, and 3.9 kg/10 a on July 18 in plot K9. **Top dressing of potassium was applied at 3.9 kg/10 a on July 18 in plot K9.

extracted chlorophyll content in acetone was determined using a spectroscope (UV-1600, Shimazu, Japan) and quantified by the method proposed by Arnon (1949). The chlorophyll content in rice leaf blade was expressed in terms of fresh weight (mg/g). Sampling and measurement were performed every two weeks throughout the entire rice development cycle.

Results

Different rates of nitrogen fertilizer yielded rice communities with wide-ranging leaf blade chlorophyll content. The change in SPAD values over the course of the entire rice development cycle was as follows: the highest SPAD values were observed in the early stage; SPAD values gradually decreased until fertilization with top dressing at the panicle formation stage, resulting in higher SPAD values; and SPAD values gradually decreased from ripening until harvest. The intra-seasonal changes in SPAD values observed in this study were consistent with those observed in the past (Inada 1994c).

Linear models for Nipponbare at each development stage were applied to the relationship between SPAD value and leaf blade chlorophyll content. Table 5 lists the linear models based on both parameters using 22 to 61 data points at each developmental stage. The relationship between both parameters were in good agreement. Coefficients of determination for each model ranged from 0.65 to 0.90 (Table 5). Table 6 lists the linear models for these parameters for Kinuhikari. High coefficients of determination for linear models using 15 to 30 data points at each developmental stage were observed except for the data set on June 17 (Table 6).

The relationship between the two parameters—SPAD value and leaf blade chlorophyll content—were investigated using all data sets throughout all development stages.

Table 3. Cultivation schedule for Nipponbare in 2000.

Cultivar	Seeding	Transplanting	Top dressing	Heading time	Maturity
Nipponbare	Apr. 27	May. 25	Jul. 20	Aug. 12	Sep. 25
	(117)	(145)	(201)	(224)	(268)

Figures in parenthesis denote the days of the year.

Table 4. Cultivation schedule for Kinuhikari in 2002.

Cultivar	Seeding	Transplanting	Top dressing			Heading time	Maturity
Kinuhikari	Apr. 30	May. 22	Jun. 12*	Jun. 26*	Jul. 18*,**	Aug. 5	Sep. 19
	(120)	(142)	(163)	(177)	(199)	(217)	(262)

* Top dressing of nitrogen was applied three times (on June 12, June 26 and July 18), and potassium was applied on July 18 in plot K9. ** Top dressing of nitrogen and potassium was applied on July 18 in plots K6 and K3. Figures in parenthesis denote the days of the year.

Table 5. Linear models at each rice development stage for Nipponbare.

Development stage	Model for each stage	Number of samples	SPAD range	Coefficient of determination (r^2)
Jun. 17 (169)	$Chl_{Ni} = 0.086\ Sp - 0.299$	47	29.3-47.0	0.73
Jul. 8 (190)	$Chl_{Ni} = 0.125\ Sp - 1.921$	60	24.6-45.6	0.90
Jul. 20 (202)	$Chl_{Ni} = 0.085\ Sp - 0.682$	59	23.2-41.4	0.79
Aug. 3 (216)	$Chl_{Ni} = 0.066\ Sp - 0.289$	59	23.8-39.2	0.65
Aug. 16 (229)	$Chl_{Ni} = 0.113\ Sp - 1.476$	61	22.9-37.2	0.85
Sep. 3 (247)	$Chl_{Ni} = 0.070\ Sp - 0.431$	59	11.5-31.0	0.79
Sep. 22 (266)	$Chl_{Ni} = 0.054\ Sp - 0.171$	22	11.1-23.0	0.83

Chl_{Ni} is the leaf blade chlorophyll content for 'Nipponbare' (mg/g, fresh weight); Sp is the SPAD value. Figures in parenthesis denote the days of the year.

The linear models provided a better fit to the data for both cultivars, as illustrated in Fig. 1. Subsequently, nonlinear models were applied to these two parameters. Though deviations were observed in the actual measurement data from the data generated on Jun 17th and July 1st by the nonlinear model for Kinuhikari (Fig. 1), nonlinear models improved the relationship between SPAD value and leaf blade chlorophyll content as compared with linear models (Table 7). The chlorophyll content in the rice leaf blade as generated by the linear model was underestimated relative to the actual chlorophyll content in the high and low SPAD ranges, while these underestimations were improved by

Table 6. Linear models at each rice development stage for Kinuhikari.

Development stage	Model for each stage	Number of samples	SPAD range	Coefficient of determination (r^2)
Jun. 17 (168)	Chl_{Ki} = 0.057 Sp + 1.448	21	34.4-47.5	0.41
Jul. 1 (182)	Chl_{Ki} = 0.114 Sp − 1.074	30	29.4-46.1	0.75
Jul. 14 (195)	Chl_{Ki} = 0.101 Sp − 1.119	28	23.1-42.1	0.93
Jul. 30 (211)	Chl_{Ki} = 0.087 Sp − 0.713	29	24.5-38.6	0.76
Aug. 14 (226)	Chl_{Ki} = 0.096 Sp − 1.093	29	13.5-35.6	0.91
Aug. 27 (239)	Chl_{Ki} = 0.085 Sp − 0.613	21	12.6-32.2	0.94
Sep. 7 (250)	Chl_{Ki} = 0.044 Sp − 0.066	15	6.5-22.2	0.89

Chl_{Ki} is the leaf blade chlorophyll content for 'Kinuhikari' (mg/g, fresh weight); Sp is the SPAD value. Figures in parenthesis denote the days of the year.

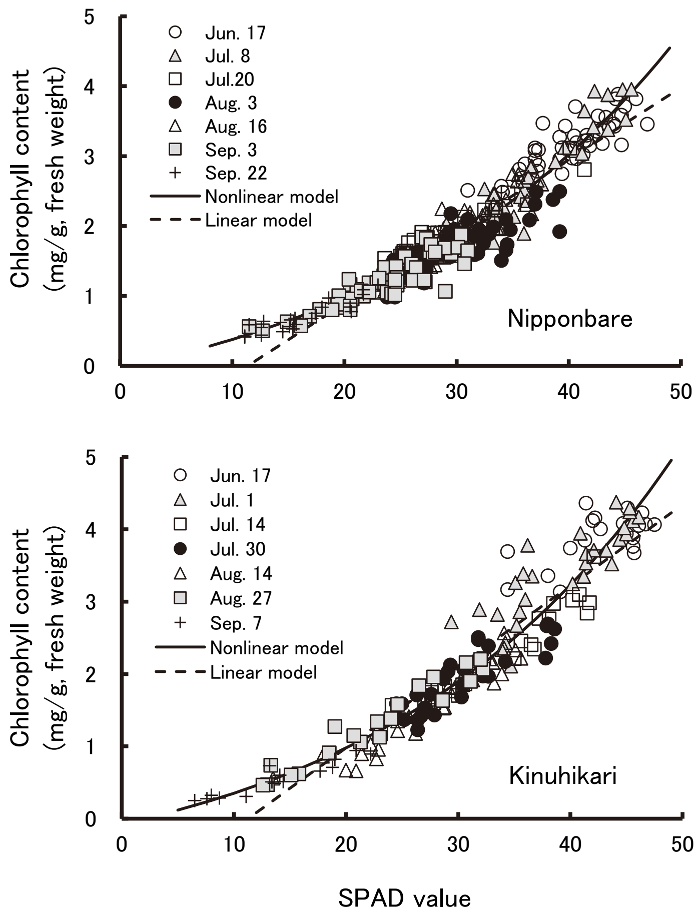

Fig. 1. Relationship between SPAD values and leaf blade chlorophyll content through all stages of rice development for 'Nipponbare' and 'Kinuhikari'.

using nonlinear models (Fig. 1).

Discussion

Linear models related to SPAD value and rice leaf blade chlorophyll content at each rice developmental stage were obtained with high coefficients of determination. This observation supports the results of previous studies by Takebe et al. (1990) and Inada (1994a). Moreover, this study confirmed the possibility of applying a nonlinear model to these parameters by using all data sets from the early to ripening stages in rice with higher coefficients of determination than those of the linear models (Table 7).

Markwell et al. (1995) proposed an exponential function on a type of nonlinear model, as a calibration equation for evaluating the leaf chlorophyll content of soybean and maize based on SPAD values, citing a good fit to their data. Uddling et al. (2007) also proposed an exponential calibration equation for the relationship between SPAD value and leaf chlorophyll content for wheat based on a similarly high goodness of fit. The results of this study are consistent with those reported by these earlier studies (Markwell et al. 1995, Uddling et al. 2007).

The reason for a better fit by a nonlinear model to the relationship between the SPAD value and chlorophyll content in the rice leaf blade in this report and past reports (Markwell et al. 1995, Uddling et al. 2007) might be due to the range of SPAD values and measurements at different crop developmental stages. SPAD values in this study ranged widely, from 6.5 to 47.5, and in the previous studies by Markwell et al. (1995) and Uddling et al. (2007), the values also ranged widely, from 2 to 65 and from 6 to 48, respectively. In contrast, SPAD values in the studies by Takebe et al. (1990) and Inada (1994a), who applied linear models, spanned narrower ranges of 23-41 and 8-37, respectively. In addition, the data sets for this study and the report by Uddling et al. (2007) not only include the data set for a specific stage but also those for different developmental stages. Conversely, Takebe et al. (1990) and Inada (1994a) conducted studies using only a data set of a specific development stage (panicle formation). The better fit of the nonlinear model might also be related to nonuniform chlorophyll distribution in the leaf blade and multiple scatterings of light emitted from the LED, a component of the SPAD meter, which might cause deviation from linearity in the high and low SPAD ranges (Markwell et al. 1995, Uddling et al. 2007).

There are few reports concerning the influence of fertilizer rate, genotype, and cultivation environment on the relationship between SPAD value and rice leaf blade chlorophyll or nitrogen content. Jhanji and Sekhon (2011) reported that the relationship between SPAD value and rice leaf blade chlorophyll content varied with the fertilizer rate. Jinwen et al. (2009) reported that rice leaves become thicker with a higher nitrogen fertilizer rate. Peng et al. (1993) discussed the effect of rice leaf thickness on the relationship between SPAD value and leaf blade nitrogen content using different genotypes with varying nitrogen fertilizer rates and environments, and reported that the relationship was improved by taking leaf thickness into account. These reports indicate that the relationship between SPAD value and rice leaf blade chlorophyll or nitrogen content varies with genotype, fertilizer rate, and environment.

Though different cultivars were used, the coefficients of equation between the two nonlinear models for Nipponbare and Kinuhikari were almost the same. Cultivars used in this study were only the japonica type. Therefore, investigations should be conducted by applying the nonlinear models obtained to other rice genotypes, including the indica type.

In this study, the relationship between SPAD values and rice leaf blade chlorophyll content from the early to ripening stages was expressed by a nonlinear model for each cultivar, though the fertilizer rate was varied over seven levels in Nipponbare and four levels in Kinuhikari. However, remarkable differences were observed between the measured data and data generated by the nonlinear model for Kinuhikari on June 17th and July 1st in 2002 in the high SPAD value range (Fig. 1). The plotted data in

Table 7. Nonlinear and linear models for the relationship between SPAD values and leaf blade chlorophyll content through all stages of rice development for 'Nipponbare' and 'Kinuhikari' cultivars.

Cultivar	Model type	Model	Number of Samples	SPAD range	Coefficient of determination (r^2)
Nipponbare	Nonlinear	$Chl_{Ni} = 0.965 \exp(0.036\ Sp) - 1$	367	11.1-47.0	0.93
	Linear	$Chl_{Ni} = 0.103\ Sp - 1.170$	367	11.1-47.0	0.90
Kinuhikari	Nonlinear	$Chl_{Ki} = 0.925 \exp(0.038\ Sp) - 1$	173	6.5-47.5	0.94
	Linear	$Chl_{Ki} = 0.112\ Sp - 1.260$	173	6.5-47.5	0.90

Chl_{Ni} and Chl_{Ki} are the leaf blade chlorophyll contents for 'Nipponbare' and 'Kinuhikari' cultivars (mg/g, fresh weight), respectively, and Sp is the SPAD value.

Fig. 1 indicate that the actual chlorophyll content of the first and second upper leaves in fertilizer treatments K3 and K0 tended to be distributed near or coinciding with the data generated by the nonlinear model, while the actual chlorophyll content in the third and fourth lower leaves in fertilizer treatments K3 and K0 tended to deviate from the data generated by the nonlinear model (data not shown). There was a time lag of five days from the day top dressing was applied on June 12th and June 26th to the day measurements of SPAD values and actual chlorophyll content were conducted (June 17th and July 1st, respectively, in 2000). In cases where the time lag was longer than 12 days, such as on July 14th (18 days) and July 30th (12 days) in 2002, when SPAD values and actual leaf blade chlorophyll content were measured in Kinuhikari, and on August 3rd (14 days) in 2000 in Nipponbare, no remarkable differences were observed between the measured data and data generated by the nonlinear model. The reason why these data were obtained is not apparent. Further study of the relationship between SPAD values and rice leaf blade chlorophyll content should be undertaken by taking time lags and the leaf blade position on stems into account.

There have been a few reports regarding precautions on measuring plant leaf chlorophyll content using the SPAD meter. Kinefuchi and Hamamura (2000) reported observing diurnal fluctuations in SPAD values in melons. Their results suggested that the fluctuations were caused by water stress and new chlorophyll formation due to solar radiation. Huang and Peng (2004) identified differences in SPAD readings derived from instrument error using different SPAD 502 chlorophyll meters. In this case, a way of correcting SPAD values to eliminate instrument error between chlorophyll meters must be developed. Precautions pointed out by these reports (Kinefuchi and Hamamura 2000, Huang and Peng 2004) should be taken for improving the nonlinear model relative to the relationship between SPAD values and rice leaf blade chlorophyll content.

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