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Using Chlorophyll (SPAD) Meter Reading and Shoot Fresh Weight for Recommending Nitrogen Topdressing Rate at Panicle Initiation Stage of Rice

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Abstract

Nitrogen management at the panicle initiation stage (PI) should be fine-tuned for securing a concurrent high yield and high quality rice production. For calibration and testing of the recommendation models of N topdressing rates at PI for target grain yield and protein content of rice, three split-split-plot design experiments including five rice cultivars and various N rates were conducted at the experimental farm of Seoul National University, Korea from 2003 to 2005. Data from the first two years of experiments were used to calibrate models to predict grain yield and milled-rice protein content using shoot fresh weight (FW), chlorophyll meter value (SPAD), and the N topdressing rate (Npi) at PI by stepwise multiple regression. The calibrated models explained 85 and 87% of the variation in grain yield and protein content, respectively. The calibrated models were used to recommend Npi for the target protein content of 6.8%, with FW and SPAD measured for each plot in 2005. The recommended N rate treatment was characterized by an average protein content of 6.74% (similar to the target protein content), reduced the coefficient of variation in protein content to 2.5% (compared to 4.6% of the fixed rate treatment), and increased grain yield. In the recommended N rate treatments for the target protein content of 6.8%, grain yield was highly dependent on FW and SPAD at PI. In conclusion, the models for N topdressing rate recommendation at PI were successful under present experimental conditions. However, additional testing under more variable environmental conditions should be performed before universal application of such models.

Key words: grain yield, protein content, SPAD reading, fresh weight, nitrogen fertilizer.

Introduction

Nitrogen fertilization is a crucial determinant of grain yield and quality of rice. Based on the critical stages of determination of rice yield and its components, the application of N fertilizer has been recommended in several splits at transplanting for crop recovery at active tillering stage for increasing tiller and panicle number, at PI for increasing number of spikelets per panicle, and at heading for increasing grain weight and ripening percentage (De Datta 1981). The N split application for ordinary rice cropping in Korea has been recommended to be 50% at transplanting, 20% at tillering stage, 20% at PI, and 10% at full heading until it was changed recently to 50, 20, 30, and 0%, respectively. N topdressing at full heading stage is not recommended because it increases the protein content of milled-rice, leading to poor eating quality. Milled-rice protein content has been reported to have a negative correlation with the palatability of cooked rice as high protein content in rice grain increases the hardness and decreases the stickiness of cooked rice (Chikubu et al. 1985; Ishima et al. 1974; Taira 1995). Presently, more emphasis is placed on improving rice quality than on increasing grain yield since Korean rice should compete with the imported rice in the domestic market. This situation makes it necessary to reevaluate the existing nitrogen management practices that have been pursued mainly on increasing grain yield.

Nitrogen application at different growth stages exerts different effects on grain yield and quality of rice (Matsushima 1995; Nguyen et al. 2006). Nguyen et al. (2006) reported that an increase of the N topdressing rate at PI increased grain yield and milled-rice protein content while an increase in the N topdressing rate at the tillering stage increased grain yield but not milled-rice protein content. Matsushima (1995) also reported that N absorbed from late N topdressing after PI resulted in much higher grain protein content in comparison to that from earlier N topdressing as the former could be translocated directly into the grain. Nguyen et al. (2006) found that the higher shoot N and biomass accumulation at PI resulted in the higher rice grain yield while resulted in the lower milled-rice protein

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content possibly due to the dilution effect. These results indicate that for achieving high grain yield and good quality rice with low protein content it is necessary to fine-tune the N management at PI according to the diagnosis of crop growth and N nutritional status.

Different crop growth and N nutritional status indicators have been used for recommending N topdressing management at PI (Dobermann et al. 2002; Peng et al. 1996). Of these indicators, shoot dry weight, shoot N concentration, and shoot N accumulation were most frequently used. However, characterization of these parameters is laborious and time consuming. Although prediction of shoot N density and concentration by canopy reflectance has been previously reported as a reliable, fast, and non-destructive method for wheat (Hansen and Schjoerring 2003) and rice crops (Nguyen and Lee 2004), it is still difficult to introduce it into the actual farming practices. Alternatively to directly measuring leaf N concentration, the chlorophyll meter (SPAD) provides a simple, quick, and non-destructive method for estimating N concentration of rice leaves (Chubachi et al. 1986; Takebe and Yoneyama 1989; Takebe et al. 1990; Peng et al. 1995, 1996). Therefore, SPAD values have been used to assess crop N status and to determine the plant's need for additional N fertilizer by Turner and Jund (1991), Peng et al. (1995, 1996), and Singh et al. (2002) who used N management tactics to apply N topdressings only when SPAD values of the index leaves fell below a predetermined threshold. These SPAD-based N fertilizer managements resulted in a higher N use efficiency (NUE) and agronomic NUE than the fixed-rate treatment. However, SPAD values do not indicate how much N should be applied. They only indicate the need for additional N (Turner and Jund 1994). SPAD values are needed to be used in combination with other crop growth and/or soil variables to determine the N topdressing rate congruent to N demand for a target grain yield and/or grain quality.

The objectives of our studies were (1) to quantify the response models of grain yield and milled rice protein content to the applied N rate at PI in relation to FW and SPAD values, and (2) to apply the models for N topdressing rate recommendation at PI for a target grain yield and milled-rice protein content.

Materials and Methods

Three experiments (2003, 2004, and 2005) were conducted on a rice field with an area of 3,000 m^2 at the experimental farm of Seoul National University, Suwon, Korea. The field had a sandy clay loam soil texture, CEC of 11.9 cmol(+) kg^{-1}; organic matter of 14.4 mg g^{-1}, total N of 0.75 mg g^{-1}, and pH of 5.4. The 2003 experiment included ten nitrogen (N) treatments and four rice cultivars of medium maturity: Hwaseongbyeo (V1), SNU-SG1 (V2), Juanbyeo (V3), and Surabyeo (V4). The 2004 and 2005 experiments included 12 and nine N treatments, respectively, with two rice cultivars: Hwaseongbyeo (V1) and Daeanbyeo (V5). The details of experiments are presented in Tables 1a-c.

It is noted from Table 1b that the purpose of the variable N-rate treatments (treatments 3 and 4) in the 2004 experiment was to widen and increase the variability of the N testing rate for the calibrating models to predict crop variables for the 2005 experiment. PI was defined here as a common date (24 days before heading) for N application at panicle initiation stage in Korea. The experiments were conducted according to a split-split-plot design, with the N rate at tillering stage, PI, and rice cultivars randomly assigned into main plots, sub-plots and sub-sub-plots, respectively. Sub-sub-plot size was 24 m^2 in 2003, and 30 m^2 in 2004 and 2005. Rice was transplanted manually at a spacing of 0.15 m x 0.30 m with three 30-day-old seedlings per hill on May 20th in 2003, May 23rd in 2004, and May 30th in 2005. The whole experimental field was fertilized with the same amount of 80 kg P_2O_5 + 48 kg K_2O ha^{-1} at transplanting and 24 kg K_2O ha^{-1} at PI. Other management techniques for the whole field such as land preparation, weeding, water supplies, etc., were applied homogenously according to the standard cultivation practices in Korea.

Table 1a: Design of the 2003 experiment.

Treatment	N rates[a] at transplanting	N rates at tillering	N rates at PI	Rice cultivar[b]
1	0	0	0	V1, V2, V3, V4
2	48	0	0, 36, 72	V1, V2, V3, V4
3	48	36	0, 36, 72	V1, V2, V3, V4
4	48	72	0, 36, 72	V1, V2, V3, V4

[a] Applied N (kg ha^{-1}), [b] V1-V4 were Hwaseongbyeo, SNU-SG1, Juanbyeo, and Surabyeo, respectively.

Table 1b: Design of the 2004 experiment.

Treatment	N rates[a] at transplanting	N rates at tillering	N rates at PI	Rice cultivar[b]
1	48	0, 36, 72	0	V1, V5
2	48	0, 36, 72	36	V1, V5
3	48	0, 36, 72	Variable N rates[c]	V1, V5
4	48	0, 36, 72	Variable N rates	V1, V5

[a] Applied N (kg ha^{-1}), [b] V1 and V5 were Hwaseongbyeo and Daeanbyeo, respectively.
[c] N rates at PI for treatments 3 and 4 was 15.1±16.7 and 15.0 ±13.8 kg ha^{-1}, respectively.

Table 1c: Design of the 2005 experiment.

Treatment	N rates[a] at transplanting	N rates at tillering	N rates at PI	Rice cultivar[b]
1	48	0, 36, 72	0	V1
2	48	0, 36, 72	36	V1
3	48	0, 36, 72	Variable N rates[c]	V1
4	48	0, 36, 72	Recommended N rate[d]	V1

[a] Applied N (kg ha^{-1}), [b] V1 and V5 was Hwaseongbyeo and Daeanbyeo, respectively.
[c] N rates at PI for treatment 3 was 42.3±112.1 kg ha^{-1}, and this treatment was used only for model valdation..
[d] Details for recommended N rate at PI, SPAD, and shoot fresh weight were presented in the next section..

Plant sampling and measurements

On the dates of N topdressing at PI of each year (July 19th in 2003, July 22nd in 2004, and July 21st in 2005), five rice hills per plot were randomly selected. From these five hills, a chlorophyll meter (SPAD 502, Minolta Co. LTD, Japan) reading (SPAD) was taken for the five most fully-expanded leaves per hill. Immediately following SPAD measurement, the five hills were sampled and weighed for shoot fresh weight (FW). At harvest (October 12th 2003, October 16th 2004, and October 9th 2005), 72 hills for grain yield and protein content measurement were sampled from each plot, and a whole-plant sample of five rice hills per plot was collected for plant N concentration analysis by Kejeltec Auto 1035 System (Tecator, Sweden).

Model calibration for N topdressing rate recommendation

Data collected from the first two years (2003-2004) was used to examine the relationship of grain yield and protein content with FW, SPAD, and applied N rates at PI (Npi). Models were calibrated by stepwise multiple linear regression (SMLR) in SAS 8.1 (SAS Inc., USA) to predict rice grain yield and protein content using Npi, FW, and SPAD (linear and quadratic) as independent variables.

Model validation

The calibrated models were used to calculate grain yield and protein content using crop parameters measured at PI in 2005. The performance of the calibrated models was evaluated by the coefficient of determination (R^2) and the root mean square error in prediction (RMSEP), an indicator of the average error in the analysis as expressed in the original measurement unit. RMSEP was calculated using Eq. 1:

$$\text{RMSEP} = \left[\frac{1}{n} \sum_{i=1}^{n} (y_i - \hat{y}_i)^2 \right]^{0.5} \qquad (1)$$

where y_i and $\hat{y}_i$ were actual and calculated values of crop parameters at harvest (grain yield and protein content, respectively), and n is the number of samples in the dataset. Another parameter used was the relative error of prediction (REP) which shows the predictive ability of the model as calculated by Equation 2:

$$\text{REP}(\%) = \frac{100}{\bar{y}} \left[\frac{1}{n} \sum_{i=1}^{n} (y_i - \hat{y}_i)^2 \right]^{0.5} \qquad (2)$$

Similar to Equation 1, y_i and $\hat{y}_i$ were actual and calculated values of crop parameters, n was the number of samples in dataset, and $\bar{y}$ was mean of the actual values of crop variables.

Testing the recommended nitrogen rate

In the 2005 experiment, a treatment (treatment 4) was added, managed homogenously, and data for crop parameters were collected at all stages as in the other treatments described in the above sections, with an exception that Npi was calculated by the model equations calibrated in the previous step. Based on the SMLR equations, which show the dependence and interdependence of grain yield and protein content on FW, SPAD, and Npi, Npi for each plot of treatment 4 in 2005 was calculated for the target protein content of 6.8% with the measured FW and SPAD.

Data analysis

Grain yield and protein content of the recommended N topdressing treatment were reported by treatment means in comparison to treatments 1 and 2 (with no N at PI as the control treatment) and 36 kg N ha^{-1} applied at PI (the commonly recommended N rate at PI in Korea), respectively.

Results and Discussion

Model calibration

For the N topdressing rate recommendation based on FW and SPAD, grain yield and protein content response to Npi in combination with FW and SPAD requires quantification. To quantify the responses of grain yield and protein content to Npi in the plots with different FW and SPAD, a stepwise multiple linear regression with forward selection at the probability of <0.05.was used. The Input variables included linear and quadratic terms of Npi, FW, and SPAD measured from the 2003 and 2004 experiments. The quadratic effect of Npi, biomass, and shoot N concentration on grain yield has been previously reported by Kim (2004). The stepwise multiple regression model to predict grain yield and protein content using Npi, FW, and SPAD (Table 2) indicated that both grain yield and protein content have a quadratic relationship with Npi and FW, but a linear relationship with SPAD (Eq. 3 and 4 in Table 2).

Table 2: Calibration models for grain yield and milled-rice protein content prediction.

Model equations (n = 66)	R^2
Grain yield (kg ha^{-1}) = 590.3 + 5.45Npi^{a} – 0.485Npi^2 + 3.38FW – 7.75 x10-4FW^2 + 62.4SPAD (Eq. 3)	*0.85*
$Protein^{b}$ = 6.47 + 0.0112Npi + 0.000115Npi^2 - 0.00133FW + 3.09 x10-7FW^2 +0.035SPAD (Eq. 4)	*0.87*

[a] Npi, FW and SPAD were applied N rate (kg ha^{-1}), shoot fresh weight (g m^{-2}), and SPAD values at PI.
[b] Protein was milled-rice protein content (%).

Equation 3 allows one to calculate the optimum values of Npi and FW for the maximum grain yield. The calculated optimum values of the Npi and FW for the maximum grain yield of rice were 56 kg N ha^{-1} and 2180 g m^{-2}, respectively. The calculated 56 kg N ha^{-1} was almost twice the commonly recommended Npi in Korea. The quadratic effect of shoot biomass on grain yield may be a result of the negative effect of shoot biomass accumulated during the vegetative growth stage on the rice harvest index as discussed by Kumura (1995). Similarly, according to Equation 4, the optimum value of FW for minimum protein content was 2150 g m^{-2}, a value quite similar to the optimum FW of 2180 g m^{-2} for maximum grain yield.

Validation for the calibrated models

The coefficient of determination (R^2), RMSEP, and REP using the calculated and actual values of grain yield and protein content in the calibration (2003 and 2004 experiments) and validation (2005 experiment) datasets are presented in Table 3. The stepwise multiple regression models using the Npi, FW, and SPAD explained 85 and 87% of the variation in grain yield and protein content in the calibration data set, respectively. In addition, REPs were small: 6.3 and 3.0% in grain yield and protein content predictions, respectively. However, the R^2 in the validation dataset decreased significantly compared to the calibration dataset while the RMSEP and REP increased slightly. These results are indicative of the decrease in precision of the models for grain yield and protein content prediction, although the accuracy in the validation set did not vary. The decrease in precision for the model may be partially due to the narrow range of grain yield and protein content in the validation dataset (Table 3 and Fig. 1), as it included the treatment recommended for the target protein content of 6.8%.

Recommended N topdress rate at PI

To determine the N topdressing rate recommendation at PI in the 2005 experiment using the models quantified from the 2003-2004 experiments, FW and SPAD values for nine plots (three replicates of three levels of applied N rates: 0, 36, and 72 kg N ha^{-1}) were measured. The actual values of FW and SPAD are presented in Table 4.

Table 3. Performance parameters of the predictive models for grain yield and milled-rice protein content.

Crop variables	Descriptive statistics			Model performance parameters[b]			
	Mean	CV (%)	Min	Max	R^2	RMSEP	REP (%)
Calibration dataset (n = 81), 2003 and 2004 experiment							
Yield[a] (kg ha^{-1})	6450	15.6	3588	8458	0.85	405	6.3
Protein (%)	6.76	8.5	5.86	8.34	0.87	0.20	3.0
Validation dataset (n = 24), 2005 experiment							
Yield (kg ha^{-1})	6837	12.4	5254	8580	0.68	472	6.9
Protein (%)	6.68	4.6	6.21	6.99	0.43	0.24	3.6

[a] Yield and Protein were grain yield and milled-rice protein content (%) at harvest, respectively.
[b] R^2, RMSEP and REP were coefficient of determination, root mean square error in prediction and relative error of prediction, respectively.

The application of 36 or 72 kg N ha^{-1} increased FW from 1,236 g m^{-2} (control) to 1,870 and 1,988 g m^{-2}, respectively. The coefficients of variation in the FW were 16.2% in the control treatment (no N applied at tillering stage), and 26.4 and 32.2% in the 36 and 72 kg N ha^{-1} applications at tillering stage, respectively. On average, the FW for the nine recommended N plots was 1,698 g m^{-2} with a coefficient of variation of 31.7%. Similarly, the application of N at tillering stage increased SPAD values from 28.2 (no N application) to 29.8, and 31.7 for the 36 and 72 kg N ha^{-1} treatments, respectively. The average value of SPAD for the nine recommended N plots was 29.9 with a coefficient of variation of 9.9%. A higher within-field spatial variation of rice biomass than SPAD and shoot N concentration has previously been reported (Nguyen et al, 2006).

Based on the measured values of FW and SPAD of the nine N topdressing recommendation plots, it is possible to compute an N topdressing rate at PI for a target grain yield or protein content using Equation 3 or 4, respectively. However, Equation 4 was used to recommend N rate at PI for the target protein content due to the recent emphasis on producing high quality rice with lower protein content. Protein content has been reported to have a significantly negative correlation with rice grain quality, especially for taste of cooked rice (Ishima et al. 1974; Chikubu et al. 1985; Taira 1995). Moreover, the result from the analysis of variance indicated that yearly variation significantly affected grain yield, not protein content, and that both were significantly affected by Npi (Nguyen 2005). Therefore, the equation for protein content prediction is more adaptable for year to year variation and thus more reliable for N topdressing recommendation. The calculated Npi for the target protein content of 6.8% (0% moisture basis) based on the measured FW and SPAD as calculated by Equation 4 is presented in Table 4. The Npi for the target protein content of 6.8% was 35.5, 40.3, and 37.3 kg N ha^{-1} for the treatments with 0, 36, and 72 kg N ha^{-1} applied at tillering stage, respectively. It should be noted that the 72 kg N ha^{-1} treatment had higher values of FW and SPAD but required less N at PI than the 36 kg N ha^{-1} treatment. This reflects the quadratic effect of FW on protein content in Equation 4. Fig. 2 indicates that the N topdressing rate at PI required for protein content of a target value varies greatly depending on plant biomass and SPAD and Npi should be adjusted based on their values. For example, it is desirable to increase Npi with the increasing plant biomass up to a certain level in order to satisfy the target protein content and achieve higher grain yield as well, even though rice plant at PI has the same N nutrition status (the same SPAD value).

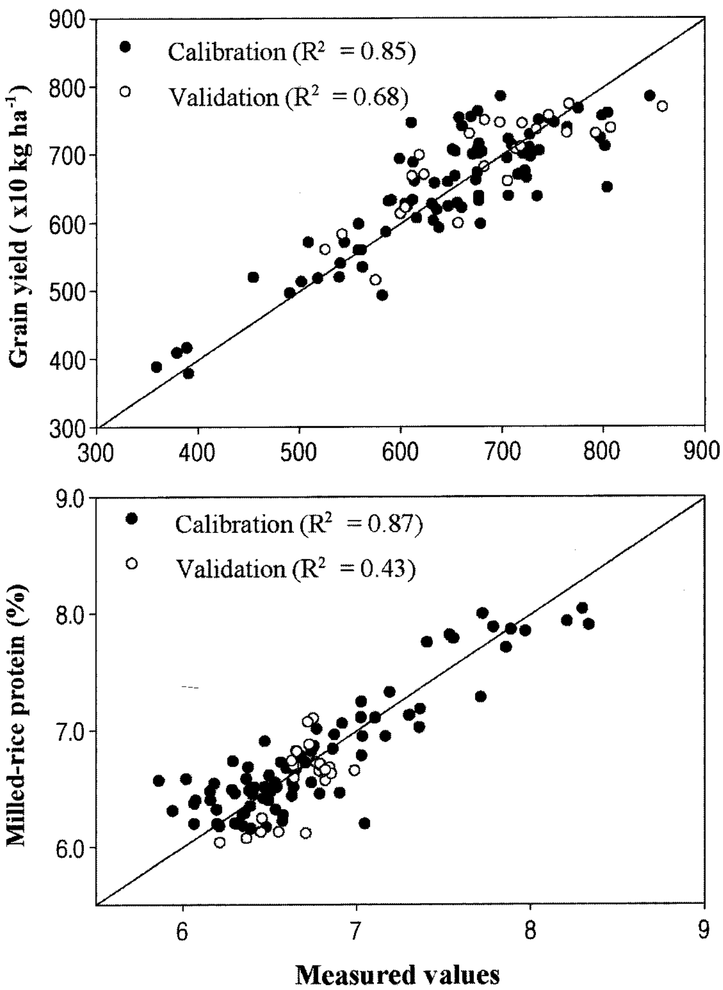

Fig. 1. The measured versus calculated values of grain yield and milled-rice protein content by Equations 1 and 2, respectively. Solid line is 1:1 line.

Table 4. Parameters of the recommended N rate treatment at PI for the target milled-rice protein content of 6.8% in cv. Hwaseongbyeo in the 2005 experiment.

N rate at tillering (kg ha^{-1})	Shoot fresh weight (g m^{-2})				SPAD values				Recommended N rate[a] (kg ha^{-1})			
	Mean	CV (%)	Min	Max	Mean	CV (%)	Min	Max	Mean	CV (%)	Min	Max
0	1236	16.3	1093	1378	28.2	2.8	27.6	28.7	35.5	9.3	33.2	37.8
36	1870	26.4	1440	2409	29.8	12.8	26.7	34.0	40.3	7.2	37.0	42.6
72	1988	32.2	1422	2684	31.7	8.2	28.9	33.9	37.3	2.1	36.7	38.2
Average	1698	31.7			29.9	9.9			37.7			

[a] Recommended N rate were calculated by Eq. 4 for target milled-rice protein content of 6.8%.

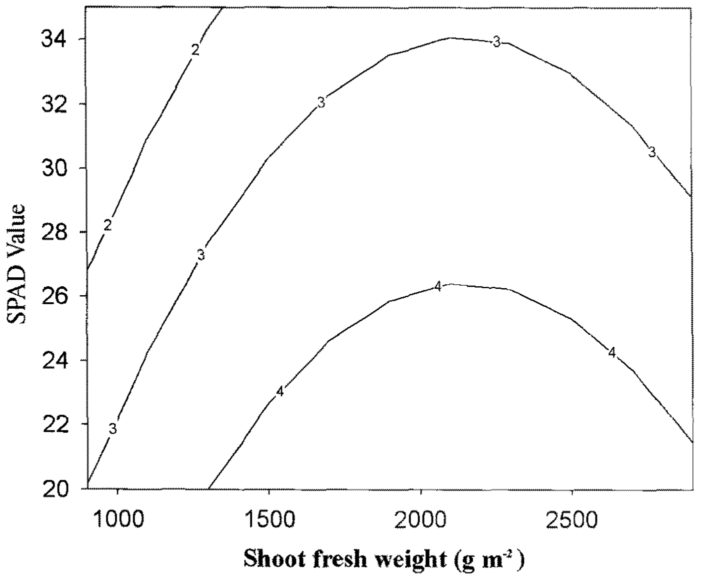

Fig. 2. Isopleths of the recommended N rate (number placed on the line of graph; kg 10a^{-1}) at PI for the target milled-rice protein content of 6.8% based on shoot fresh weight and SPAD values measured at PI. Graph was drawn by Equation 4 in Table 2.

Test of N topdressing rate recommendation

The recommended N topdressing rates presented in Table 4 were applied to nine plots for the 2005 experiment. The measured grain yield and protein content of the recommended N rate treatment (PRT) compared to the fixed N rate treatment of 36 kg N ha^{-1} at PI (FRT) and the control treatment with no N applied at PI are presented in Table 5.

Table 5. Descriptive statistics of grain yield and milled-rice protein content of the recommended N rate treatment (PRT), fixed rate treatment (FRT, 36 kg ha^{-1}), and no N treatment at PI of cv. Hwaseongbyeo in the 2005 experiment.

N rate at tillering (kg ha^{-1})	Treatment	Grain yield (kg ha^{-1})				Milled-rice protein content (%)			
		Mean	CV (%)	Min	Max	Mean	CV (%)	Min	Max
0	No N	5254	6.5	4868	5504	6.55	6.5	6.44	6.72
0	FRT	6184	8.3	5596	6563	6.65	8.3	6.27	7.09
0	PRT	6229	10.1	5756	6947	6.65	10.1	6.59	6.75
36	No N	5422	9.3	4925	5928	6.36	9.3	6.02	6.65
36	FRT	6678	5.3	6277	6953	6.64	5.3	6.22	7.01
36	PRT	6977	4.5	6733	7331	6.77	4.5	6.58	7.07
72	No N	6042	11.9	5240	6615	6.45	11.9	6.25	6.65
72	FRT	6826	1.3	6757	6924	6.84	1.3	6.71	7.10
72	PRT	7458	2.1	7312	7624	6.79	2.1	6.68	6.96
Pooled	No N	5572	10.6	4868	6615	6.46	10.6	6.02	6.73
	FRT	6563	6.6	5596	6953	6.72	6.6	6.23	7.10
	PRT	6888	9.4	5756	7624	6.74	9.4	6.58	7.07

According to the results shown in Table 5, the mean value of protein content in the PRT was 6.74%, similar to the target protein content of 6.8%. The variation in protein content decreased from 4.6% in the FRT to 2.5% in the PRT. The lower variation and narrower range of protein content in the PRT (compared to in FRT) indicated the potential application of the PRT for target protein content in rice production. Moreover, the PRT had higher rice grain yield than observed in the FRT; grain yield increased on average from 6563 kg ha^{-1} in the FRT to 6888 kg ha^{-1} in the PRT. The higher grain yield in the PRT may have resulted from the higher N application rate (Table 4) and the increased response of grain yield to the applied N at PI of the PRT, as the N rate calculation reflected crop growth and N nutritional status prior to the N application.

Dependence of grain yield on shoot fresh weight and SPAD value at PI

The grain yield value measured in the recommended N plots was indicative of the high dependence of grain yield on FW and SPAD in the PRT for the target protein content of 6.8% (as in Fig. 3). These results imply that the vegetative growth and N nutrition status should be secured to a certain level through crop management prior to application of the recommended N topdressing at PI to ensure concurrent high yield and quality production of rice. The maximum yield was attained at 2178 g fresh weight m^{-2} and a SPAD value of 33.9. This optimum value of FW was consistent with the optimum value of 2180 g fresh weight m^{-2} for maximum grain yield, as determined by Equation 3, calibrated according to data collected in 2003 and 2004.

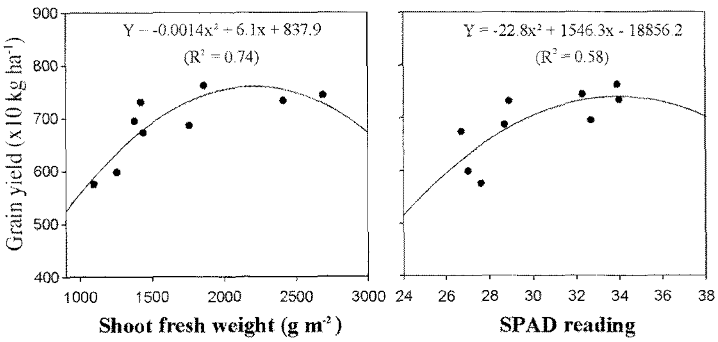

Fig. 3. Relationship between crop parameters measured at PI and grain yield of the recommended N rate treatment plots for the target protein content of 6.8% in cv. Hwaseongbyeo.

Conclusions

Stepwise multiple regression using FW, SPAD, and Npi as predictor variables explained 85 and 87% (in the calibration dataset using data from 2003 and 2004), and 68 and 43% (in the validation dataset using data from 2005) of the variation in grain yield and protein content, respectively. Application of N topdressing as recommended by the calibrated models for target protein content of 6.8% were characterized by an average protein content of 6.74%, with a range of 6.58 to 7.07%. The recommended N topdressing reduced the variation of protein content and significantly increased grain yield as compared to the FRT of 36 kg ha^{-1}, regardless of the rate of N application at the tillering stage. In the PRT for the target protein content of 6.8%, grain yield was highly dependent upon FW and SPAD values at PI, with optimal FW and SPAD values of approximately 2180 g m^{-2} and 33.9 for maximum yield, respectively. In conclusion, the model for N topdressing rate recommendation at PI was successful under the present experimental conditions. However, additional testing under more variable environmental conditions should be performed before universal application of these models.

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