Hindawi Publishing Corporation
International Journal of Agronomy
Volume 2010, Article ID 863605, 11 pages
doi:10.1155/2010/863605

Research Article

Effect of Abiotic Stresses on the Nondestructive Estimation of Rice Leaf Nitrogen Concentration

Stephan M. Haefele,[1] Joel D. L. C. Siopongco,[2] Serafin T. Amarante,[1] and To Phuc Tuong[1]

[1] *Crop and Environmental Sciences Division, International Rice Research Institute (IRRI), Los Baños, Laguna, DAPO Box 7777, Metro Manila, Philippines*
[2] *Department of Rice Research, National Institute of Crop Science, 2-1-18, Kannondai, Tsukuba, Ibaraki, 305-8518, Japan*

Correspondence should be addressed to Stephan M. Haefele, shaefele@cgiar.org

Received 25 December 2009; Revised 17 May 2010; Accepted 17 May 2010

Academic Editor: Rodomiro Ortiz

Decision support tools for non-destructive estimation of rice crop nitrogen (N) status (e.g., chlorophyll meter [SPAD] or leaf color chart [LCC]) are an established technology for improved N management in irrigated systems, but their value in rainfed environments with frequent abiotic stresses remains untested. Therefore, we studied the effect of drought, salinity, phosphorus (P) deficiency, and sulfur (S) deficiency on leaf N estimates derived from SPAD and LCC measurements in a greenhouse experiment. Linear relations between chlorophyll concentration and leaf N concentration based on dry weight (N_{dw}) between SPAD values adjusted for leaf thickness and N_{dw} and between LCC scores adjusted for leaf thickness and N_{dw} could be confirmed for all treatments and varieties used. Leaf spectral reflectance measurements did not show a stress-dependent change in the reflectance pattern, indicating that no specific element of the photosynthetic complex was affected by the stresses and at the stress level applied. We concluded that SPAD and LCC are potentially useful tools for improved N management in moderately unfavorable rice environments. However, calibration for the most common rice varieties in the target region is recommended to increase the precision of the leaf N estimates.

1. Introduction

For more than a decade, considerable efforts have been made to develop and establish improved nutrient management options for rice (*Oryza sativa* L.). The goals were concepts and tools allowing site- and season-specific (i.e., real-time) adaptation of nutrient management recommendations [1]. These efforts resulted in technologies contributing considerably to increased productivity of Asian rice farmers, improved use efficiency of scarce and expensive resources, and reduced detrimental environmental effects [2]. However, these developments are mostly limited to irrigated systems because the improved nutrient management technologies did not target rainfed rice systems. But recent developments show that, because of the increasing availability of input responsive varieties for rainfed lowlands [3], these environments are characterized by the fastest yield growth rates, often enabled by increasing fertilizer use. Therefore, improved fertilizer technologies are also needed for rainfed lowlands in order to increase the productivity of rice farming in these environments to maximize the efficiency of fertilizer use and to minimize negative environmental side effects of such practices.

Nitrogen (N) as the most commonly used fertilizer element and the one limiting growth in the majority of environments is the most adequate target for site- and season-specific nutrient management. In irrigated environments, portable field tools to monitor crop N status are increasingly used for the fine-tuning of N management throughout the season. Widely used tools in rice are the chlorophyll meter (SPAD) and the leaf color chart (LCC) [4–7]. Both tools are based on the relation between leaf chlorophyll concentration, which largely determines leaf color, and is an important parameter determining photosynthetic rate and biomass production and leaf N concentration (e.g., [8]). However, it is well known from field observations that abiotic stresses, which are much more common in rainfed rice environments than in irrigated systems, can affect leaf color [9]. Widespread abiotic stresses in rainfed lowlands are drought, submergence, salinity, P deficiency, and soil acidity; less common stresses are K, S, Zn, and Fe deficiency and Fe toxicity [3, 10]. However, little is known whether and how such leaf color changes in rice affect estimates of crop N status based on tools such as SPAD or the LCC.

Therefore, the objective of our study was to investigate in a greenhouse trial (i) the effect of selected abiotic stresses on chlorophyll and leaf N concentration and (ii) to evaluate whether leaf N estimates based on SPAD or LCC measurements would still be reliable under these conditions. A set of different rice varieties were used in the study to represent typical germplasm grown in rainfed environments and also to include potentially different varietal response to abiotic stresses.

2. Materials and Methods

We conducted a greenhouse experiment at the end of the dry season/beginning of the wet season in 2005 (April to July) at the International Rice Research Institute (IRRI) in Los Baños, Laguna, Philippines (14°11′ N, 121°15′ E; elevation 21 m). Treatments were designed to investigate the effect of four different abiotic stresses: drought (±W), salinity (±SAL), phosphorus deficiency (±P), and sulfur deficiency (±S). All treatments and varieties used are shown in Table 1. The varieties used were chosen to represent recently developed, modern germplasm (IR72, IR65192-4B-10-3, PSBRc9) as well as older or even traditional cultivars (Mahsuri, Swarna, Dular). Two of these cultivars were reported to have a higher stress tolerance, IR65192-4B-10-3 for salinity [11] and Dular for P deficiency [12]. Each treatment × variety combination was replicated three times, and each replication consisted of four pots with three individual rice plants (except for salinity stress, for which only two plants per pot were used). Inner pot diameter was 0.15 m for salinity treatments (including the nonsaline control) and 0.25 m for all other treatments. Soil column depth was 0.45 m for the water stress treatments, 0.2 m for S and P treatments, and 0.12 m for the salinity treatments.

Table 1: Average grain weight, straw weight, and panicle number at maturity for all treatments and all varieties used in the greenhouse experiment, including results of the analysis of variance conducted separately for each stress treatment.

Variety	Treatment[(b)]	Grain weight (g per pot)	Straw weight (g per pot)	Panicle number (per pot)
IR72	+W	48.5	69.5	30.7
IR72	–W	65.1	91.9	39.7
Mahsuri	+W	46.8	207.4	28.1
Mahsuri	–W	60.6	217.2	34.4
PSBRc9	+W	45.4	64.0	17.3
PSBRc9	–W	67.0	91.8	23.1
Swarna	+W	52.9	87.5	27.7
Swarna	–W	87.6	117.6	38.6
Treatment [(a)]		***	***	***
Variety		*	***	***
T×V		ns	ns	ns
IR65192-4B-10-3	–Sal	61.2 b	132.9 a	16.2 a
IR65192-4B-10-3	+Sal	1.2 d	90.1 c	5.0 c
IR72	–Sal	71.0 a	113.5 b	15.5 a
IR72	+Sal	12.5 c	82.9 c	11.1 b
Treatment [(a)]		***	**	***
Variety		***	ns	Ns
T×V		Ns	ns	Ns
IR72	+S	18.9 ns	31.2 a	13.3 ns
IR72	–S	19.3 ns	28.7 b	12.5 ns
Treatment [(a)]		ns	*	ns
Dular	+P	18.4	23.2	9.7
Dular	–P	14.3	16.4	8.5
IR72	+P	21.6	26.1	14.0
IR72	–P	14.6	15.3	11.4
Treatment [(a)]		***	***	**
Variety		ns	ns	Ns
T×V		ns	⋆⋆	Ns

[(a)] Significance levels indicated for the ANOVA are ns: $P > .05$; *: $.05 \leq P \leq .01$; **: $.01 \leq P \leq .001$; ***: $P < .001$.

[(b)] Note that the nonstressed control treatments are indicated by +W, +S, and +P, but by –Sal.

Drought stress was applied by lowering the soil water potential to around −50 kPa at 15 cm depth from 34 to 46 days after seeding (DAS) and from 75 to 84 DAS (monitored daily with ceramic cup tensiometers following the protocol described in [13]). According to Bouman and Tuong [14], this soil moisture potential represents considerable drought stress for most rice varieties. In all nondrought treatments, a water layer of 0.05 m depth was maintained from early seedling stage (10 DAS) to harvest. For the salinity treatment, perforated pots were used, and all pots of one replication were placed in a large tub. For the saline treatment, water conductivity in the tub was maintained at $0.3\,\mathrm{S\,m^{-1}}$ in the early phase (21 to 40 DAS) and at $0.6\,\mathrm{S\,m^{-1}}$ in the later growth stages (41 DAS to maturity), which was monitored and adjusted daily (modified from [15]). Soils used for the experiment were collected from the IRRI experimental farm at Los Baños, Laguna, for the drought and salinity treatments, from a known P-deficient site in Pangil, Laguna (60 km east of IRRI), and from a known S-deficient site in San Juan, Batangas (70 km southwest of IRRI). Respective analytical soil results are given in Table 2. Inorganic fertilizer quantity per pot was calculated based on the number of plants per pot, the medium rate of 60-40-40 kg N-P_2O_5-K_2O per hectare, and the average plant density in the field of 20 plants per m^2. Fertilizers used were urea ($460\,\mathrm{g\,N\,kg^{-1}}$), triple superphosphate ($460\,\mathrm{g}\ P_2O_5\mathrm{kg^{-1}}$), muriate of potash ($600\,\mathrm{g}\ K_2O\,\mathrm{kg^{-1}}$), and ammonium sulfate ($210\,\mathrm{g\,N\ kg^{-1}}$,

Table 2: Some characteristics of the soils used for the four different abiotic stresses tested.

Soil property	Unit	Soil used for Drought and salinity	Sulfur deficiency	Phosphorus deficiency
pH (1:1)	(-)	5.8	7.1	5.1
C_{org}	(g kg^{-1})	20	16	11
Total N	(g kg^{-1})	2.0	1.5	1.1
Olsen P	(mg kg^{-1})	41	25	2.4
CEC	(cmol kg^{-1})	24.5	34.5	19.6
SO_4^{-2} S	(mg kg^{-1})	nd	0.6	nd
Sand	(g kg^{-1})	280	110	30
Silt	(g kg^{-1})	360	430	370
Clay	(g kg^{-1})	360	460	600

240 g SO_2 kg^{-1}). The nutrient amount applied per plant was 300 mg N, 87 mg P, 166 mg K, and 87 mg S (but the ammonium sulfate fertilizer was only used in the +S treatment). No P and no S were applied in the P-stress and the S-stress treatments, respectively. All fertilizers were incorporated before seeding.

For P, S, and salinity treatments, leaf chlorophyll and leaf N parameters were determined at 29, 50, and 68 DAS. For the drought treatment, the same parameters were determined before the stress period (34 and 75 DAS), at the end (47 and 85 DAS), and 10 days after the stress period (57 and 95 DAS). At each sampling date and for each treatment, measurements started with a chlorophyll meter (SPAD-502, Minolta) and a leaf color chart reading (LCC, IRRI standard). SPAD and LCC readings were conducted for 12 leaves (one youngest fully expanded leaf [Y-leaf] of each plant in a pot); for measurement procedures see Peng et al. [16] and Balasubramanian [17]. All measured Y-leaf blades were detached and immediately stored on ice. Six of these Y-leaves were used for further determination of leaf spectral reflectance (spectrophotometer CM-3700d, Minolta), leaf area (LI-3000, LiCOR, Lincoln, NE, USA), dry weight, and total N. Reflectance was measured in 10-nm bandwidths from 400 to 700 nm wavelength (three scans per leaf blade). Dry weight was determined after oven-drying at 70°C to constant weight. Specific leaf weight (SLW) was calculated as the ratio of dry weight to leaf area. Total leaf N was analyzed by micro-Kjeldahl digestion and distillation [18]. Leaf N concentration was expressed on a dry weight (N_{dw}) and leaf area (N_a) basis. The other six Y-leaves were used for the determination of chlorophyll content according to Mackinney [19]. Chlorophyll concentration was also expressed on a dry weight (Ch_{dw}) and leaf area (Ch_a) basis. At maturity, all above-ground biomass was harvested and analyzed for panicle number, grain yield, grain moisture content, and straw yield.

For each stress, all pots with a different treatment x variety combination were clustered in a block, and all blocks were oriented perpendicular to the southern side of the greenhouse to ensure a homogeneous exposure to the light gradient along the greenhouse wall. Distance between the pots was about 0.15 m. The experimental layout within each block represented a randomized complete block design. Standard ANOVA and linear regression analysis, including the F-test for homogeneity of regression coefficients, were applied for the statistical analysis using Jandel Scientific Software SigmaStat 2.0 (SPSS Science, Chicago, IL, USA).

3. Results

3.1. General Indicators and Effects of the Applied Stresses. In the drought stress treatment (−W), the water tension at 0.15 m depth was fluctuating slightly around the target of −50 kPa, and the observed average soil water tension was −47 kPa during both drought phases. But, contrary to expectations, drought stress consistently and significantly increased grain yield, straw yield, and tiller number per pot (Table 1). Mahsuri had a significantly higher straw yield, and Swarna had the highest average grain yield. Salinity (+Sal) as well as P deficiency (−P) significantly reduced grain yield, straw yield, and tiller number, but only straw weight was significantly affected by the sulfur treatments (±S). Average P concentrations of the Y-leaf measured at 29, 50, and 68 DAS in both P treatments differed significantly only at 29 DAT (1.1 g kg^{-1} and 1.3 g kg^{-1} for IR72 and the –P and +P treatment, resp.; 0.9 g kg^{-1} and 1.1 g kg^{-1} for Dular and the –P and +P treatment resp.), but all P concentration values were close to the critical lower value of 1 g kg^{-1} P, a threshold suggested by Dobermann and Fairhurst in [9]. Y-leaf S concentrations also differed significantly at 29 DAS (2.4 g kg^{-1} and 2.6 g kg^{-1} for IR72 and the –S and +S treatment, resp.), but both values were above the critical lower value of 1.6 g kg^{-1} S [9]. However, the observed S concentrations were at or even below this threshold at later samplings (1.6 g kg^{-1} at 50 DAS, 1.3 g kg^{-1} at 68 DAS) but no differences between treatments could be detected anymore.

3.2. Stress Effect on Non-Destructive Leaf-N Estimation. The relationship between Y-leaf chlorophyll and N concentration based on dry weight (Ch_{dw} and N_{dw}) for all individual measurements is shown in Figure 1, which includes data from samplings of all treatments, varieties, and crop growth stages ($n = 210$). Although the values cover a considerable range, a linear relationship is strongly indicated and highly significant. Linear regression analysis for the individual treatments (average of all varieties in one treatment) showed homogeneity of regression coefficients with the exception of the +W treatment. Because the latter was caused only by a few early measurements for variety Swarna (low N_{dw} and high CH_{dw} values, see Figure 1), the regression was calculated for the complete data set. In Figure 2, SPAD and LCC values were adjusted for leaf thickness (specific leaf weight (SLW)) because of the previously described effect of leaf thickness on the relation between leaf color measured by SPAD or the LCC and leaf N based on dry weight [20]. Data from all samplings (including all treatments, varieties, and crop growth stages; $n = 210$) were used for calculating the linear

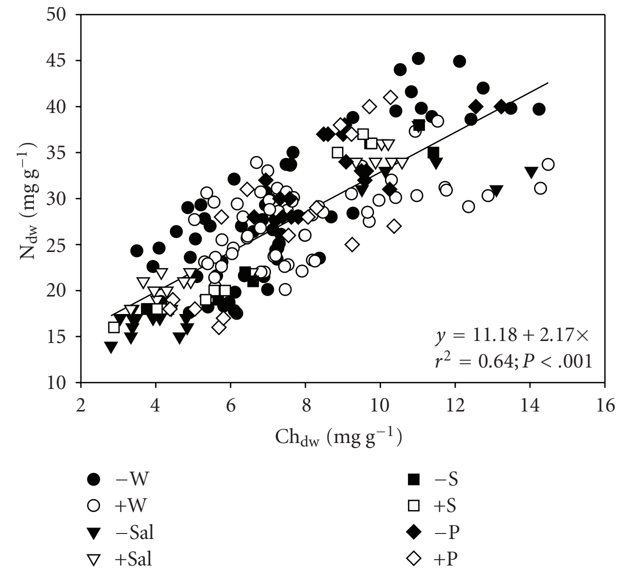
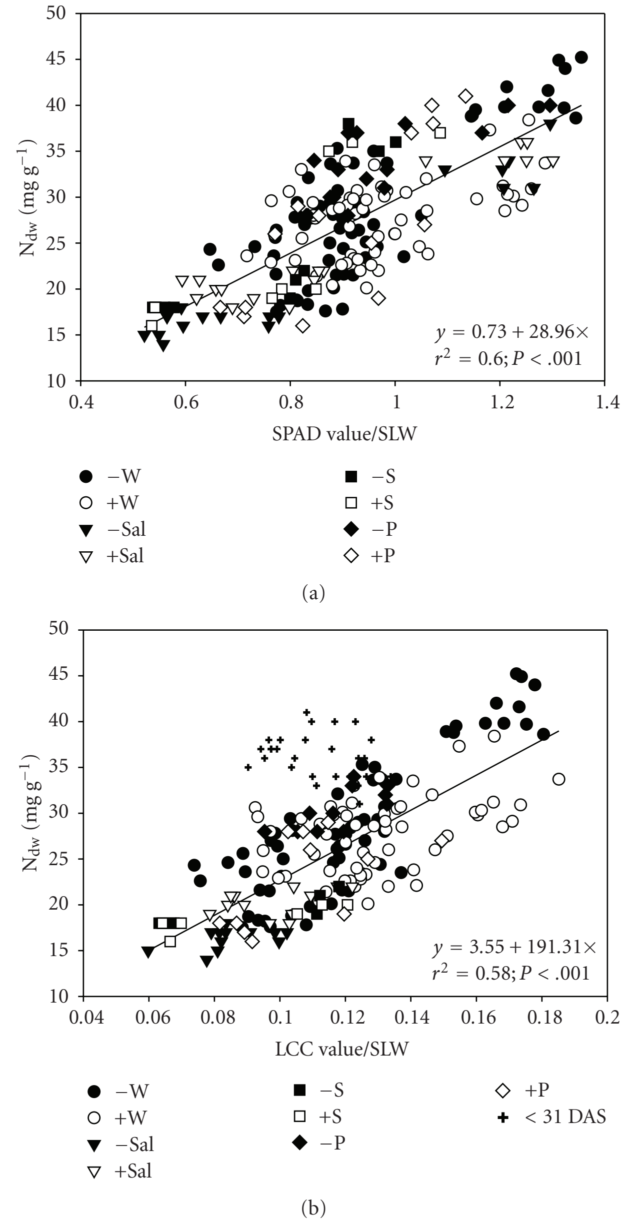


FIGURE 1: Leaf chlorophyll concentration (Ch_{dw}) versus leaf N concentration (N_{dw}), both based on dry weight, in a 2005 greenhouse trial. Data from all samplings and treatments ($n = 210$) were included for the linear regression analysis.



(a)

(b)

FIGURE 2: Leaf N concentration based on dry weight (N_{dw}) versus (a) chlorophyll meter (SPAD) and (b) leaf color chart (LCC) values, both adjusted for specific leaf weight (SLW). Data from all samplings and treatments ($n = 210$) were used for the linear regression in (a) whereas value pairs from measurements at very early crop growth stages (≤31 DAS) in (b) were not included.

regression of SPAD/SLW versus N_{dw} in Figure 2(a) whereas value pairs from measurements at very early crop growth stages (≤31 DAS) in Figure 2(b) (LCC/SLW versus N_{dw}) were not included based on a significant difference indicated by the *F*-test for homogeneity of regression coefficients. These measurements constituted a distinct group of outliers in the relation presented (Figure 2(b)) and when comparing LCC with SPAD values (data not shown). With this exception, N_{dw} was linearly related to SPAD and LCC measurements adjusted for SLW given the conditions of the experiment.

Leaf spectral reflectance scans were conducted to verify whether the reflectance patterns were influenced by the applied stresses. Figure 3 shows representative scans for all stress and control treatments at 29 DAS (for salinity, P and S deficiency) and at 47 DAS (at the end of the first drought phase for the water stress setup). Although there were differences in reflectance between treatments and varieties, the reflectance pattern over the scanned wavelength interval remained stable. The same observation applied to all other scans conducted at the other sampling dates (data not shown). The field observation of a comparatively dark leaf color for the variety Swarna was confirmed by the observed low reflectance values (Figure 3). Darker leaf colors were also indicated by the low reflectance values for the stress treatments +Sal and –P, which also confirms field observations of darker leaf colors for stressed plants. In contrast, S deficiency is characterized by lighter leaf colors, fitting to the high reflectance values at 550 nm for this treatment. To test the hypothesis that reflectance is a better indicator of leaf N than SPAD or LCC because it is less influenced by leaf thickness [21], we compared the reflectance at 550 nm (maximum reflectance for all scans conducted) with leaf N_{dw} for all treatments, varieties, and crop growth stages ($n = 210$). Reflectance does increase with decreasing N_{dw}, but the same reflectance indicates much higher N_{dw} values in early plant development compared with later development stages (Figure 4). Within each sampling period, no significant effects of applied stresses on reflectance versus N_{dw} relations could be detected using the *F*-test for homogeneity of regression coefficients.

3.3. Abiotic Stresses and Their Effect on the Dynamics of Leaf N Characteristics. Tables 3 and 4 show the results of the analysis of variance and mean treatment values for

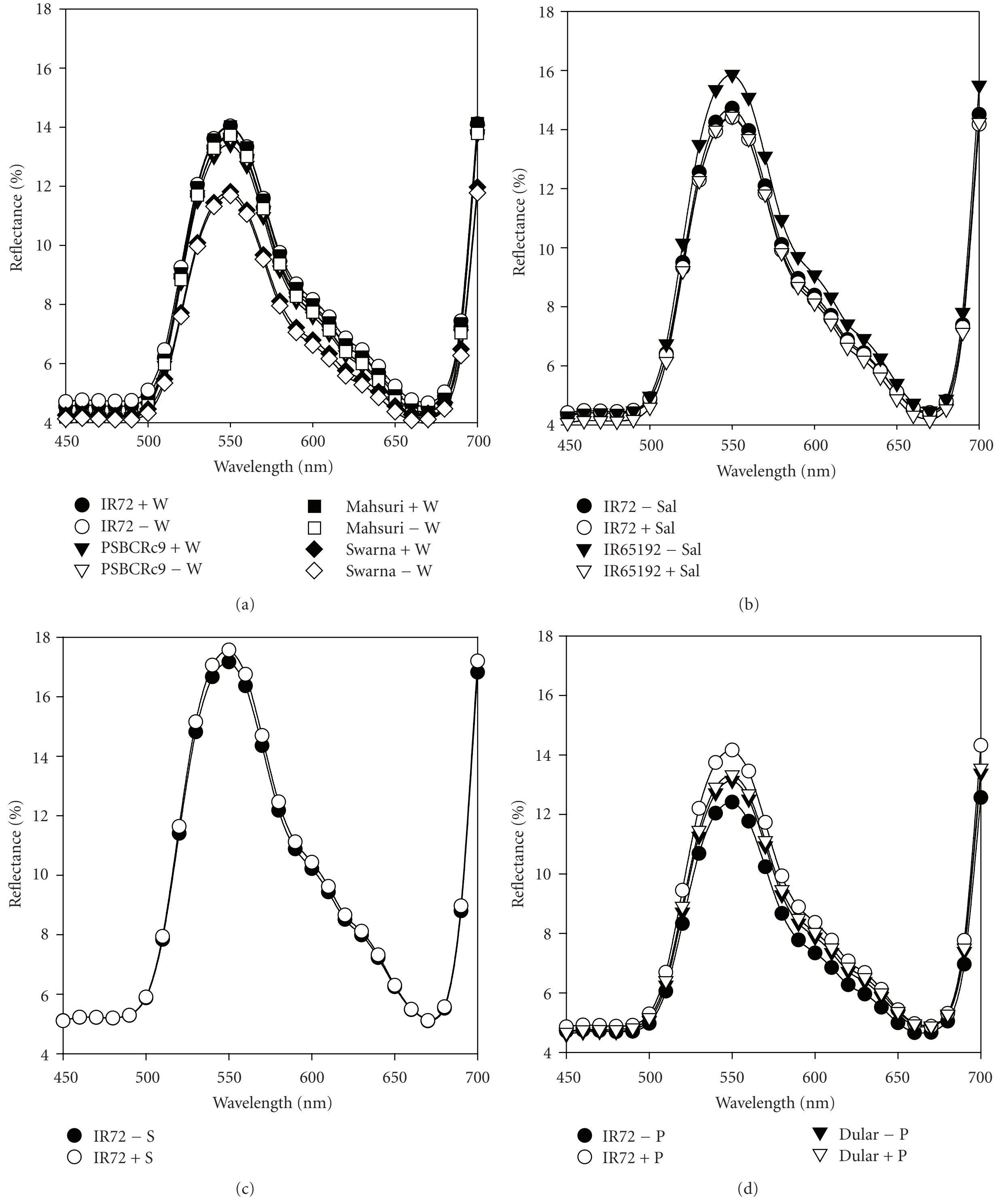


Figure 3: Leaf spectral reflectance scans for all stress and control treatments at 29 DAS (for salinity [±Sal], phosphorus [±P] deficiency, and sulfur [±S] deficiency) and at 47 DAS (at the end of the first drought phase for the water stress setup [±W]). Each curve represents the average of three replications.

the parameters chlorophyll and leaf N concentration based on leaf area (Ch_a and N_a) and dry weight (Ch_{dw} and N_{dw}). Although not always statistically significant, some general trends can be observed. Stress treatments influenced chlorophyll concentration (Ch_a and Ch_{dw}) significantly in almost all cases whereas the effects on N concentration were harder to detect. Drought caused a lower mean chlorophyll concentration and a stable or increased leaf N concentration

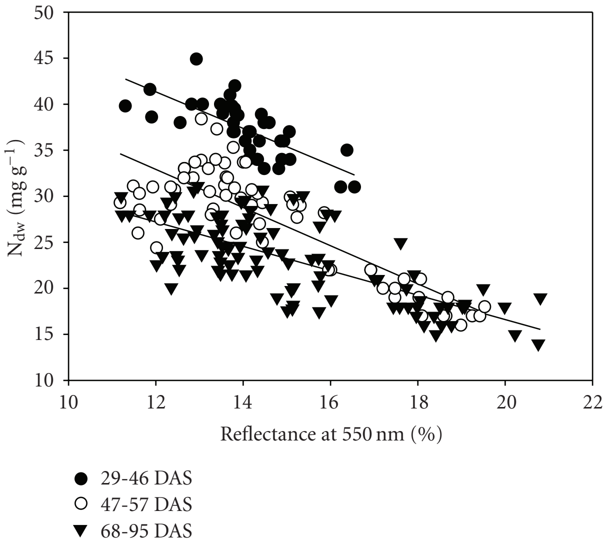


Figure 4: Reflectance at 550 nm versus leaf N concentration based on dry weight (N_{dw}) for all samplings and treatments ($n = 210$). Linear regression was conducted separately for three sampling periods: 29 to <47 days after seeding (DAS), 47 to 57 DAS, and 68 to 95 DAS.

Table 3: Analysis of variance for the effect of water treatment, variety, and sampling date on specific leaf weight (SLW) and on leaf chlorophyll and N concentration (Ch_a and N_a: area based; Ch_{dw} and N_{dw}: dry weight based). Included are mean treatment values across varieties and sampling dates for the two water treatments (±W).

	SLW g m^{-2}	Ch_a g m^{-2}	N_a g m^{-2}	Ch_{dw} mg g^{-1}	N_{dw} mg g^{-1}
Source of variation$^{(a)}$					
Treatment (T)	ns	***	*	***	*
Variety (V)	***	***	***	***	***
Sampling (S)	***	***	***	***	***
T×V	ns	ns	ns	ns	ns
T×S	ns	*	***	*	***
V×S	ns	**	***	*	***
T×V×S	ns	ns	**	ns	*
Treatment mean$^{(b)}$					
+W	42.2 a	0.35 a	1.20 b	8.48 a	28.8 b
–W	42.9 a	0.33 b	1.24 a	7.89 b	29.5 a

$^{(a)}$ Significance levels indicated for the ANOVA are ns: $P > .05$; *: $.05 \leq P \leq .01$; **: $.01 \leq P \leq .001$; ***: $P < .001$.

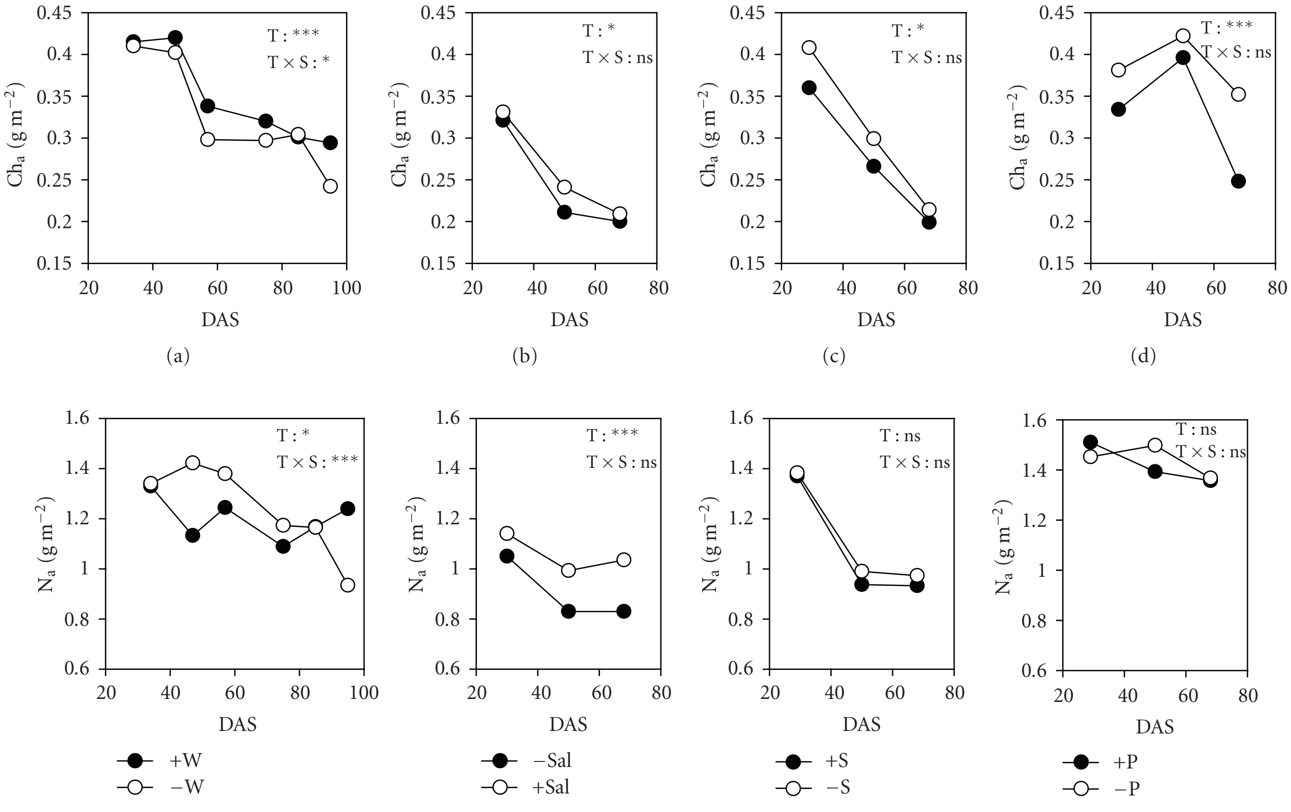
$^{(b)}$ Values in a column followed by the same letter are not significantly different at $P > .05$ according to the Tukey test.

for all varieties (Table 3). Contrary to this observation, the other stresses increased both chlorophyll and leaf N concentration (Table 4). Although there was a considerable varietal effect on the parameters presented, treatment × variety effects were, with only one exception (SLW in the P treatments), never significant, indicating that varieties reacted in the same way to each particular stress applied. The always significant effect of sampling time illustrates that all parameters are dynamic during crop development. Observed SLW values did differ between varieties and sampling times but stress treatments caused only very small and inconsistent SLW changes (Table 4). The trends for chlorophyll and leaf N concentration based on dry weight (Ch_{dw} and N_{dw}) are presented in detail in Table 5. This clearly shows the considerable range both values take across all samplings although all plants received the same amount of N. However, it also indicates that most of the variability is explained by sampling time whereas a smaller part is due to the stress treatment and variety used (data for the varieties Mahsuri and PSBRc9 are not shown).

The dynamics of Ch_a and N_a for all four stresses is shown in Figure 5. Because varieties reacted largely in the same way to the stresses (no significant T × V interaction; Tables 3 and 4), only treatment means across varieties are shown. According to Peng et al. [5], N_a decreases only a little in early crop development and tends to be stable thereafter. For the treatments +W, ±Sal, and ±S, we observed a similar dynamics although the initial drop in N_a is considerable for the ±S treatments. Contrary to this development, drought-stressed plants were characterized by constantly decreasing N_a values and both P treatments by continuously high N_a concentrations. Chlorophyll concentration (Ch_a) dynamics were similar to the dynamics of N_a, except for both P treatments, in which Ch_a peaked at the sampling 50 DAS. Figure 5 also shows that the stresses applied increase N_a and Ch_a simultaneously in all treatments except for drought, which has opposite effects on N_a and Ch_a.

4. Discussion

Different indicators were used to verify successful stress application in the various treatments. The stress level in the drought phases of the –W treatment was established by maintaining an average soil water potential of −47 kPa at 15 cm soil depth. According to Bouman and Tuong [14], this represents considerable water stress and usually causes rice yield reductions of 10% to 40% if occurring regularly throughout the cropping season. The applied drought stress had significant effects on leaf chlorophyll and N concentrations (Table 3) and influenced leaf N concentration dynamics over time (Figure 5). But, in contrast to the expected consequences the –W treatment increased biomass production at harvest, similar to reports in which midseason drainage increased yields in field experiments [14, 22]. Possible explanations are that the temporary aerobic conditions increased the average soil N supply or induced increased root development, or both, thus compensating for reduced growth during drought phases. Unlike for drought, biomass at harvest was a good indicator for the applied stress in the salinity (+Sal) and P-deficiency (–P) treatment (Table 1). Additional evidence for the applied stress in the –P treatment was the low soil P availability (Table 2) and the low leaf P concentration. But, the latter indicated that



(a) (b) (c) (d)

(e) (f) (g) (h)

Figure 5: Seasonal dynamic of area-based leaf chlorophyll concentration (Ch_a) and leaf N concentration (N_a) for all treatments. Stress treatments applied were drought (–W), salinity (+Sal), sulfur deficiency (–S), and phosphorus deficiency (–P). Shown are mean treatment values for three replications and all varieties used because no significant interaction was detected. Significance levels for treatment (T) effects and treatment by sampling time interaction (T×S) are ns = $P > .05$; * : $.05 \le P \le .01$; ** : $.01 \le P \le .001$; *** : $P < .001$ (further details in Tables 3 and 4).

even the +P treatment was still limited by P, explaining the comparatively low biomass production (Table 1). The dominant P limitation also explains the continuously high leaf N_a (Table 4, Figure 5). Because P limited growth, it was diluted as much as possible in the plant tissue (source limitation) whereas sufficient N was available for the limited growth, causing plant N accumulation due to the sink limitation [23]. High stress in the salinity treatment was clearly shown by the almost complete yield loss in the +Sal treatments. Less obvious was the stress level in the ±S treatments. Leaf S concentrations at the early sampling (29 DAS) did not indicate S deficiency, but the observed values were at or below the threshold value at later samplings. Sulfur deficiency was also suggested by the significant treatment differences for the Y-leaf S concentrations at 29 DAS, for straw weight at harvest, and for the leaf chlorophyll concentration (Tables 1 and 4). However, the comparatively low total biomass for both S treatments and the low leaf S concentration at both later samplings indicated that most of the applied S in the +S treatment was not plant available, causing considerable S deficiency in both S treatments. The reason for the missing S response in the +S treatment remained unclear. Limited response to S application was reported for alkaline soils and when the S was applied too late in the season but both conditions do not really apply in this experiment.

Leaf photosynthetic rate and leaf N concentration are closely related (e.g., [8]), as most of the leaf N is bound in enzymes associated with chlorophyll. Because it was unclear whether and to what extent the applied stresses could affect the close interaction between chlorophyll and leaf N concentration, both parameters were determined for all treatments. Figure 1 indicates a linear relation between both parameters within the observed range but the values scatter considerably around the linear regression line. This might be mostly caused by the measurement of these parameters from two different leaf samples (both measurements were destructive and not possible for the same sample) and imprecision of the analysis. However, a small and not significant effect of the applied stresses on the chlorophyll versus N-concentration ratio cannot be excluded. Especially for drought stress, such an uncoupling of both parameters is strongly suggested by the stress-induced decrease in chlorophyll (in mg g^{-1}) and the simultaneously stable or slightly increasing N_{dw} (Table 3, Figure 5). Nang [24] described the same phenomenon for stress phases with −80 kPa soil water tension but found no effect at −20 kPa soil water tension (both measured at 0.15 m

Table 4: Analysis of variance for specific leaf weight (SLW), chlorophyll concentration, and leaf N concentration (Ch_a and N_a: area based; Ch_{dw} and N_{dw}: dry weight based). The analysis was conducted separately for salinity (±SAL), sulfur (±S), and phosphorus (±P) treatments. Included are mean treatment values across varieties and sampling dates, separated for each stress treatment.

	SLW g m^{-2}	Ch_a g m^{-2}	N_a g m^{-2}	Ch_{dw} mg g^{-1}	N_{dw} mg g^{-1}
Salinity					
Treatment (T)(a)	ns	*	***	ns	***
Variety (V)	***	ns	***	ns	***
Sampling (S)	***	***	***	***	***
T×V	ns	ns	ns	ns	ns
T×S	ns	ns	ns	**	*
V×S	ns	ns	ns	ns	ns
T×V×S	ns	ns	ns	ns	ns
Treatment mean(b)					
–SAL	44.9 a	0.24 b	0.90 b	6.34 a	22.0 b
+SAL	44.3 a	0.26 a	1.06 a	6.37 a	25.1 a
Sulfur					
Treatment (T)(a)	ns	*	ns	***	ns
Sampling (S)	***	***	***	***	***
T x S	ns	ns	ns	ns	ns
Treatment mean(b)					
+S	46.6 a	0.28 b	1.08 a	6.22 b	24.3 a
–S	44.7 a	0.31 a	1.12 a	6.97 a	25.0 a
Phosphorus					
Treatment (T)(a)	ns	***	ns	***	**
Variety (V)	ns	*	ns	ns	ns
Sampling (S)	***	***	*	***	***
T×V	**	ns	ns	ns	ns
T×S	ns	ns	ns	ns	***
V×S	*	ns	ns	*	*
T×V×S	ns	ns	ns	ns	ns
Treatment mean(b)					
+P	45.0 a	0.33 b	1.42 a	7.39 b	31.7 b
–P	44.0 a	0.39 a	1.44 a	8.89 a	33.1 a

(a) Significance levels indicated for the ANOVA are ns: $P > .05$; *:$.05 \le P \le .01$; **:$.01 \le P \le .001$; ***: $P < .001$.
(b) Values in a column followed by the same letter are not significantly different at $P > .05$ according to the Tukey test.

depth). A similar effect was not detected for any of the other stresses applied (Table 4).

Past research on the relation between Y-leaf N_{dw} and SPAD readings in rice showed repeatedly that the linear regression equation describing this relation was influenced considerably by growth stage and genotype [20, 21, 25]. Peng et al. [20] demonstrated that these differences were largely due to differences in leaf thickness (SLW) and that the prediction of N_{dw} from SPAD readings across genotypes and growth stages was improved markedly by adjusting SPAD values for SLW. Yang et al. [6] confirmed the same mechanism for LCC measurements. Accordingly, acceptable regression coefficients were achieved for our data set when SPAD and LCC readings were adjusted for SLW (Figure 2), but closer relations (i.e., higher regression coefficients) were repeatedly reported in studies using fewer varieties or more homogeneous trial conditions [6, 16]. Lower precision was also reported by Witt et al. [21], who included a large number of different varieties in their study. It is most likely that, in our study, varietal differences *and* abiotic stresses contributed to a larger scatter even though an uncoupling of chlorophyll and leaf N was indicated only for the drought stress. Peng et al. [26] described slightly increased SPAD values at the same N_{dw} level resulting from P deficiency in early growth stages, but the same study could not confirm any effect of K nutrition. Why the LCC failed to predict N_{dw} at early growth stages (≤ 30 DAS) in our study (Figure 2(b)) remained unclear; Shukla et al. [7] previously reported good correlation between SPAD values and LCC scores even at very early growth stages.

Leaf spectral reflectance was measured for two reasons. First, we hypothesized that any damage to the photosynthetic

TABLE 5: Descriptive statistics (mean, standard deviation, and coefficient of variation) for chlorophyll and leaf N concentration based on dry weight (Ch_{dw} and N_{dw}) dependent on sampling time (days after seeding: DAS), stress treatment, and variety used.

Sampling	Treatment	Variety	Ch_{dw} (mg g^{-1})			N_{dw} (mg g^{-1})		
DAS			Mean	STDEV	CV	Mean	STDEV	CV
29	P−	Dular	8.7	0.3	3	37.0	0.0	0
50	P−	Dular	9.4	0.3	3	33.0	1.0	3
68	P−	Dular	7.3	0.6	8	28.7	1.2	4
29	P−	IR72	11.6	2.2	19	39.3	1.2	3
50	P−	IR72	8.9	1.7	19	32.0	1.0	3
68	P−	IR72	7.5	0.1	2	28.7	1.2	4
29	P+	Dular	7.6	1.2	16	38.7	0.6	1
50	P+	Dular	8.3	2.0	24	28.7	2.1	7
68	P+	Dular	5.3	0.7	14	17.5	2.1	12
29	P+	IR72	9.7	0.5	5	39.3	2.1	5
50	P+	IR72	8.4	0.9	10	26.7	2.1	8
68	P+	IR72	5.1	0.7	14	17.7	0.6	3
29	S−	IR72	10.7	0.9	8	36.3	1.5	4
50	S−	IR72	6.2	0.5	8	20.7	1.5	7
68	S−	IR72	4.0	0.3	8	18.0	0.0	0
29	S+	IR72	9.4	0.5	5	36.0	1.0	3
50	S+	IR72	5.6	0.3	5	19.7	0.6	3
68	S+	IR72	3.7	0.7	19	17.3	1.2	7
30	SAL−	IR65192	10.9	1.9	18	31.7	1.2	4
50	SAL−	IR65192	3.7	0.4	11	16.7	0.6	3
68	SAL−	IR65192	3.8	0.9	24	15.3	1.5	10
30	SAL−	IR72	12.2	1.6	13	35.0	2.6	8
50	SAL−	IR72	3.7	0.9	25	17.0	0.0	0
68	SAL−	IR72	3.9	0.9	22	16.3	1.5	9
30	SAL+	IR65192	10.1	0.6	6	34.0	0.0	0
50	SAL+	IR65192	5.4	1.1	20	19.7	2.1	11
68	SAL+	IR65192	3.9	0.5	13	19.0	1.0	5
30	SAL+	IR72	10.0	0.2	2	35.3	1.2	3
50	SAL+	IR72	4.7	0.4	10	21.7	0.6	3
68	SAL+	IR72	4.1	0.6	14	20.7	0.6	3
47	W−	IR72	10.4	1.1	10	39.1	0.4	1
57	W−	IR72	5.6	1.1	19	28.7	0.9	3
75	W−	IR72	6.4	0.9	13	26.7	0.3	1
85	W−	IR72	6.8	0.9	13	24.3	1.0	4
95	W−	IR72	5.7	0.7	12	17.6	0.2	1
47	W−	Swarna	12.2	1.3	11	40.0	1.5	4
57	W−	Swarna	7.0	0.2	2	28.1	3.3	12
75	W−	Swarna	7.5	0.7	10	22.3	1.9	9
85	W−	Swarna	5.8	0.5	9	20.5	2.0	10
95	W−	Swarna	5.1	1.0	19	20.0	1.4	7
47	W+	IR72	10.7	0.9	9	30.4	0.7	2
57	W+	IR72	7.9	0.7	9	29.1	0.9	3
75	W+	IR72	7.4	0.5	7	22.7	3.0	13
85	W+	IR72	6.6	0.6	8	24.7	1.0	4
95	W+	IR72	7.2	0.4	5	30.2	1.3	4
47	W+	Swarna	13.2	1.0	8	30.2	1.0	3
57	W+	Swarna	9.1	1.0	11	27.3	1.3	5
75	W+	Swarna	6.8	1.1	16	22.2	0.3	1
85	W+	Swarna	6.1	1.1	18	22.0	1.4	6
95	W+	Swarna	6.3	0.8	13	23.1	1.4	6

complex by abiotic stress would change the reflectance pattern. But, the results indicated that, although there were differences in the intensity of reflectance between varieties and treatments (see Figure 3), the basic reflectance pattern did not change. This confirms results of Witt et al. [21] and indicates that the applied stresses affected photosynthesis as a whole rather than affecting individual components of the photosynthetic complex. But, the finding of Witt et al. [21] that SLW had no influence on light reflectance measurements could not be confirmed (Figure 4). By using all spectral data for the prediction of leaf N content as was done by Witt et al. [21], this influence became smaller. However if only the wavelength with maximum reflectance was used (550 nm ± 5 nm in Figure 4), sampling date, and thereby SLW, did affect the reflectance versus N_{dw} relations. The most likely explanation is that leaf thickness did affect the ratio of reflected and penetrating light during the scan, that is, younger, thinner leaves had a higher reflectance value at the same N/chlorophyll content because of lower light penetration/transmission.

Compared with N_{dw}, rice leaf N concentration based on area (N_a) is much more stable throughout the growing period because the decreasing leaf N_{dw} is compensated for by increasing leaf thickness. Nevertheless, Peng et al. [5] showed that N_a decreases slightly in early crop development (until about 45 DAS) and increases slightly in later crop development (from about 75 DAS). This pattern was largely reconfirmed in the treatments +W, ±Sal, and ±S (Figure 5). However, leaf N concentration was continuously high for both P treatments perhaps, as indicated above, because both treatments were P deficient (Figure 5, Table 4). Peng et al. [26] also observed increased leaf N concentration under P deficiency at midtillering, and the higher leaf N and chlorophyll concentration could also explain the dark green color of P-deficient plants/leaves. Drought seemed to disrupt the above-described normal N_a development, leading to an irregular decrease in N_a from the first drought phase onward. More detailed samplings by Nang [24] demonstrated the same effect, but his study did not continue measurements beyond 50 DAS and used only a single stress phase.

We concluded that abiotic stresses affected leaf N and chlorophyll concentrations but did not change leaf reflectance patterns or the general linear correlation between leaf N and leaf chlorophyll concentration at the stress levels tested. Observed SPAD and LCC measurements adjusted for leaf thickness were linearly correlated with leaf N and chlorophyll concentration, but applied abiotic stresses and varietal differences decreased the precision of the relationship. Therefore, both measurement tools could not be used to accurately estimate leaf N. However, less precision is needed for the use of SPAD or the LCC as decision support tools for N management. In addition, calibration for the most commonly used varieties in a target region should be used to increase the predictive value of the leaf color measurements. Severe abiotic stresses in the target area for SPAD/LCC technologies are unlikely because farmers will usually not apply significant fertilizer quantities in fields which repeatedly experienced, for example, strong drought or serious other limitations (e.g., strong P deficiency). Thus, abiotic stresses are likely to be limited, and their effect on the accuracy of leaf N predictions based on SPAD or LCC measurements will be small in most cases. We therefore believe that both tools can help to improve N management of rice crops in moderately unfavorable environments. The next steps towards such a technology are on-farm trials determining the agro-economic value and feasibility of these tools in rainfed rice systems.

References

[1] A. Dobermann and P. F. White, "Strategies for nutrient management in irrigated and rainfed lowland rice systems," *Nutrient Cycling in Agroecosystems*, vol. 53, no. 1, pp. 1–18, 1999.

[2] A. Dobermann, C. Witt, and D. Dawe, *Increasing Productivity of Intensive Rice Systems through Site-Specific Nutrient Management*, Science Publishers, Enfield, NH, USA; International Rice Research Institute (IRRI), Los Baños, Philippines, 2004.

[3] S. M. Haefele, G. Atlin, S. P. Kam, and D. E. Johnson, "Improving farmers' livelihood in rainfed rice-based lowlands of Asia," in *Proceedings of the Deutscher Tropentag*, Humboldt University, Berlin, Germany, October 2004, www.tropentag.de/2004/abstracts/full/128.pdf.

[4] F. T. Turner and M. F. Jund, "Chlorophyll meter to predict nitrogen topdress requirement for semidwarf rice," *Agronomy Journal*, vol. 83, pp. 926–928, 1991.

[5] S. Peng, F. V. Garcia, R. C. Laza, A. L. Sanico, R. M. Visperas, and K. G. Cassman, "Increased N-use efficiency using a chlorophyll meter on high-yielding irrigated rice," *Field Crops Research*, vol. 47, no. 2-3, pp. 243–252, 1996.

[6] W.-H. Yang, S. Peng, J. Huang, A. L. Sanico, R. J. Buresh, and C. Witt, "Using leaf color charts to estimate leaf nitrogen status of rice," *Agronomy Journal*, vol. 95, no. 1, pp. 212–217, 2003.

[7] A. K. Shukla, J. K. Ladha, V. K. Singh et al., "Calibrating the leaf color chart for nitrogen management in different genotypes of rice and wheat in a systems perspective," *Agronomy Journal*, vol. 96, no. 6, pp. 1606–1621, 2004.

[8] S. Yoshida and V. Coronel, "Nitrogen nutrition, leaf resistance, and leaf photosynthetic rate of the rice plant," *Soil Science and Plant Nutrition*, vol. 22, no. 1, pp. 207–211, 1976.

[9] A. Dobermann and T. Fairhurst, *Rice: Nutrient Disorders and Nutrient Management*, Potash and Phosphate Institute of Canada and International Rice Research Institute, Los Baños, Philippines, 2000.

[10] S. M. Haefele and R. J. Hijmans, "Soil quality in rice-based rainfed lowlands of Asia: characterization and distribution," in *Science, Technology, and Trade for Peace and Prosperity. Proceedings of the 26th International Rice Conference*, P. K. Aggarwal, J. K. Ladha, R. K. Singh, C. Devakumar, and B. Hardy, Eds., pp. 297–308, International Rice Research Institute, Indian Council of Agricultural Research, and National Academy of Agricultural Sciences, Manila, Philippines, 2007.

[11] F. Moradi and A. M. Ismail, "Responses of photosynthesis, chlorophyll fluorescence and ROS-scavenging systems to salt stress during seedling and reproductive stages in rice," *Annals of Botany*, vol. 99, no. 6, pp. 1161–1173, 2007.

[12] M. Wissuwa, N. Ae, and S. S. Jones, "Genotypic variation for tolerance to phosphorus deficiency in rice and the potential for its exploitation in rice improvement," *Plant Breeding*, vol. 120, no. 1, pp. 43–48, 2001.

[13] J. C. O'Toole and M. A. Maguling, "Greenhouse selection for drought resistance in rice," *Crop Science*, vol. 21, pp. 325–327, 1981.

[14] B. A. M. Bouman and T. P. Tuong, "Field water management to save water and increase its productivity in irrigated lowland rice," *Agricultural Water Management*, vol. 49, no. 1, pp. 11–30, 2001.

[15] L. Zeng, J. A. Poss, C. Wilson, A.-S. E. Draz, G. B. Gregorio, and C. M. Grieve, "Evaluation of salt tolerance in rice genotypes by physiological characters," *Euphytica*, vol. 129, no. 3, pp. 281–292, 2003.

[16] S. Peng, M. R. Laza, F. V. Garcia, and K. G. Cassman, "Chlorophyll meter estimates leaf area-based nitrogen concentration of rice," *Communications in Soil Science and Plant Analysis*, vol. 26, no. 5-6, pp. 927–935, 1995.

[17] V. Balasubramanian, "Use of the leaf color chart (LCC) for N management in rice," in *CREMNET Technology Brief No. 2*, International Rice Research Institute, Los Baños, Philippines, 1999.

[18] J. M. Bremner, "Nitrogen-total," in *Methods of Soil Analysis: Chemical Methods. Part 3*, D. L. Sparks, Ed., Soil Science Society of America Book Series No. 5, pp. 1085–1122, Soil Science Society of America and American Society of Agronomy, Madison, Wis, USA, 1996.

[19] G. Mackinney, "Absorption of light by chlorophyll solutions," *The Journal of Biological Chemistry*, vol. 140, pp. 315–322, 1941.

[20] S. Peng, F. V. Garcia, R. C. Laza, and K. G. Cassman, "Adjustment for specific leaf weight improves chlorophyll meter's estimate of rice leaf nitrogen concentration," *Agronomy Journal*, vol. 85, no. 5, pp. 987–990, 1993.

[21] C. Witt, J. M. C. A. Cabrera-Pasuquin, R. Mutters, and S. Peng, "Nitrogen content of rice leaves as predicted by SPAD, NIR, spectral reflectance and leaf color charts," in *Proceedings of the ASA-CSSA-SSSA Annual Meetings on Changing Sciences for a Changing World: Building a Broader Vision*, Denver, Colo, USA, November 2003.

[22] S. K. De Datta, *Principles and Practices of Rice Production*, IRRI, Los Baños, Philippines, 1981.

[23] B. H. Janssen, F. C. T. Guiking, D. van der Eijk, E. M. A. Smaling, J. Wolf, and H. van Reuler, "A system for quantitative evaluation of the fertility of tropical soils (QUEFTS)," *Geoderma*, vol. 46, no. 4, pp. 299–318, 1990.

[24] N. D. Nang, *Nitrogen uptake, leaf nitrogen, chlorophyll content and leaf color of rice (Oryza sativa L.) as affected by drought stress*, thesis, University of the Philippines, Los Baños, Philippines, 2004.

[25] M. Takebe and T. Yoneyama, "Measurement of leaf chlorophyll scores and its implication to nitrogen nutrition of rice plants," *Japan Agricultural Research Quarterly*, vol. 23, pp. 86–93, 1989.

[26] S. Peng, A. L. Sanica, F. V. Garcia, et al., "Effect of leaf phosphorus and potassium concentration on chlorophyll meter reading in rice," *Plant Production Science*, vol. 2, no. 4, pp. 227–231, 1999.


Scientifica
Veterinary Medicine International
International Journal of Food Science
Journal of Botany
The Scientific World Journal
International Journal of Agronomy
International Journal of Ecology
International Journal of Biodiversity
Applied & Environmental Soil Science
International Journal of Forestry Research
Journal of Nutrition and Metabolism
International Journal of Evolutionary Biology
International Journal of Plant Genomics
Advances in Agriculture
Biotechnology Research International
International Journal of Cell Biology
International Journal of Genomics
International Journal of Microbiology
Psyche