Measurement of Leaf Color Scores and Its Implication to Nitrogen Nutrition of Rice Plants

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Introduction

In rice (*Oryza sativa* L.) cultivation, top-dressing with fertilizers, particularly, with nitrogen during the panicle-formation stage is an important technique to obtain high grain yields. Koshihikari and Sasanishiki are the japonica cultivars well known for their high eating quality. These are susceptible to excess nitrogen which leads to lower quality. High nitrogen concentration in rice plants caused by excess nitrogen is also a potential factor of lodging and blast. Therefore, appropriate diagnosis of nitrogen status in leaves is necessary before application of top-dressing fertilizers. In Japan measuring the leaf green color is the widely used technique as the index of nitrogen status of the crop. Machines for measuring the green score of leaves have developed. Comparison of leaf color intensity with a standard green color chart (color scale method) and measurement of light transmission have been the basis of leaf green measurement. These methods are very simple and do not require any destructive analysis. The color scale method is applied to a single leaf and also to canopy green assessment. The diagnosis of nitrogen status by green color measurement of individual leaves is a unique technique of Japan, and a few reports[4,8] have introduced it abroad.

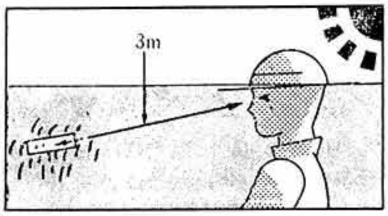
In this report, several methods of measuring leaf green color are described, and the green color scores measured are compared with chlorophyll and nitrogen contents of leaves. Applicability of these methods for diagnosis of nitrogen status of crops is also evaluated.

A historical and methodological description of leaf color measurement

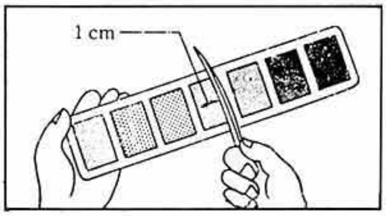
The methods of quantitative assay of leaf color so far developed are; (1) measurement of three characteristics of color by a color variation meter, (2) measurement of chlorophyll concentration after extraction from leaves with extractants like aceton, (3) comparison of leaf color with a standard color chart (color scale method), and (4) measurement of light transmission in leaves. The color scale method was commercialized by Fuji Film Co., which devised a color chart special for rice leaf color during 1978–1980. The measurement of light transmission by leaves was first devised by Inada[5] as a suitable assay of green leaf color, and portable machines to measure leaf green were produced. They are 'green meter' by Fuji Film Co. (1980), 'chlorophyll meter SPAD 501' by Minolta Co. (1983), and 'chlorophyll tester' by Fujihira Co. (1985).

1) *Fuji color scale for rice leaves*

The brightness (L value) of green is sectioned into seven from light (No. 1) to dark (No. 7). The leaf color is compared with this standard color chart under the same



(a)



(b)

Fig. 1. Measurement of green color scores of canopy (a) and a single leaf (b) by Fuji color scale

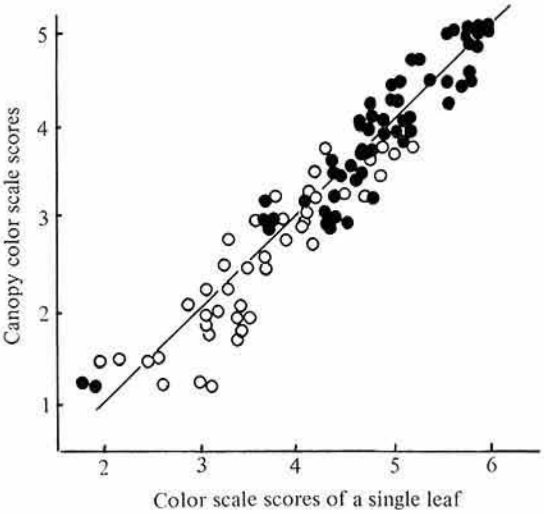


Fig. 2. Relation of canopy color scale scores to scores of a single leaf in Sasanishiki (●) and Koshihikari (○)
Regression line: $Y=1.00X-1.01$ ($r=0.960^{**}$) (Tanno et al.[11]).

environmental conditions. If the L value of leaves is between No. 3 and No. 4, it is counted as 3.5 by 0.5 unit. The color scale is made of plastic resistant to sunshine damage and less light-reflectable, and consists of many stripe color lines arranged like leaf veins. Its surface has protuberant streaks, imitating the leaf surface with leaf veins. Comparison of canopy green with the color scale is carried out at the place 3 m away from the canopy in the direction toward the sun as shown in Fig. 1. The color of a single leaf is measured by placing the leaf 1 cm above the color scale. It sometimes happens that the reading of leaf color differs with different persons engaged in the measurement. Hence, the average score by several experts is more reliable.

The color scale method can assess the canopy green as well as green of a single leaf. The readings obtained with canopy and with a single leaf differ in the same field, and relationship of the two readings is expressed by;

$$Y=1.00\ X-1.01,$$

where Y is the canopy green and X is the color of a single leaf (Fig. 2)[11].

The reading by the color scale of canopy green is more closely correlated with average chlorophyll content of three top leaves than the reading on a single leaf[11], and is also correlated with total N contents of rice plants as well as nitrogen concentration of leaf blades[9]. A fault of the color scale method is the occurrence of fairly big personal variations in the reading, but this method has an advantage that it can assess canopy green color.

2) Chlorophyll meter

Inada's finding (1963)[5] using cut pieces of rice leaves that the highest peak of attenuance spectrum of chlorophyll in fresh leaves is at 670 nm with constant minimum attenuance in the band beyond 750 nm in infrared area led him to propose that the difference of the attenuance at 670 and 750 nm in intact fresh leaves can be the index of chlorophyll content. Based on this idea, three portable machines were produced as stated before. 'Green meter' ceased soon to be produced, and 'chlorophyll tester' was not used much, but the 'chlorophyll meter SPAD 501' was most widely distributed in Japan.

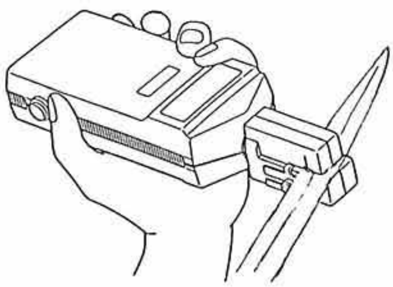


Fig. 3. Measurement of green color scores of leaves by the chlorophyll meter SPAD 501

To measure leaf green color with the SPAD 501, first a piece of leaf is inserted into the head of machine as shown in Fig. 3, and then flushed by a xenon lamp. The transmitted light is separated into infrared and red areas, and the difference in absorbance of the light transmitting in the two areas is expressed on the window of the machine ranging from 0 to 80. The suitable portion for the measurement is a central part of the second uppermost leaf blade of rice plants, which was highly related with the nitrogen status of the plants at given growth stages.

Relation of green color scores obtained with the 'color scale' to chlorophyll and nitrogen contents in leaves

Table 1 shows the result of the study conducted in Fukushima Prefecture by Tanno et al.[11]. In this study the green scores of leaves of Koshihikari and Sasanishiki were determined as described above, and the chlorophyll content was determined by measuring the absorbance at 642.5 and 660 nm using the extract of 200 mg fresh leaves in 50 ml of 85% acetone. The result shows that green color scores of canopy and individual leaves are highly correlated with the average chlorophyll content of the uppermost three or four leaves.

According to the similar study conducted by Manabe et al.[7] on relation of green color scores of rice canopy and individual leaves to leaf chlorophyll contents in Nishihomare and Reiho showed that the regressions were slightly different due to the personal variations, but the correlation was very high (Table 2) in each regression line.

Relation of green color score with nitrogen contents was also studied in many experimental stations. Tanno et al.[11] found out, with Sasanishiki and Koshihikari, a close correlation between canopy color scores and nitrogen content in the whole leaf blades (Fig. 4). Although the regression lines were different at different growth stages, the yearly variation was small at the panicle-formation stage. The investigation by Manabe et al.[7] also showed

Table 1. Relation of color scale scores to chlorophyll contents

(Tanno et al.[11])

Measurement on	Chlorophyll content in	Correlation (r)	n
Single leaf	Uppermost first leaf	0.315	42
	Uppermost two leaves	0.631**	42
	Uppermost three leaves	0.762**	42
	Uppermost four leaves	0.817**	42
Canopy	Uppermost first leaf	0.493	47
	Uppermost two leaves	0.752**	47
	Uppermost three leaves	0.837**	47
	Uppermost four leaves	0.812**	36

** Significant at 1%.

Table 2. Relation of color scale scores to chlorophyll contents

Measurement on	Persons engaged	Regression line	r^2
Canopy	A	Y=0.73X+1.71	0.98
	B	Y=0.50X+2.24	0.93
	C	Y=0.67X+1.96	0.95
	D	Y=0.60X+2.09	0.87
	E	Y=0.80X+1.50	0.85
	F	Y=0.57X+2.11	0.82
Single leaf (Uppermost third leaf)	A	Y=0.93X−1.19	0.92
	B	Y=0.82X+0.06	0.89
	D	Y=0.90X−1.10	0.83
	E	Y=1.10X−1.96	0.89
	F	Y=0.63X+0.74	0.62

Y is chlorophyll content and X is color scale value. Redrawn cited with modification from Manabe et al.[7].

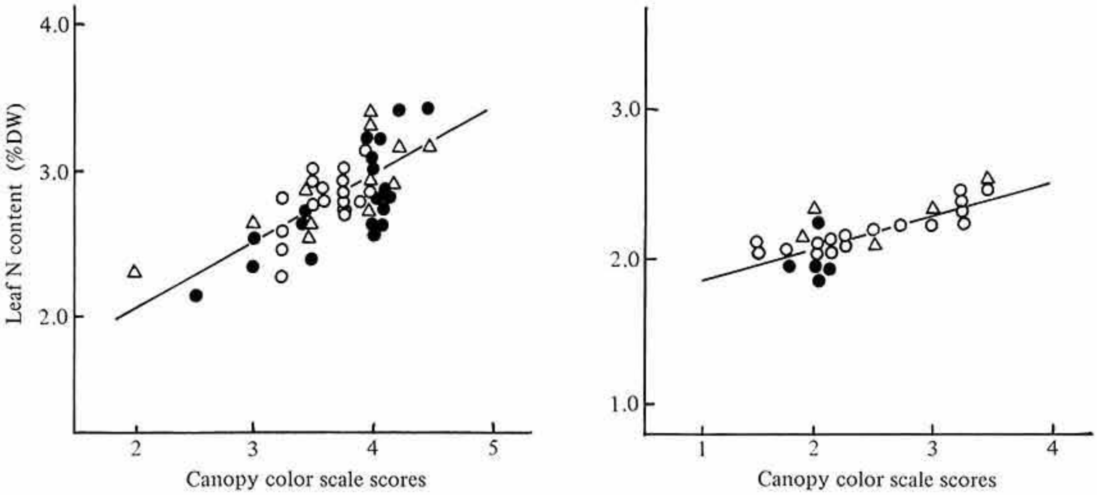


Fig. 4. Relation of canopy color scale scores of Sasanishiki (left) and Koshihikari (right) with leaf N contents (% DW) in 1979 (△), 1980 (●) and 1981 (○)
Regression lines: $Y=0.46X+1.10$ ($r=0.724^{**}$) for Sasanishiki and $Y=0.24X+1.63$ ($r=0.825^{**}$) for Koshihikari (Tanno et al.[11]).

Table 3. Estimate of leaf N or chlorophyll contents from color scale scores

Color scale score	Leaf N (% DW) (by Tanno et al.) Panicle-formation stage Whole leaves (cv. Sasanishiki & Koshihikari)		Leaf N (% DW) (by Manabe et al.) Leaf number index 75-80 Uppermost 2nd leaf (cv. Nishihomare) 1978	1979	Leaf N (% DW) (by Chiubachi et al.) Leaf number index 85 Uppermost 2nd & 3rd leaves (cv. Sasanishiki) 1978	1979—80	Chlorophyll ($mg/100\ cm^2$) (by Manabe et al.) Aug. 27. 1980 Uppermost 3rd leaf (cv. Nishihomare & Reiho)
2	2.0	2.1	1.5	1.3	2.6	1.5	3.2
3	2.5	2.4	2.2	1.9	3.0	1.9	3.9
4	2.9	2.6	3.0	2.5	3.4	2.3	4.5
5	3.4	2.8	3.7	3.1	3.8	2.7	5.2

close relation between canopy color scale values and nitrogen contents of the uppermost second leaf blades in each cultivar at different growth stages. The 1 unit difference in the color score is equivalent to 0.4 to 0.7% of nitrogen content in leaf blades. On the other hand, Chiubachi et al.[2] showed that regression lines of the relation between canopy color scale values and nitrogen contents of developed uppermost 2 to 3 leaves differed year by year; this may be due to different growth patterns of rice plants and also due to differences in nitrogen and carbohydrate metabolism under different climatic conditions.

In Table 3, values of chlorophyll and nitrogen contents corresponding to color scale values from 2 to 5 at the panicle-formation stage or nearby stages calculated from each regression line are given. In conclusion, the relation of color scale values with leaf N contents is very close, although it sometimes differ under different growth conditions.

Relation of green color scores obtained with the chlorophyll meter SPAD 501 (CM values) to chlorophyll and nitrogen contents of leaves

We studied relations between the CM values and chlorophyll and nitrogen contents in the developed uppermost second leaves of high yielding indica varieties (Suweon 258, Akenohoshi, and Nanjing 11) and a japonica variety (Musashikogane) during the period from 1984 to 1987. The chlorophyll content in leaf discs taken from the sites used for measuring CM values was determined with a spectrophotometer using the extracts prepared by extracting chlorophyll with 80% acetone for overnight following the method of Arnon et al.[1].

Fig. 5 shows the relation between CM values and chlorophyll contents in four varieties (1984) and in two varieties (1986) at the panicle-formation stage, and Fig. 6 shows the relation at the panicle-formation (July 23) and full heading (August 13) stages in two varieties (1986). The regression lines were very close although some differences were observed depending on years and growth stages. The regression line for all the data is;

$$Y=0.199\ X-2.868\ (r=0.898^{**}),$$

where Y is chlorophyll content and X is CM value.

Fig. 7 shows the relation between CM values and leaf N contents in four varieties at the panicle-formation stage of four varieties. The regression lines for Musashikogane and Suweon 258, and those for Nanjing 11 and Akenohoshi were close between the two varieties, respectively. The regression for all the data of four varieties is;

$$Y=0.062\ X+0.522\ (r=0.828^{**}),$$

where Y is leaf N content (%DW) and X is CM value. Fig. 8 shows the relation between CM values and leaf N contents in Musashikogane and Suweon 258 at the panicle-formation stage in four successive years from 1984 to 1987. The regression lines for 1984 and 1985, and for 1986 and 1987 were close each other of the two years. The regression line for all the data of four years is $Y=0.076\ X+0.073\ (r=0.815^{**})$. Fig. 9 shows

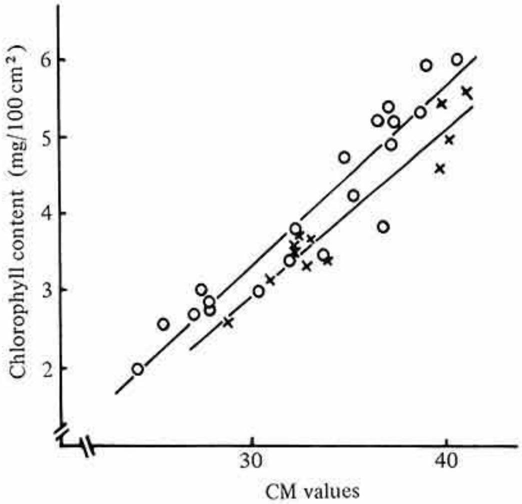


Fig. 5. Relation of CM values to chlorophyll contents at the panicle-formation stage in 1984 (○) and 1986 (×)
Regression lines: $Y=0.232X-3.632$ $(r=0.946^{**})$ for 1984 and $Y=0.214\ X-3.488$ $(r=0.960^{**})$ for 1986.

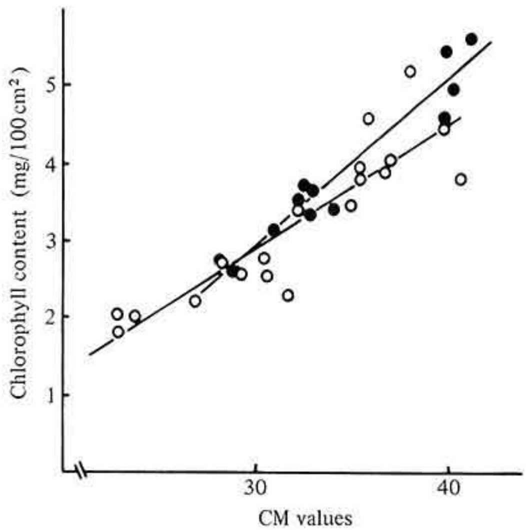


Fig. 6. Relation of CM values to chlorophyll contents on July 23 (●) and on August 13 (○) in Musashikogane and Suweon 258
Regression lines: $Y=0.159X-1.874$ $(r=0.897^{**})$ for July 23 and $Y=0.214X-3.488$ $(r=0.960^{**})$ for Aug. 13.

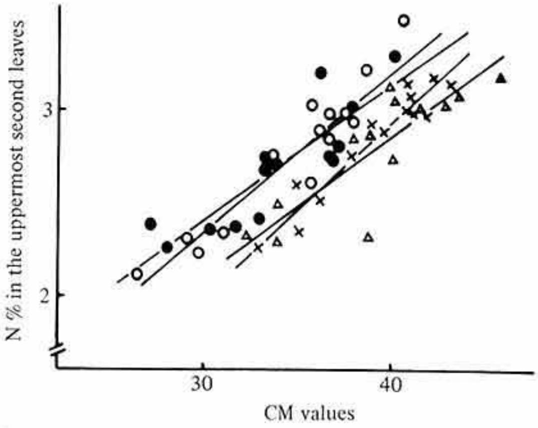


Fig. 7. Relation of CM values to N contents (% DW) in the uppermost second leaves at the panicle-formation stage in Musashikogane (○), Suweon 258 (●), Akenohoshi (×) and Nanjing 11 (△) in 1985

Regression lines: Y=0.086X−0.223 (r=0.956**) for Musashikogane, Y=0.072X+0.253 (r=0.866**) for Suweon 258, Y=0.092X−0.725 (r=0.954**) for Akenohoshi, and Y=0.075X−0.118 (r=0.847**) for Nanjing 11.

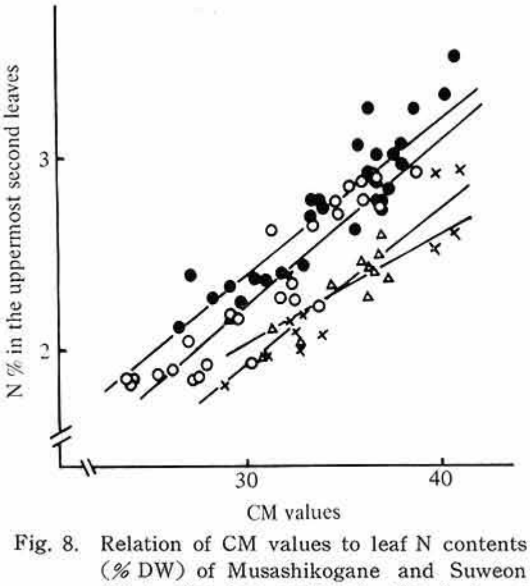


Fig. 8. Relation of CM values to leaf N contents (% DW) of Musashikogane and Suweon 258 in 1984 (○), 1985 (●), 1986 (×) and 1987 (△)

Regression lines: Y=0.084X−0.296 (r=0.938**) for 1984, Y=0.080X−0.001 (r=0.916**) for 1985, Y=0.078X−0.403 (r=0.908**) for 1986 and Y=0.056X+0.362 (r=0.824**) for 1987.

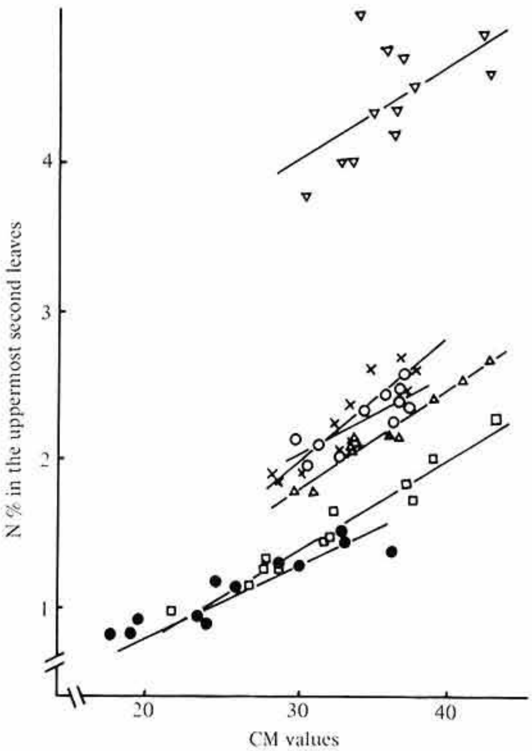


Fig. 9. Relation of CM values to leaf N contents (% DW) at different growth stages in Musashikogane and Suweon 258 in 1987

Regression lines: Y=0.064X+2.132 for June 18 (▽), Y=0.056X+0.362 for July 23 (○), Y=0.086X−0.581 for August 7 (×), Y=0.068X−0.223 for August 20 (△), Y=0.060X−0.404 for September 3 (□), and Y=0.039X+0.117 for September 16 (●).

the relation of CM values and leaf N contents of Musashikogane and Suweon 258 at different growth stage in 1987. The data for June were very different from other seasons. The data at the panicle-formation stage (July 23) and full heading (August 20) were very close. Although the N content decreased thereafter, the regression lines for September 9 and 16 were very close. The regression line is Y=0.060X+0.170 (r=0.840**) for all the data during panicle-formation (July 23) and full heading stages (Aug. 20).

Chiubachi et al.[3] studied the relation of CM values of developed uppermost second leaves to nitrogen contents in the whole leaf blades for Sasanishiki, and found that they

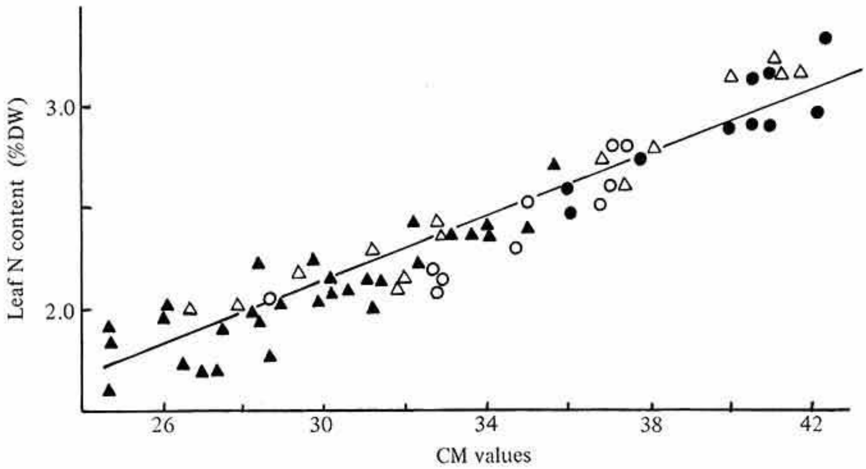


Fig. 10. Relation of CM values with leaf N contents (% DW) on June 25 (●), July 11 (○), July 19 (△), and July 31 (▲) Regression line: Y = 0.074X − 0.07 (r = 0.980**, n = 55) (Chiubachi et al.[3]).

Table 4. Estimate of leaf N and chlorophyll contents from CM values

CM value (SPAD 501)	Leaf N (% DW)			Chlorophyll (mg/100cm²)
	(by Takebe et al.) 1984–87 Panicle-formation stage Uppermost second leaf (cv. Musashikogane & Suweon 258)	(by Chiubachi et al.) July 11–31, 1984 Whole leaves (cv. Sasanishiki)	(by Kitagawa et al.) July 14–22, 1984, 1986 Whole leaves (cv. Koshihikari)	(by Takebe et al.) Panicle-formation stage —Aug. 13, 1984, 1986 Uppermost second leaf (cv. Musashikogane & Suweon 258)
30	2.4	2.2	2.0	3.1
35	2.7	2.5	2.4	4.1
40	3.1	2.9	2.9	5.1

were highly correlated on July 11 and 31 (panicle-formation stage) (Fig. 10). Kitagawa et al.[6] reported that in Toyama Prefecture the regression lines of the two values for Koshihikari differed from season to season, but the regression line for the data during the middle to end of July (panicle-formation to meiosis stages) did not differ in 1986 and 1987.

In Table 4, the CM values ranging from 30 to 40 are translated into nitrogen and chlorophyll contents at the panicle-formation stage. The estimates for Sasanishiki in Miyagi and for Koshihikari in Toyama were very close. The CM values may be a better index of N status of individual leaves than the leaf color scores determined with the color scale.

Conclusion

The green color scores determined using the color scale and CM values obtained using the chlorophyll meter SPAD 501 were highly correlated with leaf N and chlorophyll contents, but the regression lines sometimes differed depending on cultivars, sites of cultivation, and growth stages. Therefore, for the purpose of diagnosing properly the nitrogen requirement of rice plants at given sites, aiming at the ideal growth of the plants, it is necessary to know the relation of green color scores or CM values to leaf nitrogen status at each area for each variety at different growth stages.

Green color scores (CM values) measured

Table 5. Relation of CM values with chlorophyll and nitrogen contents in corn leaves

Measuring date	Relation				n
	CM vs chlorophyll		CM vs N		
June 26, 1984	$Y=0.077X+0.590$	$r=0.723^{**}$	$Y=0.052X+2.569$	$r=0.434^{*}$	35
July 19, 1984	$Y=0.166X-2.566$	$r=0.877^{**}$	$Y=0.064X-0.273$	$r=0.785^{**}$	44

Y is chlorophyll (mg/100 cm^2) or N (% DW) content, and X is CM value.
*, ** Significant at 5 %, 1 %, respectively.

with the SPAD 501 were more closely correlated with leaf N contents than color scores measured with the color scale. However, the color scale method is widely used in Japan. This reason may be because the color scale method is inexpensive, and it also gives the diagnosis for plant canopy. We are now developing a new machine for determining canopy green scores by measurement of light reflection from crop leaves in the field.

We examined the applicability of these methods to upland crops. High correlation between CM values and chlorophyll contents and rather low correlation between CM values and leaf N contents in corn leaves were found as shown in Table 5[10]. Paddy rice plants do not contain any significant amount of nitrate. This point is quite different from upland crops, which sometimes contain a large amount of nitrate. The nitrogen status of upland crops cannot be easily determined from green color scores of leaves unlike the case of paddy rice plants. In addition to green scores of leaves, measurement of nitrate in tissues may be necessary on the nitrogen diagnosis of upland crops.

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