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Development of Critical Values for the Leaf Color Chart, SPAD and Fieldscout CM 1000 for Fixed Time Adjustable Nitrogen Management in Aromatic Hybrid Rice (*Oryza sativa* L.)

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*The nitrogen (N) requirement of hybrid rice is generally greater than in conventional rice varieties. Recommendations for N monitoring at regular intervals of 7–10 days through leaf greenness are available, but farmers are accustomed to apply fertilizer N at selected growth stages only. An inexpensive leaf color chart (LCC) and nondestructive chlorophyll meters were evaluated for site-specific N management strategy in world's first aromatic rice hybrid PRH-10 at the Indian Agricultural Research Institute, New Delhi. Two field experiments were conducted on PRH-10 with four levels of N (0, 70, 140, and 210 kg ha^{-1}) during June–October of 2010 and 2011 to determine the LCC, soil–plant analysis development (SPAD), and Fieldscout CM 1000 (CM 1000) values for achieving economic optimum grain yield at three critical growth stages (tillering, panicle initiation, and flowering). Quadratic regression between N levels and grain yield were used to determine economic optimum grain yield (6427 kg ha^{-1} in 2010 and 6399 kg ha^{-1} in 2011) corresponding to optimum economical dose of 151 kg N ha^{-1} (2010) and 144 kg N ha^{-1} (2011). Nitrogen concentration in fully expanded youngest leaf correlated significantly (*P < 0.01) and positively with LCC score, SPAD value, CM 1000 value, and total chlorophyll concentration at tillering, panicle initiation, and flowering for both years. The critical LCC score, SPAD, CM 1000 values, chlorophyll concentration, and leaf N concentration obtained were at tillering 4.4, 42.3, 285, and 2.16 mg g^{-1} fresh weight and 3.29%; at panicle initiation 4.4, 43.0, 276, and 2.16 mg g^{-1} fresh weight and 3.02%; and at flowering 4.5, 41.7, 270, and 2.05 mg g^{-1} fresh weight and 2.83%, respectively. Corrective N application should be done when observed leaf N indicator values at a particular growth stage reach or go below the critical values.*

Keywords Fieldscout CM 1000, leaf color chart, nitrogen, *Oryza sativa* L., site-specific nutrient management, SPAD

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Introduction

Rice is the most important staple food for large population in India. Rice occupied 36.95 Mha of area and produced 120.62 Mt of grains with a low productivity of 3.26 t ha^{-1} against the world's average productivity of 4.37 t ha^{-1} (FAOSTAT 2011). Replacement of area under existing inbred lines by hybrids could help boost the productivity of rice in India. Rice hybrids can yield 10–44% more grain than the popular high-yielding varieties in India (Wanjari et al. 2006). Nitrogen (N) is the predominant nutrient applied to rice, and its consumption has increased significantly over the past two decades (FAOSTAT 2009). Partial factor productivity (PFP) (i.e., quantity of rice grain produced per unit of applied fertilizer N) has continuously decreased to very low values (Dobermann et al. 2002). Low N-use efficiency in irrigated rice is a result of inefficient splitting of N dose coupled with application of N in excess of crop requirement. When N is managed inefficiently, a large portion of applied N can escape the soil–plant system to reach water bodies and atmosphere, creating pollution problems (V. P. Singh et al. 2010). Farmers will benefit significantly if they can adjust N input according to actual crop conditions and nutrient requirements. Successful N management requires better synchronization between crop N demand and N supply from all sources throughout crop growing season (Cui et al. 2008). Many studies have shown that N fertilizer recommendations that are based on soil tests in flooded rice soils have not been very successful. Hence, plant need–based application of N is crucial for achieving high yield and greater N-use efficiency.

To alleviate problems associated with low N-use efficiency, one of the viable options for efficient N management is site-specific nutrient management (SSNM). The International Rice Research Institute (IRRI) has developed SSNM techniques such as real-time N management (RTNM) and fixed-time adjustable-dose N management (FTNM) to increase the fertilizer-N-use efficiency of irrigated rice. One of the key features of site-specific nutrient management is dynamic adjustment of fertilizer N using gadgets such as leaf color charts (LCC) and chlorophyll meters (B. Singh et al. 2012). Nachimuthu et al. (2007) reported 50% saving of fertilizer N by LCC-guided N management in rice. The Soil Plant Analysis Development (SPAD) meter is a portable and lightweight absorption-based instrument developed by Soil Plant Analyses Development Unit of Minolta Camera Company (Konica Minolta Business Solutions, Tokyo, Japan). It can be used to measure relative chlorophyll levels of leaves. Another hand-held portable reflectance-based gadget, Fieldscout CM 1000 (CM 1000) developed by Spectrum Technologies Ltd. (Spectrum Technologies PLC, Bridgend, Wales, UK), is based on point-and-shoot technology to instantly estimate relative chlorophyll concentration. Recently, Lopez-Bellido et al. (2012) reported successful use of chlorophyll meter (CM) 1000 for N management in creeping bentgreen golf grass. However, there are no reports on the use of CM 1000 for N management in rice; so, the present research also focuses on standardization of this instrument for N management in rice. Use of chlorophyll meter to monitor plant N status in situ and to determine right time of N topdressing in rice has already been established (Huang et al. 2008; Golizadeh et al. 2009). Initially guidelines for using tools such as LCC and chlorophyll meters were based on real time management in which leaves were monitored every 7–10 days (V. Singh et al. 2007; Y. Singh et al. 2007). Oftentimes, farmers do not rely on frequent monitoring of leaf color and spectral properties as they are strongly accustomed to applying fertilizer N at critical growth stages as per blanket recommendations. In view of this, an alternative fixed-time option involving monitoring leaf color with LCC and chlorophyll meters only at critical growth stages such as active tillering around 21 days after transplanting (DAT), panicle initiation at around 42 DAT, and a week before flowering is more practical (B. Singh et al. 2012).

Many studies have evaluated and developed LCC and chlorophyll meters' threshold values for traditional rice varieties but their use for prescribing fertilizer N in aromatic hybrid rice remains an open question because the N requirement of hybrid rice is expected to be greater than traditional inbred cultivars (Gupta et al. 2011). Thus, the present investigation emphasizes use of LCC and chlorophyll meters (SPAD and CM 1000) for fixed-time adjustable site-specific fertilizer N management in transplanted irrigated aromatic hybrid rice. Aim of the study was to compute optimum and critical values of LCC, chlorophyll meter (SPAD and CM 1000), leaf chlorophyll concentration, and leaf N concentration to be maintained at different critical growth stages to achieve economical optimum grain yield.

Materials and Methods

Description of the Experimental Site

Field experiments were carried out during the wet season (June–October) of 2010 and 2011 at the research farm of the Indian Agricultural Research Institute, New Delhi, India (latitude 28.4° N, longitude 77.1° E), which is 250 m above the mean sea level. The climate of the Delhi region is semi-arid and subtropical characterized by dry summers and cold winters. The soil was sandy loam in texture with predominance of illitic type of clay minerals, which is taxonomically categorized under the great group *Typic Haplustept* (old alluvium). Soil of the experimental field had 172 kg ha^{-1} alkaline potassium permanganate–oxidizable N (Subbiah and Asija 1956), 14 kg ha^{-1} 0.5 *N* sodium bicarbonate–extractable phosphorus (P) (Olsen et al. 1954), 146 kg ha^{-1} neutral *N* ammonium acetate–exchangeable potassium (K) (Hanway and Heidel 1952), 0.43% organic carbon (Walkley and Black 1934), pH 8.0, and electrical conductivity of 0.43 dS m^{-1} in 1:2.5 soil-to-water suspension ratio (Jackson 1973).

Experimental Details and Field Techniques

Because India is entering an era of hybrid rice cultivation, a medium-duration (110–120 days) aromatic rice hybrid, Pusa Rice Hybrid-10 (PRH-10), was selected for conducting the field experiment. PRH-10 is world's first aromatic rice hybrid developed by the Indian Agricultural Research Institute, New Delhi. The treatments (4) consisted of three levels of N (70, 140, and 210 kg ha^{-1}) plus one absolute control (no N) and were replicated three times in a randomized block design (RBD). The experimental field was plowed by tractor-drawn disc harrow and then levelled once using a laser leveller before start of the experiment in 2010. The levelled field was puddled twice using tractor-drawn puddler in standing water in both years. Seedlings were transplanted in the first week of July in 2010 and the second week of July in 2011 at a spacing of 20 cm × 15 cm in plots 6.2 m × 2.3 m. Three doses of N, 70, 140, and 210 kg ha^{-1}, were applied in four equal splits at basal, tillering, panicle initiation, and flowering stages of rice growth. Basal doses of P (26 kg ha^{-1}), K (50 kg ha^{-1}), and sulfur (S) (30 kg ha^{-1}) were applied before transplanting in all the plots in both the years. Sources of nutrients used in the experiment were different combinations of urea, single superphosphate, potassium dihydrogen phosphate, and muriate of potash. Twenty-two-day-old seedlings, two per hill, were transplanted in standing water after puddling. Standing water of 5 cm was maintained throughout the growing season in all the plots. Dikes were sufficiently high and compacted enough to prevent seepage, overflow of water, and transfer of nutrients into and from adjacent plots. Weed management and plant protection measures were followed uniformly in all plots as and when required. The crop was harvested in the second week of October in 2010 and third week of October in 2011.

The LCC, SPAD, and CM 1000 Measurements

Swain and Sandip (2010) and later B. Singh et al. (2012) reported three important growth stages of rice crop (tillering, panicle initiation, and flowering) for FTNM where the crop should not suffer from any stresses in order to achieve the target yield. That is why three critical growth stages (tillering, panicle initiation, and flowering) were targeted for LCC, SPAD, CM 1000, and leaf chlorophyll measurements (leaf N indicators). Observations were recorded at 6–8 days after top dressing of fertilizer N. The youngest fully expanded leaves of 10 plants in a plot were used for measurement of these parameters. The observations were made on sunny days. The LCC measurement was done by matching six colored strips of LCC with youngest fully expanded leaf in the shadow of the observer. The SPAD readings were taken on one side of the midrib of the leaf blade, midway between the leaf base and tip. Observations by using CM 1000 were made by the point-and-shoot technique such that the sun is at the user's back and the line between the sample and the sensor is approximately parallel to the sun's rays. Mean of 10 readings per plot was taken as the measured LCC and chlorophyll meters value of that plot.

Plant Sampling and Chemical Analysis

The ten youngest fully expanded leaves (Y leaves) from the top of rice crop at tillering and flag leaves at panicle initiation and flowering stages were sampled from each plot, leaving two border rows. Leaf samples were then cleaned and washed with distilled water to remove surface contamination. On the same day of the sampling, total chlorophyll concentrations of leaves were estimated by the dimethyl sulfoxide method (Hiscox and Israelstam 1979). Crop was harvested at 18% grain moisture content. In each plot, a 1-m^2 area was harvested where plant sampling was not done earlier during crop growth. Grain and straw were separated by threshing. Grain yield was converted to 14% grain moisture content. Leaf samples were kept for oven drying at 60 ± 2 °C until constant biomass was attained. Oven-dried samples were powdered by grinding and analyzed for total N concentration using the micro-Kjeldahl method (Yoshida et al. 1976).

Statistical Analysis

Correlations among LCC score, SPAD value, CM 1000 value, chlorophyll concentration, and N concentration were tested at the 1% level of significance (Gomez and Gomez 1984). Two kinds of regression analysis, linear and polynomial, were performed to test the treatment effects on grain yield and best fits were selected based and coefficient of determination (R^2) and significance level. The polynomial functions were found most appropriate in the present investigation. Moreover, the optimum dose of fertilizer cannot be computed from linear regression equation ($y = a + bx$), but it can be worked out from a quadratic (polynomial) equation ($y = a + bx + cx^2$) when it is significant. Maximum yield dose of

N was calculated by equating first-order derivative of quadratic equation with zero or simply by using formula –b/2c where b stands for linear regression coefficient and c stands for quadratic regression coefficient of a quadratic regression equation. About 90% of maximum yield is generally obtained at an economical N dose (Fageria and Baligar 2001; Fageria, Slaton, and Baligar 2003; Fageria, Santos, and Cutrim 2008). In the present study, economical optimum dose computed by equating quadratic regression equation with price ratio (unit cost of N to unit cost of rice grain = 1.1) was approximately equal to N dose corresponding to 90% of maximum yield. Hence, we estimated economical optimum dose of N at 90% of maximum obtainable grain yield.

Regression equations among LCC score, SPAD, CM 1000 value, chlorophyll concentration, and N concentration in the youngest fully expanded leaf and grain yield were developed. Values of N indicators in youngest fully expanded leaf corresponding to optimum grain yield were considered as their respective optimum value at a particular growth stage. The LCC score, SPAD, CM 1000 value, chlorophyll concentration, and N concentration in youngest fully expanded leaf at three critical growth stages corresponding to critical yield (96% of economic optimum yield) were considered as critical values of respective parameters for N management.

Results

Effects of N Levels on Rice Biomass, N Indicators, and Leaf N Concentration

Regression analysis of applied N with grain yield revealed that grain yield significantly increased with increasing N application levels (Figure 1). Maximum grain yields of 6484 and 6497 kg ha^{-1} were obtained in 2010 and 2011, respectively, with application of 140 kg N ha^{-1}. Grain yield increases of 110.5% in 2010 and 110.6% in 2011 by application of 140 kg N ha^{-1} over control were recorded. In both years, perfect quadratic response between grain yield and applied N was found. Adjustment of such quadratic type curve was highly significant ($P < 0.01$). By using regression equations [$y = -0.121x^2 + 40.69x + 3041$ ($R^2 = 0.98$) for 2010 and $y = -0.133x^2 + 40.69x + 3063$ ($R^2= 0\ .98$) for 2011], maximum grain yield (6461 kg ha^{-1} in 2010 and 6497 kg ha^{-1} in 2011) were obtained at 168 (Figure 1a) and 160 kg N ha^{-1} application level (Figure 1b). Furthermore, economical optimum N doses were computed as 151 (economical optimum yield = 6427 kg ha^{-1}) in the first year and 144 kg N ha^{-1} (economic optimum yield = 6394 kg ha^{-1}) in the second year. Increases in grain yield using regression equations at optimum N dose of 151 and 144 kg N ha^{-1} were 108.6% in 2010 and 107.3% in 2011 over control (no-N) treatment. Grain yields recorded in control were 3080 and 3084 kg ha^{-1} in 2010 and 2011, respectively. Significant ($P < 0.01$) quadratic increase in straw yield was observed with increasing N application level in both years (Figure 2). Unlike grain yield, straw yield was greatest at greater level of N application (i.e., 210 kg ha^{-1}).

Several leaf N indicators were observed throughout the growing season of the rice crop. Results of regression analysis between N application levels and leaf N indicators are furnished in Table 1. In general, leaf N indicator values increased with increasing levels of N application. Highly significant ($R^2 \geq 0.76$, $P < 0.01$) linear and quadratic relationships were observed between N application levels and leaf N indicators. Maximum values of all N indicators were achieved at greater N application level of 210 kg N ha^{-1}. Perusal of data on N concentration in leaf revealed that translocation of N from soil to younger leaves through plant increased significantly ($R^2 \geq 0.86$, $P < 0.01$) and positively with increasing N application levels at all critical growth stages studied.

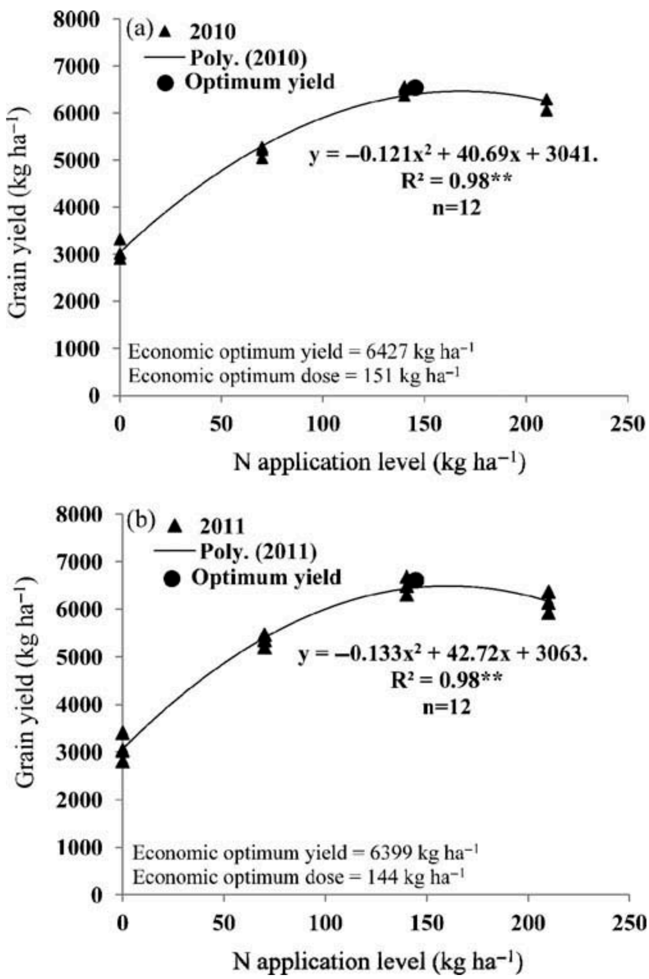

Figure 1. Relationship between N application levels and grain yield of aromatic hybrid rice in (a) 2010 and (b) 2011. **Significant at $P < 0.01$.

Relationship between Leaf N Indicators and Leaf N Concentration

Correlation matrices of different N indicators and leaf N concentration at panicle initiation stage for 2010 and 2011 are presented in Table 2. All leaf N indicators were strongly and significantly correlated ($r \geq 0.83$, $P < 0.01$) with each other and leaf N concentration during both years. Thus, results prove that LCC score, SPAD, and CM 1000 readings are an effective surrogate indicator and predictor of chlorophyll concentration and thereby leaf N concentration at different critical growth stages of hybrid rice. Two chlorophyll meters, SPAD and CM 1000, used for measuring relative chlorophyll concentration in rice crop could be calibrated with each other. The SPAD values increased significantly with increasing CM 1000 values at panicle initiation. Both meters supply different ranges of readings or values, but they were found to be significantly ($P < 0.01$) and linearly correlated with each other (Table 2). Relationship between CM 1000 and SPAD based on range of observations recorded over period of growing season of rice for 2 years could be calibrated as

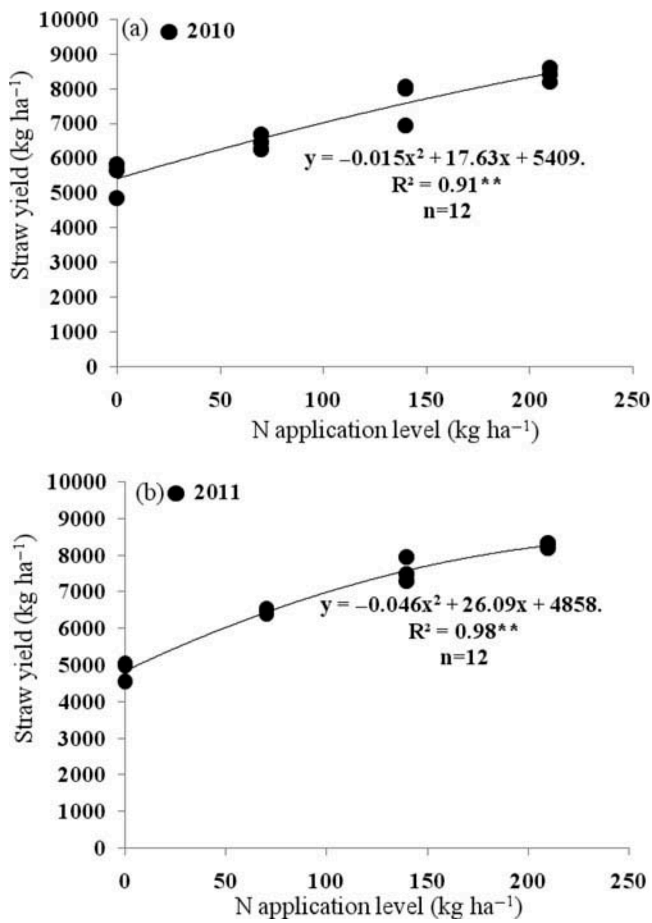

Figure 2. Relationship between N application levels and straw yield of aromatic hybrid rice in (a) 2010 and (b) 2011. **Significant at $P < 0.01$.

CM 1000 reading = 10.71 SPAD – 195.6 ($R^2 = 0.84$, $P < 0.01$) (Figure 3). This enables interconversion of readings between two chlorophyll meters using linear equation.

Relationships among Leaf N Indicators, Leaf N Concentration, and Grain Yield

To translate observations of N indicators into fertilizer recommendations, the relationships among leaf N indicators such as LCC score, SPAD, CM 1000 value, chlorophyll concentration, and N concentration in leaf with grain yield at three critical growth stages were evaluated using regression analysis. Graphical presentation of relationship between mean leaf N indicator (average of three critical growth stages) and grain yield is done in Figure 4. Grain yield showed a significant ($P < 0.01$) linear response with increasing LCC scores in first year (y = 2020x – 2497, $R^2 = 0.86$) (Figure 4a) and second year (y = 2277x – 3610, $R^2 = 0.73$) (Figure 4b) of experimentation. Significant ($P < 0.01$) linear (in 2010) and quadratic (in 2011) responses of grain yield with increasing SPAD values were observed in the present investigation (Figures 4c and 4d). Quadratic response of grain yield with CM

Table 1
Effects of N application levels on leaf N indicators (LCC, SPAD, CM 1000 meter values, chlorophyll concentration) and leaf N concentration at different critical growth stage of PRH-10

Treatment	LCC score		SPAD value		CM 1000 meter value		Chlorophyll content (mg g^{-1} fresh weight)		Leaf nitrogen concentration (%)	
(kg N ha^{-1})	2010	2011	2010	2011	2010	2011	2010	2011	2010	2011
Tillering										
0	3.2	3.3	32.8	31.7	134.6	138.3	0.93	0.91	1.67	1.64
70	3.7	3.4	38.0	37.0	214.5	220.6	1.59	1.48	2.48	2.46
140	4.2	4.2	42.3	42.1	271.7	250.0	1.99	1.86	3.23	3.05
210	4.6	4.8	46.1	46.1	294.7	293.7	2.54	2.53	3.58	3.32
Mean	3.9	3.9	38.9	39.2	228.9	225.7	1.76	1.70	2.74	2.62
R^2 (linear)	0.96**	0.88**	0.76**	0.90**	0.95**	0.94**	0.89**	0.98**	0.98**	0.95**
R^2 (quadratic)	0.96**	0.92**	0.83**	0.91**	0.99**	0.97**	0.89**	0.98**	0.99**	0.99**
Panicle initiation										
0	2.9	3.2	35.1	32.5	131.7	145.2	1.12	1.15	1.55	1.68
70	3.7	3.5	38.1	38.1	204.3	230.3	1.57	1.58	2.26	2.49
140	4.3	4.2	41.8	41.2	263.7	261.5	1.92	1.93	2.62	2.72
210	4.7	4.7	43.8	43.9	288.3	298.1	2.27	2.58	2.97	3.12
Mean	3.9	3.9	39.7	40.5	222.0	233.7	1.72	1.83	2.28	2.50
R^2 (linear)	0.92**	0.90**	0.92**	0.94**	0.92**	0.93**	0.88**	0.96**	0.89**	0.86**
R^2 (quadratic)	0.93**	0.91**	0.92**	0.94**	0.99**	0.97**	0.89**	0.97**	0.89**	0.98**
Flowering										
0	3.0	3.3	32.6	30.5	122.0	128.7	0.85	1.05	1.51	1.55
70	3.5	3.4	37.5	36.5	198.7	221.0	1.25	1.45	2.22	2.37
140	4.2	4.2	39.9	42.4	247.7	253.7	2.00	1.86	2.32	2.93
210	4.7	5.0	43.4	46.6	277.0	288.1	2.91	2.45	2.84	3.25
Mean	3.8	4.0	37.8	39.0	211.3	222.8	1.75	1.71	2.22	2.53
R^2 (linear)	0.89**	0.90**	0.82**	0.96**	0.96**	0.92**	0.93**	0.98**	0.91**	0.95**
R^2 (quadratic)	0.89**	0.95**	0.87**	0.97**	0.98**	0.98**	0.95**	0.99**	0.92**	0.99**

R^2, regression coefficient between N application level and leaf N indicators.
**Significant at the 0.01 level.

Table 2
Correlation matrix of LCC score, SPAD, CM 1000 value, chlorophyll concentrations, and N concentration in flag leaf at panicle initiation of aromatic hybrid rice PRH-10 in 2010 and 2011

N indicator	Year	LCC score	SPAD value	CM 1000 value	Chlorophyll concentration (mg g^{-1} fresh weight)	Flag leaf N concentration (%)
LCC score	2010	1.00	0.97**	0.97**	0.95**	0.95**
	2011	1.00	0.94**	0.85**	0.93**	0.83**
SPAD value	2010		1.00	0.93**	0.95**	0.90**
	2011		1.00	0.96**	0.95**	0.95**
CM 1000	2010			1.00	0.92**	0.92**
Value	2011			1.00	0.94**	0.97**
Chlorophyll	2010				1.00	0.89**
content (mg g^{-1} fresh weight)	2011				1.00	0.88**
Flag leaf N	2010					1.00
concentration (%)	2011					1.00

**Significant at $P < 0.01$.

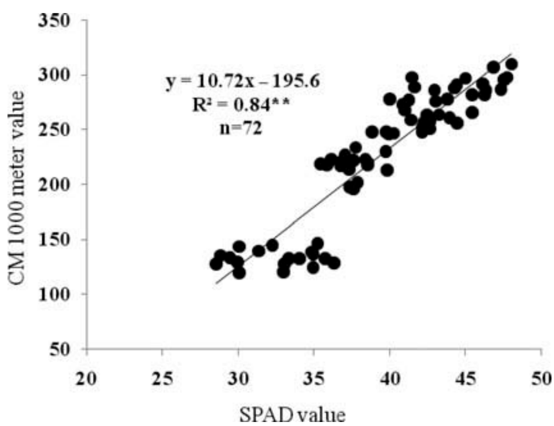

Figure 3. Relationship between Minolta SPAD and Fieldscout CM 1000 readings of youngest fully expanded leaf at tillering, panicle initiation, and flowering over two seasons. **Significant at $P < 0.01$.

1000 meter values having capacity to record greater ranges of values (0–999) is evident in Figures 4e and 4f. Response of grain yield to increasing leaf N concentration was similar to that of chlorophyll concentration and CM 1000 meter values during both years of experimentation (Figures 4g and 4h).

Optimum and Critical Values of Leaf N Indicators and N Concentration

The relationship between leaf N indicators and grain yield was utilized to compute growth-stage-specific and average optimum and critical leaf N indicator values. Data were

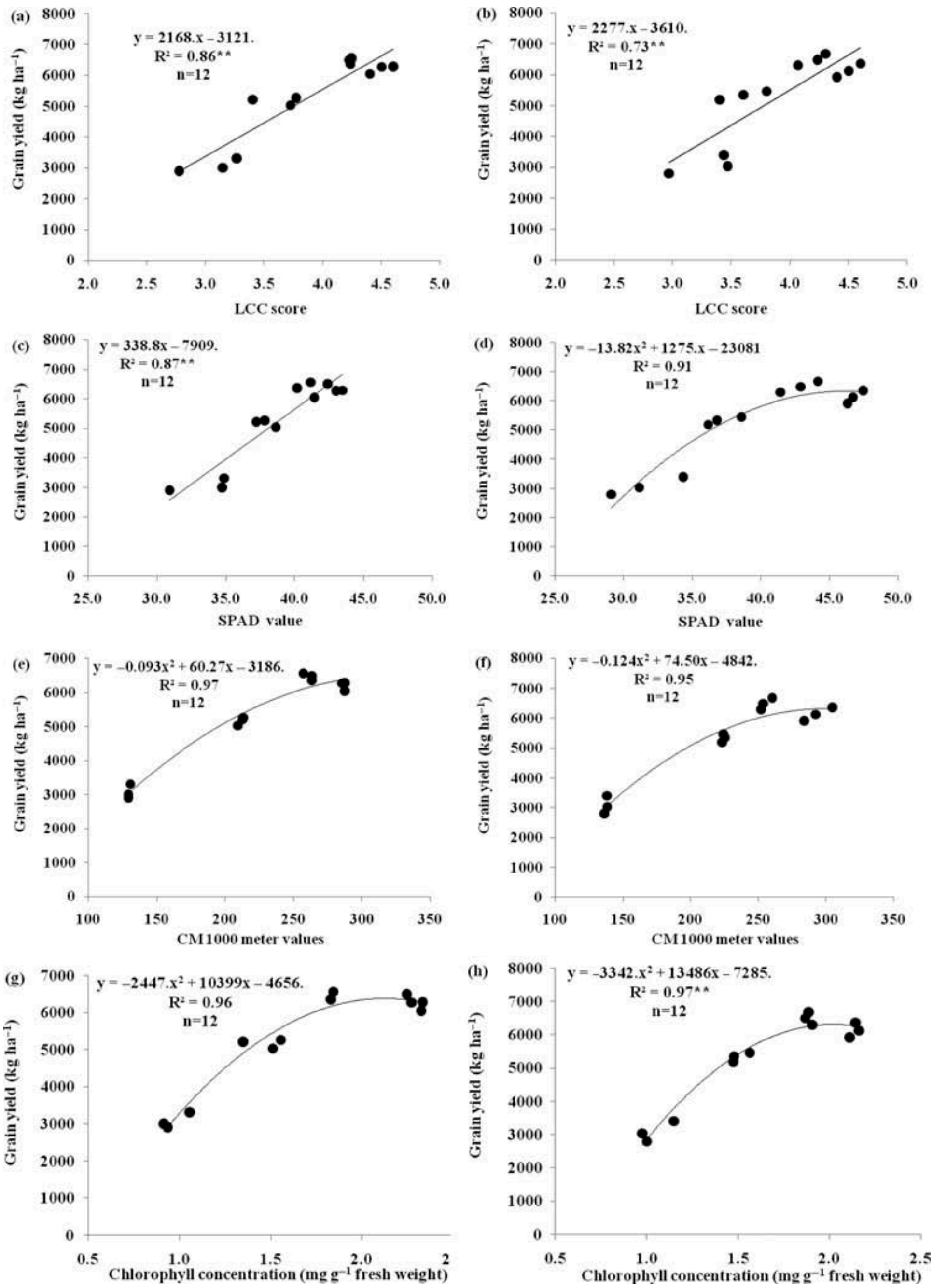

Figure 4. Relationship of grain yield with mean LCC score in (a) 2010 and (b) 2011, SPAD values in (c) 2010 and (d) 2011, CM 1000 in (e) 2010 and (f) 2011, and chlorophyll concentrations in (g) 2010 and (h) 2011. **Significant at $P < 0.01$.

analyzed across year for this purpose. Leaf N indicator value corresponding to mean economic optimum grain yield of 6411 kg ha^{-1} was considered as the optimum value of leaf N indicator.

Using this approach average optimum LCC score, SPAD, CM 1000 values, chlorophyll concentration, and leaf N concentration to obtain economic optimum yield were found to be 4.4, 44.0, 284, and 2.21 mg g^{-1} fresh weight and 3.15%, respectively (Table 3). Immediate insignificantly lower grain yield (96% of the economic optimum grain yield) was the critical grain yield. Values of leaf N indicator corresponding to critical grain yield calculated using regression relationship (Table 3) were considered as critical values of leaf N indicator. The critical growth-stage-specific LCC score, Minolta SPAD, CM 1000 values, chlorophyll concentration and leaf N concentration observed in the present investigation were at tillering 4.4, 42.3, 285, 2.16 mg g^{-1} fresh weight and 3.29%, at panicle initiation 4.4, 43.0, 276, 2.16 mg g^{-1} fresh weight and 3.02%, and at flowering 4.5, 41.7, 270, 2.12 mg g^{-1} fresh weight and 2.83%, respectively (Table 3). Average critical leaf N indicator values of LCC, SPAD, CM 1000 values, chlorophyll concentration and leaf N concentration were 4.3, 42.2, 273, 2.05 mg g^{-1} fresh weight and 3.02%, respectively (Table 3). Because values of LCC score, SPAD, CM 1000 showed variations at different growth stages, growth-stage-specific optimum and critical values of N indicators were calculated using regression relationship between leaf N indicators and grain yield at that particular growth stage (Table 3).

Discussion

Effects of N Levels on Rice Biomass, N Indicators and Leaf N Concentration

Response of the grain yield to applied N followed quadratic response function. In the present experiment there was a response to the successive increasing N doses, but once optimal dose (140 kg N ha^{-1}) was reached, a maximum yield was obtained and yield did not increase with further increase of N fertilizer application (Figure 1). This observation was similar to those by B. Singh et al. (2002), Nachimuthu et al. (2007), Huang et al. (2008), Chen, Huang, and Tang (2011), and Swain and Sandip (2010). Swain and Sandip (2010) reported an increase in grain yield of rice variety Swarna up to 150 kg N ha^{-1} application level. In yet another study by Nachimuthu et al. (2007), rice variety CO47 showed a quadratic response function between grain yield and applied N. In the present experiment, grain yield of rice increased with increasing N application up to 140 kg ha^{-1}. Optimal N application caused vigorous root development ensured exploration of soil by roots and greater vegetative growth may have caused absorption of macro- and micronutrients optimally. This might be due to greater growth rate rice from panicle initiation to ripening stage of crop this hybrid, and this observation was found to be concordant with those of Huang et al. (2008), who reported greater total biomass of popular rice hybrid LYP9 in China due to rapid growth during flowering and ripening phase. However, further increase in application of N (210 kg ha^{-1}) showed a decreasing response with grain yield. At very high application levels of N, greater N uptake caused greater leaf area index, which enhanced carbohydrate loss via dark respiration (Shi and Akita 1993) and thereby decreased grain yield. Increase in values of N indicators such as LCC, chlorophyll meter, and chlorophyll concentration with graded doses of N is an expected result. The greatest values of N indicators were achieved at application of 210 kg N ha^{-1}. Progressive increases in LCC score, SPAD, and CM 1000 meter values were observed with graded doses of N (Table 1). Huang et al. (2008) reported linear and significant ($r = 0.77–0.95$, $P < 0.05$) increase in SPAD values in two rice varieties with increasing N application rate as observed in the present experiment. Absorption of radiations at red (640 nm) and infrared (950 nm) wavelength

Table 3
Relationship of leaf N indicators with grain yield at three critical growth stages of PRH-10 and their corresponding optimum and critical values

Days after transplanting	Critical growth stage	Relationship	R^2	Optimum value	Critical value
		LCC score			
21	Tillering	$y = 1982x - 2542$	0.66**	4.5	4.4
52	Panicle initiation	$y = 1760x - 1606$	0.74**	4.5	4.4
75	Flowering	$y = 1517x - 703.3$	0.62**	4.6	4.5
	Mean	$y = 2213x - 3327$	0.79**	4.4	4.3
		SPAD readings			
21	Tillering	$y = -9.908x^2 + 991.2x - 18093$	0.84**	44.1	42.3
52	Panicle initiation	$y = -14.40x^2 + 1384x - 26741$	0.84**	44.8	43.0
75	Flowering	$y = -14.21x^2 + 1310x - 23730$	0.86**	43.4	41.7
	Mean	$y = -13.10x^2 + 1251x - 23325$	0.87**	44.0	42.2
		CM 1000 readings			
21	Tillering	$y = -0.120x^2 + 72.62x - 4624$	0.96**	297	285
52	Panicle initiation	$y = -0.092x^2 + 61.19x - 3624$	0.95**	287	276
75	Flowering	$y = -0.111x^2 + 66.26x - 3495$	0.95**	281	270
	Mean	$y = -0.107x^2 + 66.55x - 3910$	0.95**	284	273
		Chlorophyll concentration (mg g^{-1} fresh weight)			
21	Tillering	$y = -1785x^2 + 8073x - 2710$	0.91**	2.25	2.16
52	Panicle initiation	$y = -2558x^2 + 11583x - 6517$	0.91**	2.25	2.16
75	Flowering	$y = -1514x^2 + 6500x - 2005$	0.86**	2.14	2.05
	Mean	$y = -2661x^2 + 1120x - 5418$	0.95**	2.21	2.12
		N concentration (%)			
21	Tillering	$y = -1054x^2 + 7234x - 6057$	0.98**	3.43	3.29
52	Panicle initiation	$y = -1581x^2 + 9571x - 8163$	0.88**	3.15	3.02
75	Flowering	$y = -1612x^2 + 9519x - 7705$	0.92**	2.95	2.83
	Mean	$y = -1370x^2 + 8634x - 7271$	0.96**	3.15	3.02

R^2, regression coefficient between N application level and leaf N indicators.
**Significant at the 0.01 level.

increases with increase in chlorophyll concentration. As the absorbance increases, so does the reading by SPAD meter. Likewise as the the absorbance increases, the reflectance decreases, which is reflected by greater digital values shown by the CM 1000 chlorophyll meter at greater N application level. The response of N indicators and leaf N concentration to increasing N application at tillering and flowering stages was moreover similar to that at panicle initiation stage of rice crop (data not shown).

Relationship between N Indicators and Leaf N Concentration

Being component of chlorophyll, N validates our result of strong correlation among LCC score, chlorophyll meter, chlorophyll concentration, and leaf N concentration in rice hybrid. These observations are concordant with those by Nachimuthu et al. (2007), Gholizadeh et al. (2009), and Cabangon, Castillo, and Tuong (2011). There is no report on use of CM 1000 for N management and its optimization for fetching optimum yield of rice. We found positive and linear relationship of CM 1000 values with chlorophyll and N concentration in leaf at different growth stages (Table 2). This is because N is an important component of chlorophyll and chlorophyll is a lead pigment in absorbing radiation at peak wavelength of 650 and 940 nm. The CM 1000 provides greater range of readings (0–999) compared to SPAD-502 (0–99.9). Reproducibility or precision of either of these instruments can be tested using validation experiments in future. A major limitation associated with such type of chlorophyll meter is that it requires bright sunshine for accurate measurement of reflectance. Brightness (BRT) range of this instrument is 0–9. In the present study observations were recorded at BRT values of 3 to 6. Observations recorded at very lower BRT values are misleading. Besides this limitation, use of such reflectance-measuring chlorophyll meter could be promoted for N management in lowland rice. Further, more field studies are needed to calibrate and validate CM 1000 chlorophyll meter for its wide application in lowland rice. The LCC is an inexpensive tool for measuring of leaf N, chlorophyll meters are easy to operate but comparatively expensive, and chemical estimation of leaf N is tedious and at the same time there are many other factors that determine leaf greenness such as water stress or deficiency of nutrients other than N (V. Singh et al. 2010). Under such circumstances an easy-to-estimate indicator of leaf N, i.e., chlorophyll concentration can be used as an alternative measure of plant N status in rice wherever facilities are available for its extraction and estimation. Estimation of leaf chlorophyll using dimethyl sulfoxide (DMSO) method is relatively simple, rather than tedious N analysis that requires digestion and distillation. To estimate relative N in real time or on the day of sampling, leaf chlorophyll estimation using DMSO method could be a viable approach for N management. Considering this point, the relationship of chlorophyll concentration with leaf N concentration was assessed at different critical growth stages of rice crop, which was found to be significantly ($P < 0.01$) and positively correlated (r = 0.88–0.89, panicle initiation) throughout the growing season over both years (Table 2). Cabangon, Castillo, and Tuong (2011) showed a good and highly significant ($R^2 = 0.70$, $P < 0.01$) linear relationship between LCC and SPAD readings in rice, which is in agreement with the findings of the present study. Similar observation was made by Sen et al. (2011). Approach of calibrating two chlorophyll meters, SPAD and CM 1000 meter, was attempted on the basis of strong and highly significant ($R^2 = 0.84$) relationship (Figure 2). Arregui et al. (2006) calibrated SPAD (Minolta) with N-tester (Hydro-Agri) and reported high correlation (r = 0.98).

Relationship among N Indicators, Leaf N Concentration and Grain Yield

Scatter diagrams (Figures 4a–h) of relationship between N indicators with grain yield showed significant ($P < 0.01$) linear responses during both the years of experimentation. Leaf N concentration increased with increasing N application until vegetative growth but with advancement of reproductive growth N was further remobilized to reproductive parts of the plant. This could be a reason for progressive increase in values of leaf N indicators with increasing grain yield of rice. These relationships are of practical importance to translate information of N indicators at different critical growth stages to prescribe time and amount of N fertilizer to be applied. Nachimuthu et al. (2007) observed a significant and positive correlation (r = 0.75) of LCC with N uptake and grain yield in wet-seeded rice. Huang et al. (2008) found a significant ($R^2 = 0.79$–0.80, $P < 0.01$) relation of SPAD values with grain yield for two different popular rice varieties in China.

Optimum and Critical Values of N Indicators and Leaf N Concentration

In the present experiment, growth-stage-specific optimum values of N indicators were calculated from the relationship between leaf N indicators and grain yield at three critical growth stages (tillering, panicle initiation, and flowering) (Table 3). Growth-stage-specific optimum values of these N indicators are supposed to maintain at respective critical growth stage to obtain economical optimum grain yield of aromatic rice hybrid. Guidelines available in reported literature to date deals with a single threshold value of LCC and chlorophyll meter to prescribe fertilizer N throughout growing season. Whenever the value of LCC and chlorophyll meter falls below a single threshold value, recommendations are to apply fertilizer N in the prescribed amount. However, the requirement and uptake of N by rice crop are different at different critical growth stages. Except for LCC, other leaf N indicator values had greater variation in critical values at different growth stages (Table 3). Perusal of data presented in Table 3 reveals that LCC score of 4.5 seems to be a logical score to obtain economical optimum grain yield. Difference between optimum and critical value is only 0.1. It is important to maintain a LCC score of more than 4.0 in aromatic hybrid rice. Only one threshold value over the entire growing season for LCC is because of lower range of variation (0–6 colored strip) to quantify leaf greenness. However, at different growth stages, optimum and critical values of SPAD were more than 40.0 (Table 3). The CM 1000 meter was suitable for measuring relative chlorophyll and N in rice leaf. A single threshold value approach is suitable for LCC, but a different value approach at different growth stages should be adopted for SPAD and CM 1000. A similar kind of approach by equating first-order derivative of the response function to the price ratio was used to determine optimum N doses and thereby optimum LCC values by Nachimuthu et al. (2007), SPAD value by Swain and Sandip (2010) in rice, and Scharf et al. (2006) in corn. Swain and Sandip (2010) computed optimum SPAD values of 35.4 and 40.1 for two rice varieties, Lalat and Swarna, respectively, using a quadratic response function. They had also derived growth-stage-specific optimum as well as critical SPAD values for two different duration rice varieties. Huang et al. (2008) calculated optimum SPAD threshold 36 for SY63 and 38 for LYP9 grown under subtropical conditions for precise N management for aiming high rice grain yield and fertilizer N use efficiency. To make N management more precise, the concept of optimum values was extended further to derive critical values of N indicators. There are studies for use of critical or optimal SPAD values for attaining the desired target yield. An immediate corrective action is needed whenever the critical values of N indicators reach or go below critical value at three critical growth stages to avoid reduction in yield. For precise N management in hybrid rice, growth-stage-specific optimal and critical values of N indicators are more realistic than the average one because values of N indicators vary at different critical growth stages.

Conclusion

The world's first aromatic hybrid rice PRH–10 had a high N requirement of 151 kg ha^{-1} and 141 kg ha^{-1} for achieving economic optimum grain yield of 6427 and 6399 kg ha^{-1} in 2010 and 2011, respectively. The required levels of LCC, SPAD, CM 1000, chlorophyll concentrations, and leaf N concentration to be maintained at three critical stages (tillering, panicle initiation, and flowering) to attain economical optimum yield have been developed. The required LCC score, Minolta SPAD, CM 1000 values, chlorophyll concentration, and leaf N concentration to obtain economic grain yield of hybrid rice were at tillering 4.4, 42.3, 285, 2.16 mg g^{-1} fresh weight and 3.29%, at panicle initiation 4.4, 43.0, 276, 2.16 mg g^{-1} fresh weight and 3.02%, and at flowering 4.5, 41.7, 270, 2.05 mg g^{-1} fresh weight and 2.83%, respectively. The average LCC score, SPAD, CM 1000 value, chlorophyll concentration, and leaf N concentration that are supposed to be maintained throughout the growing season for achieving optimum yield were 4.3, 42.2, 273, 2.12 mg g^{-1} fresh weight and 3.02%, respectively. Adoption of growth-stage-specific leaf N indicator value guided fixed time N management is a more practical approach than a single threshold value approach in rice. Such growth-stage-specific optimum and critical values of N indicators have been developed in the present investigation. Two consecutive field experiments were carried out on irrigated transplanted hybrid rice on an alluvial soil (Inceptisol) under semiarid and subtropical climatic conditions, and results of the experiment are expected apply under similar situations. Because leaf greenness varies among varieties and hybrids, results would more suited to rice hybrids than traditional varieties. Experimental soils were inherently low in soil-available N. Quadratic response between grain yield and N application levels might be reproducible on N-deficient soils. A six-color-panel LCC (Pretech-IRRI) developed by International Rice Research Institute was used in the present investigation. Adoption of fixed-time adjustable N management using aforementioned LCC will ensure the same results. The present study shows that reflectance measuring CM 1000 can be used for N management in irrigated hybrid rice. Because CM 1000 meter used reradiated solar radiations, brightness variations or cloudy conditions may incorporate errors in CM 1000 measurements. Use of CM 1000 under bright sunshine is advised to manage N efficiently. Further studies are needed for its calibration and large-scale application in rice and other crops under different systems.

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