Chlorophyll Meter’s Estimate of Weight-based Nitrogen Concentration in Rice Leaf is Influenced by Leaf Thickness

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Abstract: The chlorophyll meter (SPAD) has been widely used to measure the leaf N concentration. Nevertheless, linear regression equations of SPAD readings on N concentration (based on dry weight, Nw) differ with plant age and genotype mainly due to differences in specific leaf weight (SLW), and there is a close relationship between SLW and leaf thickness. This implies that SPAD readings may be influenced by leaf thickness alone. The present paper is to testify whether SPAD readings on rice (Oryza sativa L.) are influenced by variation in leaf thickness in different plant ages or genotypes. In a paddy field trial with rice Bing 9363 and in a lysimeter trial with 9 genotypes of rice, leaf thickness was measured using a specially developed displacement sensor. Leaf N was estimated using SPAD-502 and directly determined by Dumas combustion method. At 3 growth stages in the field trial, the degree of linear fit between Nw and SPAD values was poor (R²=0.557), but it was improved (R²=0.729) by introducing leaf thickness as an independent variable. In pooled data of the lysimeter trial, the predication of Nw was also improved by introducing leaf thickness as a secondary independent variable, the coefficients being increased from 0.0114 (not significant) to 0.513. However, if the leaf N concentration was expressed based on leaf area (Na), the leaf thickness did not influence the value estimated from the SPAD reading in both trials.

Key words: Leaf thickness, N content, Rice, Specific leaf weight.

Nitrogen (N) is the major nutrient element that most frequently limits the growth and productivity of rice. Fertilizer N is habitually applied in excess of the requirement to achieve high yields of rice. This is a burden to the farmer and environment as well. Critical weight-based concentration of N (Nw), which corresponds to the minimum concentration of N necessary to achieve maximum aboveground biomass, could be used to diagnose the plant N status (Sheehy et al., 1998). Nevertheless, traditional measurement of leaf Nw by the Kjeldahl procedure is laborious, time-consuming, and costly.

The chlorophyll meter (soil-plant analysis development, SPAD) is a quick, rapid tool for estimating chlorophyll concentration in leaves nondestructively. Chlorophyll concentration in a leaf is closely correlated with leaf N concentration (Evans, 1983), so the SPAD reading can be used to estimate the leaf N status. During the past decades, SPAD has become a popular tool, facilitating researches on plant physiological ecology. It is widely used to monitor rice N status and optimize N topdressing (Peng et al., 1996; Huang et al., 2008).

The meter estimates chlorophyll concentration or Nw with satisfactory accuracy for specific genotypes of plant cultivated in a given condition, whereas the regression equations for Nw or chlorophyll concentration (based on fresh weight, chlw) and meter readings differed significantly with physiological age, genotype and growth environment (Peng et al., 1993; Chapman and Barreto, 1997; Yamamoto et al., 2002; Jifon et al., 2005; Esfahani et al., 2008). Thus, the direct use of the meter to estimate Nw is complicated by the effects of crop ages and genotypes. Campbell et al. (1990) suggested that differences in leaf thickness probably contributed to the variation in the relationship between meter reading and leaf Nw. Marenco et al. (2009) confirmed that leaf thickness needs to be taken into account when calibration equations are used to convert SPAD values into chlw. Because of the difficulty in measuring leaf thickness, it is usually substituted by specific leaf weight (SLW), which is the ratio of dry weight to area of leaf. SLW is the product of leaf density and thickness.
and treated with N at 6 rates in a randomized block design. *Oryza sativa* L.) cv. Rice Bing 9363 was planted in 2008. Rice (farm of Hangzhou Academy of Agriculture, Hangzhou in 1.

Effect on the estimation of Na by SPAD (Peng et al., 1995a). Consequently, the leaf thickness has already been incorporated into each plot on the transplanting day, and another 75 kg ha$^{-1}$ potassium chloride was top-dressed on 40 DAT (days after transplanting). Plants received N at a rate of 0, 75, 150, 225, 300, or 375 kg ha$^{-1}$ as urea, and N at each rate was divided into 4 doses and applied on 3 July (plant reviving, 20%), 10 June (tillering, 30%), 6 August (panicle initiation, 50%) and 30 August (grain filling, 20%). The paddy field has loam soil with organic matter at 35.50 g kg$^{-1}$ and total N at 2.05 g kg$^{-1}$.

2. Lysimeter experiment

A lysimeter trial was conducted in an open-air field located in Huajiachi Campus of Zhejiang University, synchronizing with the field experiment. Rice was planted in concrete-framed lysimeters. The length and width of the lysimeter was 4 and 2 m respectively, with 1-meter depth of homogeneous soil. A total of 9 genotypes of rice with various degrees of greenness were selected, 2 japonica genotypes (Guihuahuang and Nanjing 42), 1 indica genotype (Zhongzu 53), and 6 hybrid genotypes (Yongyou 6, Yongyou 9, Qianyou 1, Qianyou 63, Qianyou 0508, and Lianyoupei 9). Genotypes Nanjing 42, Liangyoupei 9, Qianyou 6, Yongyou 9, Qianyou 0508, and Lianyoupei 9. Genotypes Nanjing 42, Liangyoupei 9, Qianyou 1, Qianyou 63 and Yongyou 9 received 75 and 225 kg N ha$^{-1}$ as urea, and the remaining genotypes received only 75 kg N ha$^{-1}$. N treatments were not repeated, so there were totally 14 lysimeters. All other farming management practices were the same as in the field experiment. Soil in the lysimeters was silt loam, containing organic matter and total N at 22.50 and 1.66 g kg$^{-1}$, respectively.

3. Sampling and Measurements

In the field trial, measurements were made during 23−25 July (middle tillering, MT), 22−24 August (panicle initiation stage, PI) and 6−9 October (ripening period, RP). The paddy field was flooded to guarantee adequate irrigation for rice plants. Five leaves with sheaths removed were sampled from each plot, and were immediately submerged into water within a bottle to prevent leaf from curling.

where dry weight and area is leaf dry weight and leaf area, respectively. Peng et al. (1993) demonstrated that differences in SLW are responsible for the variations in the relationship between rice leaf N$_w$ and SPAD values. Yamamoto et al. (2002) proved that multiple regression with SPAD reading as the dependent variable, and chlw and SLW as the independent variables presented the best estimate of chlw in leaves of sorghum and pigeonpea. Therefore, the differences in leaf thickness formed in different genotypes, physiological ages or growth environments may influence chlorophyll meter’s estimate of N$_w$. However, the hypothesis has not been confirmed directly, because nondestructive measuring of the leaf thickness is affected by close protruding veins in rice leaf (Chen et al., 2007). Furthermore, SLW of each leaf was also determined to influence the change in leaf thickness, rice leaf thickness. To confirm whether the SPAD reading is not be influenced by leaf thickness, but to date this deduction has not been verified directly.

In the present study, a specific displacement sensor was applied to the nondestructive measurement of leaf thickness. To confirm whether the SPAD reading is influenced by the change in leaf thickness, rice leaf thickness of the same genotype in different growth stages and different rice genotypes were measured respectively. Furthermore, SLW of each leaf was also determined to compare its influence on the estimate of N concentration with that of leaf thickness on the estimate. We also examined whether or not the use of SPAD to estimate N$_w$ is affected by leaf thickness.

Materials and Methods

1. Field experiment

A field experiment was conducted on the experimental farm of Hangzhou Academy of Agriculture, Hangzhou in 2008. Rice (*Oryza sativa* L.) cv. Rice Bing 9363 was planted and treated with N at 6 rates in a randomized block design with 3 replications. Seedlings with 5 fully expanded leaves were transplanted on 27 June. Hill spacing was 0.23×0.13 m with 2 seedlings per hill in the 3×6 m plot. Superphosphate (225 kg ha$^{-1}$) and potassium chloride (75 kg ha$^{-1}$) were incorporated into each plot on the transplanting day, and another 75 kg ha$^{-1}$ potassium chloride was top-dressed on 40 DAT (days after transplanting). Plants received N at a rate of 0, 75, 150, 225, 300, or 375 kg ha$^{-1}$ as urea, and N at each rate was divided into 4 doses and applied on 3 July (plant reviving, 20%), 10 June (tillering, 30%), 6 August (panicle initiation, 50%) and 30 August (grain filling, 20%). The paddy field has loam soil with organic matter at 35.50 g kg$^{-1}$ and total N at 2.05 g kg$^{-1}$.

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**Figure 1.** Schematic diagram of leaf thickness measuring instrument. A: level table; B: probe (mental rod); C: displacement sensor; D: signal transferring cable.
Based on single-chip measuring system, a leaf thickness measuring instrument (YL-20010A, China Jiliang University) with a displacement sensor was used to determine leaf thickness, and the thickness was measured within 15 min after sampling (Li and Song, 2009). The instrument was composed of 4 modules: level table, movable probe (mental rod), displacement sensor and signal transferring cable (Fig. 1). Rice leaf was put on the level table horizontally, and then the probe (mental rod) was put onto the leaf surface. The pressure exerted by the probe to leaf blade surface was only 0.1 N to reduce the variability of leaf thickness. The displacement of probe is converted into electric signal by displacement sensor. The electric signal is transferred to a monitor, and the reading of leaf thickness is displayed. Leaf thickness was measured at a stable temperature of approximately 30°C to reduce the measurement error induced by fluctuation of ambient temperature (Schroeder, 1980). In the same cross section of a leaf, the thickness tended to increase closer to main vein, so to accurately compare the thickness of different leaves, we measured the thickness as near the main vein as possible. The leaf veins were carefully avoided when the leaf thickness and SPAD readings were taken.

A chlorophyll meter (SPAD-502, Konika Minolta, Japan) was applied to leaf SPAD reading. Both SPAD readings and leaf thickness were obtained from leaf base to apex for at least 10 times (according to leaf length), and averages of these values were used as the SPAD reading and leaf thickness, respectively. Leaf area was measured using a leaf area meter (AM100, ADC, UK). After drying to a constant weight at 70°C, dry weight was determined. Specific leaf weight was calculated as the ratio of dry weight to leaf area. N concentration in a single leaf was determined by Dumas combustion method (Jung et al., 2003). Before the determination of N concentration, each leaf was ground into powder and pelleted wrapped in tin foil which is nitrogen free. The N concentration was measured on a Rapid N cube (Elementar, Germany). In the lysimeter experiment, the 5 uppermost fully expanded leaves were sampled from each lysimeter during August 11-15, and all the leaves were disposed and measured in the same way as field trial.

4. Data Analysis

Simple and multiple regressions of \( N_w \) on SPAD readings, thickness, and SLW were obtained and analysis of partial correlation were also conducted. Intercepts and slopes of regression lines at different growth stages were compared using the mixed model procedures of SAS 9.2.

| Growth stages | Mean values | Simple regressions | \( R^2 \) |
|---------------|-------------|--------------------|------|
|               | Thickness   | SLW                |      |
| MT            | 93.2 ± 10.4 | 42.0 ± 3.5         | log(SLW) = 0.381 log(thickness) + 0.873 | 0.434*** |
| PI            | 146.0 ± 8.2 | 45.6 ± 4.3         | log(SLW) = 0.961 (thickness) − 0.422 | 0.459*** |
| RP            | 179.7 ± 11.6| 52.8 ± 5.6         | log(SLW) = 1.337 log(thickness) − 1.319 | 0.668*** |
| Pool          | 139.5 ± 36.6| 45.6 ± 5.5         | log(SLW) = 0.278 log(thickness) + 1.064 | 0.453*** |

*** Significant at \( P < 0.001 \).

a, b, c: Significant difference at \( P < 0.05 \) between the simple regressions formula followed by different letters.
Results

Rice leaf was uneven, and the average thickness decreased from leaf base to tip (Fig. 2a). Leaf thickness and SLW of Bing 9363 increased from middle tillering stage to ripening period (Table 1). Leaf thickness was significantly related to SLW at each growth stage, with coefficients of determination increasing from MT to RP. Intercepts of regression lines of SLW against leaf thickness at the 3 growth stage were significantly different from each other (P <0.05), and the slope at MT was significantly different from the slopes at PL and RP (P <0.05), but slopes at PL and RP were similar (P =0.229). At each growth stage, both of the leaf thickness and SLW were decreased by the increase of N application (Fig. 2b).

In the field trial, a significant correlation existed between Nw and SPAD values at each growth stage, with slopes of the regression lines decreasing as plants aged (Table 2). There is no significant difference between slopes at MT and PI (P =0.677), but the slope at RP was significantly different from those at MT and PI (P <0.01), and the intercepts at the 3 growth stages were significantly different from each other (P <0.01) (Fig. 3a). Thus, regression lines at the 3 growth stages were different from each other, which resulted in the conspicuous decrease in coefficients of determination for regression generated from pooled data of the 3 growth stages (R^2 = 0.557, Table 2). Multiple regression analysis showed that SPAD and leaf thickness or SLW were significant variables (P <0.05) for explaining variability of Nw at each growth stage and pooled data (Table 2). In the pooled data, if Nw was the control variable, the coefficients of partial correlation for SPAD readings with leaf thickness and with SLW were 0.194 (P =0.002) and 0.188 (P =0.003), respectively. The adjustment of SPAD values through dividing SPAD readings by leaf thickness (SPAD/thickness) and SLW (SPAD/SLW) improved the accuracy of chlorophyll meter’s predication of Nw across the 3 growth stages (Figs. 3b, 3c).

In the lysimeter trial, the leaf thickness and SLW ranged from 106.2 μm, 26.8 g kg\(^{-1}\) (Qianyou 63) to 137.5 μm, 43.3 g kg\(^{-1}\) (Nanjing 42), respectively. Nw is not significantly related to SPAD values when all data were pooled (R^2 = 0.0114, P =0.379; Fig. 4a). Nevertheless, when leaf thickness or SLW was introduced as independent variable, Nw is significantly related to SPAD values and leaf thickness or SLW \[Nw = 0.084 (SPAD)−0.029 (thickness)+3.638, R^2 = 0.527, P < 0.0001; Nw = 0.05 (SPAD)−0.085 (SLW)+ 4.13, R^2 = 0.656, P <0.0001\], and both SPAD values and leaf thickness or SLW were significant independent variables accounting for variability in Nw in the 9 genotype rice leaf (P <0.01). Coefficients of partial correlation of SPAD values with leaf thickness and SLW were 0.573 (P <0.001) and 0.396 (P =0.001), respectively, when Nw was control variable. If SPAD values were adjusted through dividing by leaf thickness and SLW, the values (SPAD/thickness and SPAD/SLW) were significantly related to Nw \[Nw = 117.795 (SPAD/thickness)−4.347, R^2 =0.513, n =70, P <0.0001; Nw = 20.949 (SPAD/SLW) +7.711, R^2 =0.520, n = 70, P < 0.001\] (Figs. 4b, 4c).

If leaf N concentration was expressed on a leaf area basis for the pooled data of 3 growth stages or 9 genotypes, significant regression (P <0.001) of SPAD values on Na existed in both trials, with increases of coefficients from 0.557 to 0.623 in field trial, and from 0.0114 (not significant) to 0.339 in the lysimeter trial (Figs. 3a, 4a, 4b, 5b). Partial correlation analysis indicated that when Nw was control variable, SPAD values were not significantly related

| Growth stages | Mean values | Simple or multiple regressions | n | R^2 |
|---------------|-------------|--------------------------------|---|-----|
|               | SPAD       | Nw                             |   |     |
| MT            | 40.3±3.7   | 33.0±6.6                       | 90 | 0.686*** |
|               | Nw = 1.474 (SPAD) − 26.335 |                             |   |     |
|               | Nw = 1.531 (SPAD) − 0.054 (thickness) − 23.649 |                             |   |     |
|               | Nw = 1.458 (SPAD) − 0.336 (SLW) − 11.705 |                             |   |     |
| PI            | 40.4±3.2   | 27.0±5.3                       | 90 | 0.724*** |
|               | Nw = 1.415 (SPAD) − 30.134 |                             |   |     |
|               | Nw = 1.413 (SPAD) − 0.043 (thickness) − 25.261 |                             |   |     |
|               | Nw = 1.483 (SPAD) − 0.136 (SLW) − 32.038 |                             |   |     |
| RP            | 35.5±5.6   | 17.9±4.5                       | 90 | 0.740*** |
|               | Nw = 0.605 (SPAD) − 3.615 |                             |   |     |
|               | Nw = 0.537 (SPAD) − 0.066 (thickness) + 10.470 |                             |   |     |
|               | Nw = 0.482 (SPAD) − 0.177 (SLW) + 9.325 |                             |   |     |
| Pool          |             |                                | 270| 0.557*** |
|               | Nw = 1.271 (SPAD) − 23.244 |                             |   |     |
|               | Nw = 0.953 (SPAD) − 0.112 (thickness) + 4.618 |                             |   |     |
|               | Nw = 1.016 (SPAD) − 0.435 (SLW) + 6.494 |                             |   |     |

***Significant at P <0.001.

Table 2. Mean and standard deviation of leaf N concentration of rice Bing 9363 based on dry weight (Nw) and per unit area (Na) at MT, PI and RP, and relationship of Nw with SPAD values, with SPAD and leaf thickness, and with SPAD and SLW, at each growth stage and in the pooled data at all growth stages.
Regression of leaf N per unit dry weight ($N_w$) on (a) SPAD values of 9 rice genotypes, or (b) on SPAD values adjusted for thickness (SPAD/thickness) for the same genotypes, or (c) on SPAD values adjusted for specific leaf weight (SLW) for the genotypes. YY9, □ NJ42, ● QY0508, ○ YY16, ▲ ZZ53, △ GHH, ▼ QY63 ▽ LYP9 and ◆ QY1 were abbreviations of rice genotypes Yongyou 9, Qianyou0508, Nanjing 42, Yongyou16, Zhongzhu 53, Guihuahuang, Qianyou 63, Liangyoupei 9, and Qianyou 1, respectively. "n.s." was the abbreviation of "not significant" ($P > 0.05$).

Regression of leaf N concentration expressed based on per dry weight ($N_w$) on (a) SPAD values in Bing 9363 at middle tillering (▲ MT), panicle initiation (○ PI), and ripening period (● RP), (regression equations were presented in Table 2) (b) SPAD values improved by introducing leaf thickness (SPAD/thickness) for pooled data from the 3 growth stages, or (c) SPAD values improved by introducing specific leaf weight (SPAD/SLW). The dotted line is the regression line reported by Peng et al. (1993) for IR72 rice: $Y = 6.56 + 33.66X$. 

Fig. 3. Regression of leaf N concentration expressed based on per dry weight ($N_w$) on (a) SPAD values in Bing 9363 at middle tillering (▲ MT), panicle initiation (○ PI), and ripening period (● RP), (regression equations were presented in Table 2) (b) SPAD values improved by introducing leaf thickness (SPAD/thickness) for pooled data from the 3 growth stages, or (c) SPAD values improved by introducing specific leaf weight (SPAD/SLW). The dotted line is the regression line reported by Peng et al. (1993) for IR72 rice: $Y = 6.56 + 33.66X$. 

Fig. 4. Regression of leaf N per unit dry weight ($N_w$) on (a) SPAD values of 9 rice genotypes, or (b) on SPAD values adjusted for thickness (SPAD/thickness) for the same genotypes, or (c) on SPAD values adjusted for specific leaf weight (SLW) for the genotypes. YY9, □ NJ42, ● QY0508, ○ YY16, ▲ ZZ53, △ GHH, ▼ QY63 ▽ LYP9 and ◆ QY1 were abbreviations of rice genotypes Yongyou 9, Qianyou0508, Nanjing 42, Yongyou16, Zhongzhu 53, Guihuahuang, Qianyou 63, Liangyoupei 9, and Qianyou 1, respectively. “n.s.” was the abbreviation of “not significant” ($P > 0.05$).
SLW could be taken as an effective surrogate for rice leaf thickness at each growth stage (Table 1). Nevertheless, the regression lines at different growth stage were greatly different from each other, and intercepts of the regressions are significantly different from zero. From eqn 1, it could be attributed to the differences of leaf thickness. Vile et al. (2005) estimated leaf thickness using the ratio of water-saturated leaf fresh mass to leaf area. In their study, overall slope and the intercept of regression were not significantly different from 1 and 0 respectively. It is because the leaves in their study were fully rehydrated, and leaf fresh mass per volume is approximately equal to 1 g cm⁻³. In the present study, however, the proportion of gaseous, liquid and solid phase in rice leaf was flexible since the leaf was not rehydrated. As a result, leaf thickness, namely dry mass per unit volume, was changeable during the study. The coefficients of determination increased from MT to RP, perhaps because the distance between two veins increased as plant aged, so the probe of the displacement sensor was less affected by veins and therefore the leaf thickness was measured more accurately. SLW and leaf thickness of uppermost fully expanded leaf at the 3 growth stages were considerably different from each other. Research on winter oilseed rape showed that variation in SLW at different growth stages were related to the plant demand including expansion of the stem and ramifications. To satisfy this demand, leaf reserves would be remobilized and hence SLW varied (Jullien et al., 2009). Therefore, rice leaf thickness may also be changed accompanied by the variation of SLW at different developmental stages, which means that SLW and leaf thickness at different developmental stages may be destined to be different.

In the present study, the leaf thickness influence on the SPAD readings was confirmed directly. In both trials, SPAD readings were augmented by the increase of leaf thickness on condition that Nw was control variable, and hence the predications of Nw by SPAD across different plant growth stages or different genotypes were deviated by leaf thickness. Compared with thinner leaf, thicker leaves with heavier leaf weight per unit area tend to have a lower N transmittance owning to an increased multiple scattering and optical pathlength (detour effect) through the leaf (Vogelmann, 1993). For this reason, if SPAD is applied to the estimate of dry weight-based leaf N concentration, thicker leaf leads to an overestimation of the concentration. In the development of next-generation SPAD, it is worthy to integrate the leaf thickness sensor to the meter for the simultaneous measurements of leaf thickness and SPAD reading with aim to the more accurate prediction of leaf Nw.

Fits of pooled SPAD values against pooled Nw in the 2 trials were poor on account of the change in leaf thickness in different growth stages or genotypes. The goodness of the fits, namely coefficients of determination, was greatly improved by the adjustment of SPAD through dividing by thickness, or the introduction of thickness as a second independent variable (Table 2; Figs. 3b, 4b). The eliminating of leaf thickness influence on SPAD values could also be achieved by using SLW (Table 2; Figs. 3c, 4c), which implies that SLW can be considered as a substitute for thickness in relevant research. The regression of Nw on SPAD/SLW in our research was different from the regression observed by Peng et al. (1995) for rice genotypes IR72 and IR64616H. ▲ YY9, □ NJ42, ○ QY0508, ○ Y116, ▲ ZZ53, △ GHH, ▼ QY63 ▽ LYP9 and ◆ QY1 were abbreviations of rice genotypes Yongyou 9, Nanjing 42, Qianyou0508, Yongyou16, Zhongza 53, Guihuahuang, Qianyou 63, Liangyoupei 9, and Qianyou 1, respectively.

Fig. 5. (a) Linear regression of area-based leaf N (Na) on SPAD values in rice Bing 9363 at middle tillering (▲ MT), panicle initiation (○ PI), and ripening period (● RP). (b) Linear regression of Na on SPAD values in 9 rice genotypes. Dot line is regression line reported by Peng et al. (1995b) for rice genotypes IR72 and IR64616H. ▲ YY9, □ NJ42, ○ QY0508, ○ Y116, ▲ ZZ53, △ GHH, ▼ QY63 ▽ LYP9 and ◆ QY1 were abbreviations of rice genotypes Yongyou 9, Nanjing 42, Qianyou0508, Yongyou16, Zhongza 53, Guihuahuang, Qianyou 63, Liangyoupei 9, and Qianyou 1, respectively.
protocols of data acquisition. In the study conducted by Peng et al. (1993), 3 SPAD readings were taken around the midpoint of each leaf blade, 30 mm apart, on one side of the midrib, but in the present study the SPAD reading of each leaf was an average of at least 10 SPAD readings from leaf base to apex. The SPAD readings in the same leaf were correlated with the proportional distance from the leaf base, and consistent with that in tropical maize (Chapman and Barreto, 1997). The SPAD readings against the proportional distance from the rice leaf base was also well fitted by a quadratic function in our study (data are not shown). Therefore, even in the same leaf, SPAD values were presented by two disparate ways. The overall leaf N concentration in Bing 9363 was lower than that in IR72 observed by Peng et al. (1993). Besides the impact of genotype, N concentrations in leaf were also affected by environmental factors. For instance, the N investment into the leaf blades of the rice plants grew under low irradiance was relatively great (Makino et al., 1997).

When leaf N concentration was expressed based on leaf area, the relationship between pooled SPAD values and leaf N concentration (N_a) was not affected by leaf thickness or SLW in the 2 trials, which was confirmed by the fact that thickness or SLW were not significant independent variables when they were introduced into the regression of SPAD on N_a. Consistent with previous study (Peng et al., 1995b), SPAD estimated N_a better than N_c across different growth stages, or especially across different genotypes (Figs. 3a, 4a, 5a, 5b). Regression of N_a on SPAD observed in field trials (Fig. 5a) was similar to that obtained by Peng et al. (1995b) in slopes, but was divergent in intercepts (Fig. 5a). Identical explanation to the differences of the 2 regression lines could be made as the interpretation in the prior paragraph.

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