Plant Prod. Sci. 7(1) : 97—100 (2004)

【Short Report】

Comparison and Standardization among Chlorophyll Meters in their Readings on Rice Leaves

Jianliang Huang* and Shaobing Peng**

*(*College of Science, Hunan Agricultural University, Changsha, Hunan 410128, China;*
***Crop, Soil and Water Sciences Division, International Rice Research Institute, DAPO Box 7777, Metro Manila, Philippines)*

Abstract : Six chlorophyll meters (SPAD) were compared to determine the magnitude of differences in SPAD readings on rice leaves. Correlations among the six SPAD meters were statistically significant (P<0.01) with correlation coefficients (r) ranging from 0.971 to 0.990. However, the differences in SPAD readings were statistically significant among the six meters (P<0.05) and the difference between two meters was as large as 2.7 units. Such magnitudes of discrepancy should be considered when different SPAD meters are used in the same study.

Key words : Chlorophyll meter, Leaf nitrogen content, Nitrogen management, Rice leaves, SPAD, Standardization.

The chlorophyll meter (SPAD) provides a simple, quick, portable, and non-destructive method for estimating leaf chlorophyll content (Watanabe et al., 1980). The ability to predict chlorophyll content on a leaf-area basis from SPAD readings was demonstrated in many crop species (Jiang et al., 1986; Yadava, 1986; Dwyer et al., 1991). The SPAD meter has also been used to estimate leaf N status of rice leaves with the goal of predicting the need for fertilizer-N topdressing in real-time N management (Takebe et al., 1990; Turner et al., 1991; Peng et al., 1996). It is a useful tool for field researchers, extension specialists, and crop consultants who do not have access to well-equipped laboratories. The SPAD meter is gradually being used in the germplasm screening program for high nitrogen use efficiency, abiotic stress tolerance, and slow leaf senescence because it measures a large number of entries in a few hours and because it has good repeatability and requires minimum maintenance (Jiang et al., 1986). It is safe to state that SPAD meter is one of the most popular instruments for nutritional physiology research.

In real-time N management, the SPAD meter is used to determine the timing of in-season N application in a rice crop to synchronize fertilizer N application with the actual crop demand (Peng et al., 1996). The timing of N application was determined based on the current SPAD value of the rice crop and a critical SPAD value. Fertilizer N was applied when the SPAD value fell below a critical value (i.e. 35 for the indica cultivar IR72 in the tropics). The critical SPAD value could be determined with different SPAD meters. In multi-location trials on real-time N management, different SPAD meters could be used in different locations. Under these circumstances, differences among SPAD meters should be considered. There is little information on variation in SPAD readings across different SPAD meters.

The objectives of this study were (1) to determine the correlation among six SPAD meters and (2) to determine the magnitude of differences in SPAD readings among the six SPAD meters. The methods for standardizing several SPAD meters are discussed.

Materials and Methods

This study was conducted in three field experiments in the 2002 dry season at the International Rice Research Institute (IRRI), Los Baños, Philippines. In each experiment, there were three N treatments with a different rate and timing of N applications (Table 1). Treatments were arranged in a randomized complete block design with four replicates.

Six chlorophyll meters [SPAD-502, Soil Plant Analysis Development (SPAD) section, Minolta Camera Co., Ltd., Japan] that were manufactured in different years were used for comparison (Table 2). The SPAD readings were taken first on the reading checker, which was provided by the manufacturer specifically for each meter. Five uppermost fully expanded leaves were selected for SPAD measurements from each treatment in all three experiments when rice plants reached midtillering in Exp I and panicle initiation in Exp II and III. The SPAD readings were taken on each leaf as described by Peng at al. (1993). Three SPAD readings were taken around the midpoint of each leaf blade, 30 mm apart, on one side of the midrib. The

Received 7 April 2003. Accepted 22 July 2003. Corresponding author : S. Peng (s.peng@cgiar.org, fax +63-2-845-0606).

Table 1. Nitrogen rate (kg ha^{-1}) and timing of N application in the three field experiments conducted at IRRI in the 2002 dry season.

Exp	Cultivar	N treatment	Total N rate	N rate in each application			
				BS[1)]	MT	PI	FL
I	IR72	N1	0	0	0	0	0
		N2	100	50	0	50	0
		N3	150	50	50	50	0
II	IR72	N1	0	0	0	0	0
		N2	60	18	12	18	12
		N3	200	60	40	60	40
III	Shanyou63	N1	0	0	0	0	0
		N2	120	40	40	40	0
		N3	180	60	60	60	0

1) BS=basal, MT=midtillering, PI=panicle initiation, and FL=flowering.

Table 2. Year of manufacture, serial number, range of reading checker and measured value on the reading checker for six chlorophyll meters (SPAD).

SPAD meter	Year manufactured	Serial number	Range of reading checker	Mean value of measurements[1)]
SPAD1	1996	73623075	71.9±3.0	71.8
SPAD2	1999	77923043	72.9±3.0	72.2
SPAD3	1999	81223014	75.2±3.0	75.3
SPAD4	2000	76013043	71.7±3.0	72.7
SPAD5	2001	71123097	70.1±3.0	70.1
SPAD6	2001	71123096	70.6±3.0	70.5

1) Average of 10 measurements on the reading checker.

Table 3. Chlorophyll meter readings on the rice leaves from three field experiments with six chlorophyll meters (SPAD). There were three N rates in each experiment. Each value is a mean of five leaves.

Exp	Cultivar	Growth stage[1)]	N Treat[2)]	SPAD1	SPAD2	SPAD3	SPAD4	SPAD5	SPAD6	Mean
I	IR72	MT	N1	35.4	34.1	35.0	36.9	36.3	36.5	35.7
			N2	36.9	35.8	36.5	38.4	38.1	37.9	37.3
			N3	39.6	38.5	39.5	41.1	40.9	40.9	40.1
II	IR72	PI	N1	31.2	29.4	30.7	32.3	32.3	32.4	31.4
			N2	33.8	32.6	33.7	35.1	35.1	35.0	34.2
			N3	37.1	36.6	37.5	38.9	38.1	37.9	37.7
III	SY63[3)]	PI	N1	30.4	29.4	30.2	32.4	31.8	32.6	31.1
			N2	38.5	37.2	38.0	39.8	39.7	39.8	38.8
			N3	37.7	36.4	37.4	38.9	38.5	38.7	37.9
Mean				35.6[b 4)]	34.4[c]	35.4[b]	37.1[a]	36.8[a]	36.9[a]	36.0
Deviation from mean				-0.4	-1.6	-0.6	1.1	0.8	0.9	

1) MT=midtillering, PI=panicle initiation.
2) See table 1 for N treatments.
3) SY63=Shanyou63.
4) Within a row, means followed by the same letter are not significantly different at the 0.05 probability level according to the Least Significant Difference Test.

three SPAD readings were averaged to represent the SPAD value of each leaf.

Data were analyzed following analysis of variance (SAS, 1982) and means of SPAD values by the six meters were compared based on the Least Significant Difference Test (LSD) at the 0.05 probability level. Correlations among the SPAD values of the six meters were determined by the correlation analysis.

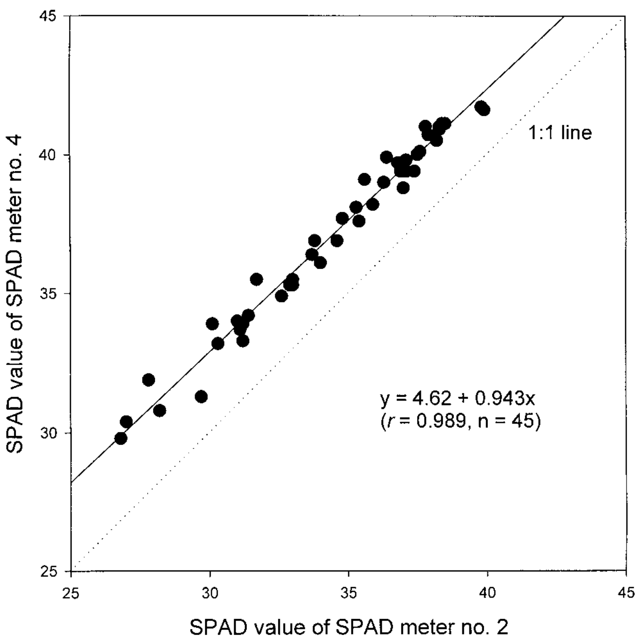

Fig. 1. Correlation in chlorophyll meter (SPAD) readings between the SPAD meter no. 2 and 4. Measurements were taken on the uppermost fully expanded leaves of rice plants from three N treatments and two cultivars in three separate field experiments.

Results and Discussion

The SPAD measurements on the reading checkers were within the suggested value provided by the manufacturer for all six SPAD meters (Table 2), suggesting that the six meters operated normally. Correlations among the six SPAD meters were statistically significant (P<0.01) with correlation coefficients (r) ranging from 0.971 to 0.990.

Due to close correlations among the six SPAD meters, the differences in SPAD values among the three N treatments were consistent across the six meters (Table 3). In general, zero-N treatment had 1.6 to 7.7 SPAD units lower than the treatments that received N applications. The differences in mean SPAD values were statistically significant across the six SPAD meters (P<0.05). The SPAD meters no. 4, 5, and 6 had the highest SPAD readings, followed by the SPAD meters no. 1 and 3. The SPAD meter no. 2 had the lowest SPAD readings among the six meters. The SPAD meters no. 1, 2, and 3 had 0.4, 1.6, and 0.6 SPAD units lower than the mean of the six meters, respectively, while the SPAD meters no. 4, 5, and 6 had 1.1, 0.8, and 0.9 SPAD units higher than the mean of the six meters, respectively. The largest discrepancy in mean SPAD value was 2.7 units and this occurred between the SPAD meter no. 2 and 4. The differences in SPAD values between the SPAD meter no. 2 and 4 were fairly consistent across a wide range of SPAD values (Fig. 1).

A close correlation was observed among the six SPAD meters, suggesting that these meters can be individually used to accurately determine the relative differences in SPAD values, chlorophyll and total N contents of rice leaves among different treatments such as N rates and cultivars. However, the differences in SPAD readings were significant among the six meters and the difference between two meters was as large as 2.7 units. Such magnitudes of discrepancy should be considered when different SPAD meters are used in the same study.

In real-time N management using an SPAD meter, the timing of in-season N application was determined based on the current value of SPAD measurement and a critical SPAD value (Peng et al., 1996). In this system, SPAD measurements were taken weekly on most recent fully expanded leaves from 15 days after transplanting to flowering. A topdressing of 30 to 45 kg N ha^{-1} was applied when the SPAD value fell below a critical value (i.e., 35 for the indica cultivar IR72 in the tropics). When a deviation as great as 2.7 units could exist among different SPAD meters, the users need to consider the deviation when they use the critical SPAD value suggested from other sources. Otherwise, there is a possibility of missing the optimum timing of N application by one week or more. Therefore, it is desirable to use the same SPAD meter to determine the critical SPAD value and to take in-season SPAD measurements for real-time N management. If several SPAD meters are used for multi-location trials on real-time N management, one should consider the possible variations in SPAD readings among different SPAD meters. It is necessary to determine the difference in SPAD readings among the meters as done in this study. If the difference is statistically significant, the SPAD meters should be standardized.

The SPAD value is determined based on the amount of light transmitted by the leaf in two wavelength regions in which the absorption of chlorophyll is different. The SPAD value has no dimension and represents a relative index for the estimation of leaf chlorophyll and total N contents. Therefore, it is impossible to compare the accuracy among the six meters tested in this study. One way of standardizing the six SPAD meters is to treat the mean of the six meters as the master unit. Compensation values are calculated as the difference between the SPAD value of each SPAD meter and the mean SPAD value of the six meters. Another way is to treat the SPAD meter no. 1 as the master unit because its values were closest to the mean values of the six meters. Compensation values are calculated as the difference between the SPAD value of each SPAD meter and the SPAD value of the SPAD meter no. 1. The compensation value can be inputted into each SPAD meter to minimize the difference among SPAD meters.

References

Dwyer, L.M. et al. 1991. Can. J. Plant Sci. 71 : 505-509.
Jiang, X.X. et al. 1986. International Rice Research Newsletter 11 : 10-11.
Peng, S. et al. 1993. Agron. J. 85 : 987-990.
Peng, S. et al. 1996. Field Crops Res. 47 : 243-252.
SAS Institute. 1982. SAS user's guide: Statistics. 4th ed. SAS Inst., Cary, NC.
Takebe, M. et al. 1990. Plant and Soil 122 : 295-297.
Turner, F.T. et al. 1991. Agron. J. 83 : 926-928.
Watanabe, S. et al. 1980. Jpn. J. Crop Sci. 49(special issue): 89-90.
Yadava, U.L. 1986. HortScience 21 : 1449-1450.