

## Calibration Update for the Measuring Total Nitrogen Content in Rice Plant Tissue Using the Near Infrared Spectroscopy

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**ABSTRACT** The aim of the present study was to update the calibration that is used for the measurement of the total nitrogen content in the rice plant samples by using the visible and near infrared spectrum. Before the equation merge, correlation coefficient of calibration equation for nitrogen content on each rice parts was 0.945 (Leaf), 0.928 (Stem), and 0.864 (Whole plant), respectively. In the calibration models created by each part in the rice plant under the various regression method, the calibration model for the leaf was recorded with relatively high accuracy. Among of those, the calibration equation developed by Partial least squares (PLS) method was more accurate than the Multiple linear regression (MLR) method. The calibration equation was sensitive based on variety and location variations. However, we have merged and enlarged various of the samples that made not only to measure the nitrogen content more accurately, but also later sampling populations became more diversified. After merging,  $R^2$  value becomes more accurate and significantly to 0.950 (L.), 0.974 (S.), 0.940 (W.). Also, after removal of outlier,  $R^2$  values increased into 0.998, 0.995, and 0.997. In view of the results so far achieved, Standard error of prediction (SEP) and SEP (C) were reduced in the stem and whole plant. Biases were reduced in the leaf, stem as well as whole plant. Slopes were high in the stem. Standard deviation reduced in the stem but  $R^2$  was high in the stem and whole plant. Result was indicated that calibration equation make update, and updating robust calibration equation from merge function and multi-variate calibration.

**Keywords** : rice, NIRS, nitrogen, calibration model, update

**NIR Spectroscopy(NIRS)** is one of the non-destructive methods used to analyze interior and exterior characteristics

of the sample. Non-destructive analysis doesn't require extraction; filtration, dilution, reagent reaction or heating of the sample and the measured sample could be recovered without damage (Clarke *et al.*, 1992; Williams *et al.*, 1991). Also, this method can observe the changes during the storage and process of same sample, and save time. this method is not use of chemical reagent, so can prevent pollution of environment. Near-infrared spectroscopy is one of the methods that analyze the sample by non-destructive method through optical characteristics of intra- and exterior sample. Near-infrared spectroscopy has been used for the rapid, easy and accurate nitrogen content analysis of rice. That analyze the sample by non-destructive an easily configured composes system for quality evaluation compared to other non-destructive analyses and doesn't require much skill to carry out analysis. Also, this method has advantage not only easy result analysis but also low expenses(Hymowitz *et al.*, 1974; Marten *et al.*, 1983; Rubenthaler & Bruinsma 1979; Shenk *et al.*, 1981).

Nutritional diagnosis and quantity analysis of chemical compounds of the plant are necessary. Spectrum of near-infrared get by light energy absorption of organic molecule, function of O-H, C-H, N-H, C=O in this absorption line appear variety chemical bond, as application can measure quickly multiple element of moisture, protein, starch and fat (Abrams *et al.*, 1987; Clarke *et al.*, 1992; Kwon *et al.*, 2005; Kwon *et al.*, 2006).

The measurement skill develop for nitrogen content in rice plant using near infrared spectrophotometer, without chemical reagent use and quickly analysis by non-destructive compare with general chemical analysis method. So it can expect large time saving and cost reduction effect. The calibration equation, which relate each other between nitrogen element and spectrum data that need for quantity analysis

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of nitrogen using a near infrared spectrophotometer. Therefore, NIRS generally used for quantity analysis of general component in plant, industry, clothes and food etc. NIRS depend upon appropriate collection of sample and good mathematics method. Accurate calibration sample must be used that represent sample for accurate calibration equation. In contrast to wet method, NIRS is an important trust degree because of indirect method. But natural products are changed by growing season, soil fertility, precipitation amount and growth condition. So it is very important that calibration equation should make update, and updating should robust calibration equation. Therefore, the aim of the present study was to develop the updated calibrations that were used for the measurement of the total nitrogen content in the merged samples by using the visible and near infrared spectrum in the rice plant.

## MATERIALS AND METHODS

Collection of spectrum was performed from 2004 to 2005. For making the calibration equation, 31 varieties (Dongjin1hobyeyo etc) of rice were transplanted at Iksan, Jeongup and Buan. The transplanting with the planting distance of 30×14 cm was made on May 30. Fertilization of N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O was the ratio of 11-4.5-5.7 kg/10a. Nitrogen was applied in three splits (basal 50 : mid-tillering 20 : panicle initiation 30). Potassium was applied in two splits (basal 70 : panicle initiation 30). However, phosphorus was applied once as a basal fertilization.

Update test use Nampyeongbyeoy that performed at experimental field of Jeollabuk-do Agricultural Research and Extension Services in 2005. Nitrogen was applied at the rate of 0, 7, 9, 11 and 15 kg/10a. Planting distance was 30×11, 13 and 15cm, respectively. However, P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O was applied at the ratio of 4.5-5.7 kg/10a. Seeding and transplanting were performed on April 30 and May 30, respectively.

Sampling was the 5 hills from maximum tilling stage to the heading stage in every 2 weeks interval. For the nitrogen content analysis, samples were dried at 90°C for 2 hours, at 70°C for 24~72 hours after picking rice from the 5 hills. The sample was divided into leaf, stem and whole plant and measured with an infrared spectrophotometer (NIR Systems, Model 6500, FOSS Inc. Sweden). The reflection

degree (Log 1/R) was obtained by fellow method. The 5 g dried rice plant was grinded and put in mini sample cup. Diffusion reflection spectrum of the sample was recorded with the use of scanning monochromatic light. The reflectance spectra were measured in the range of 400-2500 nm, with 2 nm intervals. Infracsoft international software (WinISI, Ver. 1.5 USA) was used for the driving of near infrared spectrophotometer. Reference spectrum was obtained by using standard ceramic plate. The regression analysis methods applied were multiple linear regression (MLR) and partial least square method (PLS).

## RESULTS AND DISCUSSION

The diffuse reflectance obtained in whole wavelength (400~2500 nm) of homogenized rice sample using near infrared spectrophotometer. Some of them, reflectance (Log 1/R) of 259 piece substituted in calibration equation that required component content analysis, and total nitrogen contents in rice plant analyzed.

Figure 1 shows absorbance spectra of rice plant. The whole wavelength in leaf, stem and whole plant showed

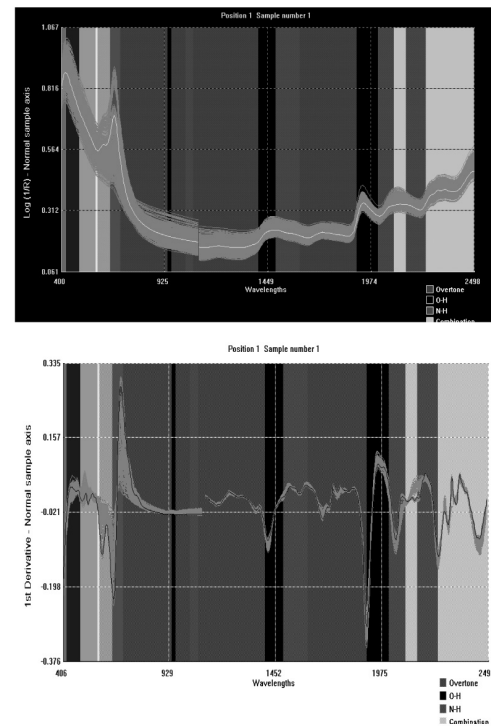
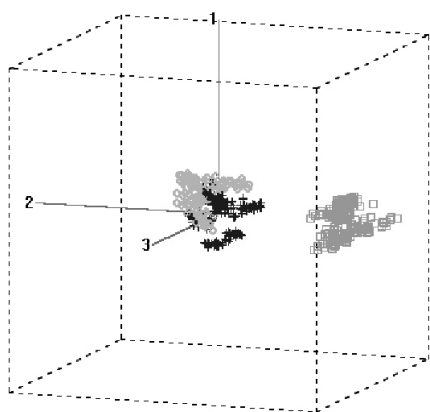


Fig. 1. Typical absorbance spectra of rice plant using NIRS.

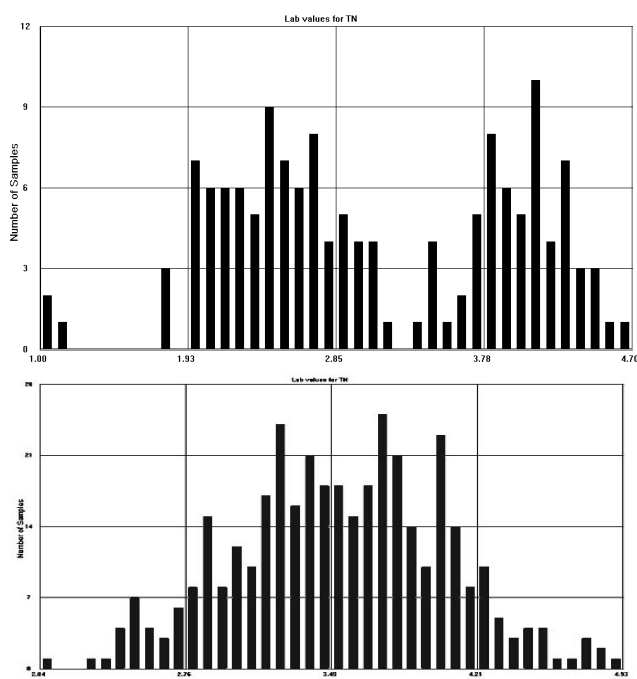
same spectrum form. The peak of absorbance in red region of visible showed high difference that is due to color of epidermis in rice plant. The absorbance in near infrared region remarkably decreased.

The basic element analysis performed using spectrum that were obtained in rice plant. Figure 2 shows that three-dimensional diagram of rice plant from three major principle component analyses. The specificity of distribution showed leaf, stem and whole plant in rice.

Figure 3, nitrogen content range of collected sample



**Fig. 2.** Three-dimensional diagram of rice plant from three major principle component analysis axes



**Fig. 3.** The ranges of the total nitrogen contents in the rice

divided into plenty and lack of nitrogen content. This result was due to difference of nitrogen absorption ability by rice variety. Many sample needs to compose the population and to explain diversity of spectrum. Also, calibration equation using right sample is important. Each part the calibration that it draws up especially before using a merge function, the sample where the nitrogen content is many with the fact that it draws up the actual condition, which is distinguished with the sample, is thought to be the natural result. The leaf, the stem, the sample of the plant it uses a merge function and the result from the picture of LSW portion of picture 3, it reports a same result was showed in comparison normal distribution (Lee *et al.*, 2001).

Calibration equation for nitrogen analysis of rice plant was developed using partial least squares(PLS). PLS method generally used when spectrum is complex and analysis is difficult by the overlapping of absorption lien. Total of 259 serum samples were measured with a NIR systems which has an available range 400~2500 nm. Calibration model was developed using a total 337 - serum spectrum.

Accuracy degree was determined by component predict regression model expressed by standard error of calibration (SEC) and coefficient of determination ( $r^2$ ) Similar to our findings, a high accuracy of regression model, low SEC, and close unit of  $r^2$  value was reported by these authors (Han & Natsuga 1996).

In the PLS model,  $r^2$  and SEC of calibration equation from leaf were 0.945 and 0.047, respectively. For making the calibration, nitrogen content of the collected sample was 1.78~4.45. In stem the  $r^2$  and SEC of calibration model were 0.928 and 0.076, respectively. However, the range of nitrogen content was 1.12~3.57, Also, the  $r^2$  and SEC of calibration model from whole plant were 0.864 and 0.087 range. The nitrogen content of collected sample was 1.16~3.37. This result showed the tendency Jimenez and Ladha (1995) report.

Total 337-piece spectrum under spectrum by each part developed calibration mode. The rest 418-piece spectrum was used by validation test. PLS method use that spectrum is complicate and analysis is different by overlapping of the absorption lines.

Test of regression model showed bias that standard error of prediction predicts value; coefficient of determination

and difference of predicts value by regression model verses with chemical analysis value average. It was described that regression model was accurate in that SEP and  $r^2$  were close to SEC, and  $r^2$  of developed regression model, and bias was close to zero (Han & Natsuga, 1996). Calibration equation created by PLS (partial least squares) method indicated high accuracy than MLR method. And nitrogen content was high in leaf, whole plant and merge calibration as well as accuracy was at the excellent level.

Multiple linear regression analysis is normally useful when composition of sample is simple and measurement component has absorbed sharply. Table (1) showed result that make a calibration equation using Multiple Linear Regression after merge. After merging the accuracy of calibration in PLS method was improved, but decreased in MLR. Therefore calibration equation developed by PLS method indicated high accuracy than the ML method.

Calibration model that coefficient of determination in leaf under MLR model was 0.908, and standard error of calibration (SEC) was 0.245. The range of nitrogen content on collected sample for calibration equation create was 1.0~4.7. Calibration model that coefficient of determination in stem was 0.893, and standard error of calibration (SEC) was 0.157. The range of nitrogen content of collected sample

for calibration created was 0.3~2.5. Calibration model that coefficient of determination in plant was 0.896, and standard error of calibration (SEC) showed 0.232. The range of nitrogen content on collected sample for calibration equation created was 0.9~3.6 (Table 2).

For NIRS analysis there is no need of these operations like: extraction, filter, dilution, reagent reaction and heating of the sample. It was described that measured sample can recover from non-damage condition. The NIRS analysis depends on wet analysis value of the lab, because we must analyzes more accurately. Figure 4 shows that relation to interaction crossing between analysis value by normal analysis method of nitrogen content and analysis value attracted by NIRS on calibration equation created. It showed high  $R^2$  0.979 and 0.962. This mean that nitrogen calibration equation created is reasonable.

Accurate calibration sample must be used that represent sample for making accurate calibration equation. In contrast to wet method, NIRS is an important trust degree because of indirect method.

Table 3 showed test result for nitrogen content of unknown sample by PLS method. Difference between wet analysis value and NIR analysis value showed 0~0.024%. This result means that NIR analysis value, as lab data was

**Table 1.** Results of calibration model and predication for determining nitrogen content by partial least squares method in different rice plant parts

Plant Parts	Calibration model				Validation test					Rating
	Nitrogen range (%)	$R^2$	SEC	No. of Sample	$R^2$	SEP	S·D	S·D/SEP	No. of Sample	
L	1.8~4.5	0.945	0.047	112	0.995	0.055	0.785	14.3	137	E
S	1.1~3.6	0.928	0.076	110	0.982	0.063	0.719	7.4	140	E
W	1.2~3.4	0.864	0.087	100	0.995	0.051	0.466	14.0	141	VG
LSW	1.1~4.5	0.883	0.123	337	0.991	0.056	0.657	11.9	418	E

L: Leaf, S: Stem, W: Whole plant, SEC: Standard error of calibration, SD: Standard deviation of range of plant % N in validation set  
\*Rating indicates the SD/SEP value E: Excellent (>10), VG: Very Good (7~10), G: Good (5~7), F: Fair (3~5)

**Table 2.** Results of calibration model and predication for determining nitrogen content by MLR method in different rice plant parts

Plant Parts	Nitrogen(%)	$R^2$	SEC	No. of Sample
L	1.0 ~ 4.7	0.908	0.245	217
S	0.3 ~ 2.5	0.893	0.157	432
W	0.9 ~ 3.6	0.896	0.232	215
LSW	0.3 ~ 4.7	0.767	0.425	431

L : Leaf, S : Stem, W : Whole plant, SEC : Standard error of calibration

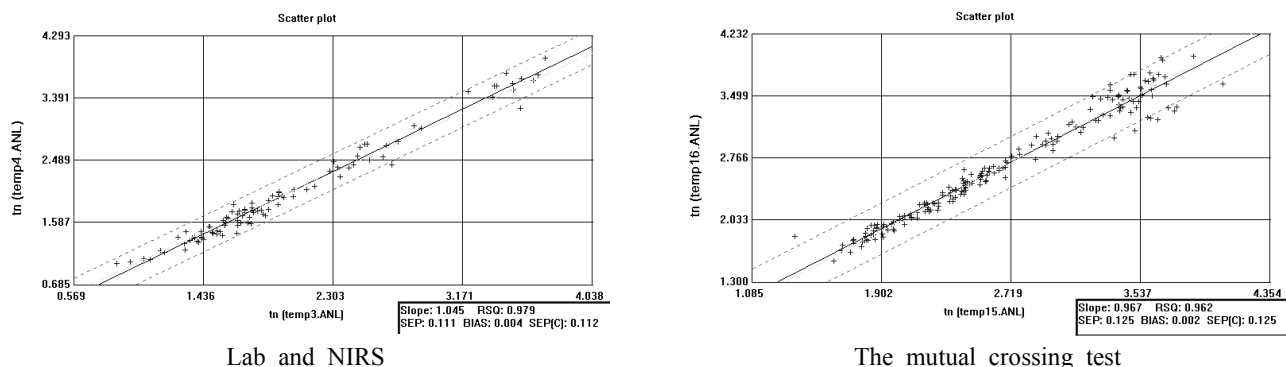


Fig. 4. Correlation between lab and NIRS measurements for TN Correlation between lab and NIRS measurements for TN

Table 3. Difference between lab and NIRS measurements by partial least squares method

Tests	Lab.	NIR	Difference	No. of sample
1	1.814	1.838	-0.024	38
2	2.902	2.902	0.000	55
3	1.052	1.042	0.010	65
4	1.755	1.758	-0.003	64
An average	1.881	1.885	0.004	-

high accuracy, it was same proof result by many methods.

Many sample needs to compose the population and to explain diversity of spectrum. Also, calibration equation using right sample is important. Calibration equation was based on the variety and location variations. However, we have merged the samples to measure the nitrogen content more accurately. Later sampling populations became more diversified. Cropping pattern i.e. transplanting and direct seeding culture variations file were also merged.

Many sample needs to compose the population and to explain diversity of spectrum. Also, calibration equation

using right sample is important. Calibration equation was based on the variety and location variations. However, we have merged the samples to measure the nitrogen content more accurately. Later sampling populations became more diversified (Fig. 5). Total 217 serum samples were updated. However, only 112 serum samples were used to make a calibration model by merging the transplanting pattern file and variation of cultivation condition.

Total 206 serum samples, after removing 11-serum outlier, were used to make a calibration equation. This result showed that SEP and Bias value were reduced. Slope value was

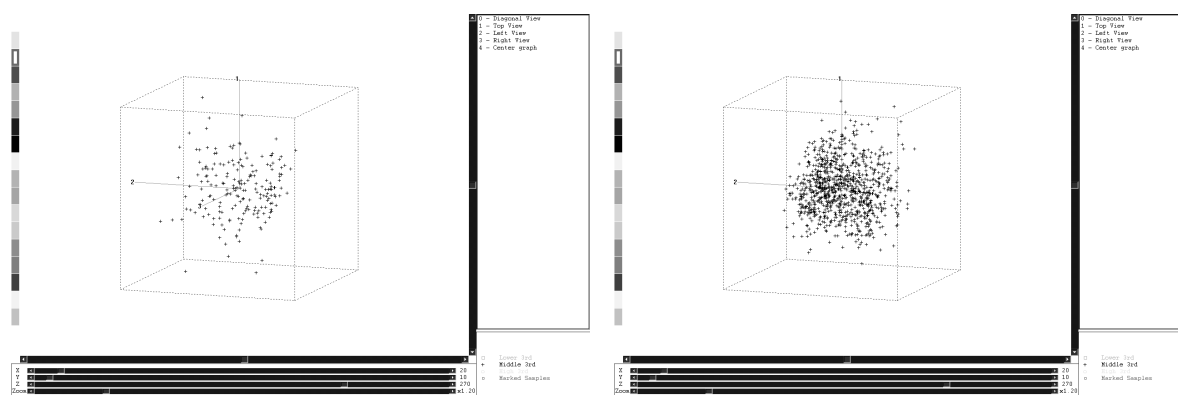


Fig. 5. Three-dimensional graphics of rice plant before and after merging.

**Table 4.** Outlier identification for update after merge

Plant Parts	Outlier removed	Bias	Slope	S·D	SEP	N·S	R <sup>2</sup>
L	B	-0.003	0.981	0.801	0.180	217	0.950
	A	0.001	1.002	0.796	0.037	206	0.998
S	B	-0.006	0.988	0.482	0.078	216	0.974
	A	0.000	1.001	0.480	0.034	211	0.995
W	B	-0.010	0.970	0.717	0.177	215	0.940
	A	0.000	1.000	0.720	0.041	204	0.997

L: Leaf, S: Stem, W: Whole plant, B: before outlier removed , A: after outlier removed,  
N·S: Number of samples, S·D: Standard deviations

**Table 5.** Results of predication for determining nitrogen content by partial least squares method in different rice plant parts

Plant Parts	R <sup>2</sup>	SEP	S·D	SD/SEP	No. of Sample	Rating
L	0.998	0.037	0.796	21.6	206	E
S	0.995	0.034	0.480	14.1	211	E
W	0.997	0.041	0.720	17.5	204	E

L: Leaf, W: Whole plant, S: Stem, SEC (p): Standard error of calibration

S·D: Standard deviation of range of plant % N in validation set

\*Rating indicates the SD/SEP value E: Excellent (>10), VG: Very Good (7~10), G: Good (5~7) , F: Fair (3~5)

close to unit and Standard Deviations value reduced but R<sup>2</sup> value was high. Similar trend was recorded in the leaf and whole plant (Table 5). Validation of regression model indicate standard error of prediction (SEP) value, r<sup>2</sup>, and bias which is the difference of prediction value by regression and chemical analysis value.

After calibration equation, file was used for unknown sample. Calibration equation was evaluated by standard error of prediction(SEP) and standard deviations value. Result showed that level of update file was excellent. R<sup>2</sup> value before update increased accuracy from 0.945, 0.928, 0.864 to 0.950, 0.970, 0.940. Also, R<sup>2</sup> after removal of outlier increased unto 0.998, 0.995, and 0.997. But, R<sup>2</sup> value after update was 0.908, 0.893, 0.896 in multiple linear regression method. The accuracy of this method was lower than PLS method. This result suggests that update of calibration equation improved accuracy. By the method above the regression update one result accuracy the Jimenez and Ladha (1995) this it compares in the result which it reports and the possibility the fact that it improves more it was.

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