Prediction from Linear Regression Equation for Nitrogen Content Measurement in Bentgrasses leaves Using Near Infrared Reflectance Spectroscopy

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근적외선 분광분석기를 이용한 잔디 생체잎의 질소 함량 측정을 위한 검량식 개발

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ABSTRACT

Near Infrared Reflectance Spectroscopy(NIRS) is a quick, accurate, and non-destructive method to measure multiple nutrient components in plant leaves. This study was to acquire a liner regression equation by evaluating the nutrient contents of 'CY2' creeping bentgrass rapidly and accurately using NIRS. In particular, nitrogen fertility is a primary element to keep maintaining good quality of turfgrass. Nitrogen, moisture, carbohydrate, and starch were assessed and analyzed from 'CY2' creeping bentgrass clippings. A linear regression equation was obtained from accessing NIRS values from NIR spectrophotometer(NIR system, Model XDS, XM-1100 series, FOSS, Sweden) programmed with WinISI III project manager v1.50e and ISIscan® (Infrasoft International) and calibrated with laboratory values via chemical analysis from an authorized institute. The equation was formulated as MPLS(modified partial least squares) analyzing laboratory values and mathematically pre-treated spectra. The

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accuracy of the acquired equation was confirmed with SEP(standard error of prediction), which indicated as correlation coefficient(r^2) and prediction error of sample unacquainted, followed by the verification of model equation of real values and these monitoring results. As results of monitoring, r^2 of nitrogen, moisture, and carbohydrate in 'CY2' creeping bentgrass was 0.840, 0.904, and 0.944, respectively. SEP was 0.066, 1.868, and 0.601, respectively. After outlier treatment, r^2 was 0.892, 0.925, and 0.971, while SEP was 0.052, 1.577, and 0.394, respectively, which totally showed a high correlation. However, r^2 of starch was 0.464, which appeared a low correlation. Thereof, the verified equation appearing higher r^2 of nitrogen, moisture, and carbohydrate showed its higher accuracy of prediction model, which finally could be put into practical use for turf management system.

Key word : Bentgrass leaves, calibration, NIRS, Nitrogen analysis

INTRODUCTION

Near Infrared Reflectance Spectroscopy(NIRS) is well reported as an analysis tool for measuring nutrient composition and contents in crop plants and foliage such as nitrogen, protein, carbohydrates, oil, moisture, minerals and so on(Eckman et al., 1983; Halgerson et al., 1995; Hattey et al., 1994; Moore et al., 1990; Roy et al., 1993). NIRS technology is based on near infrared absorption properties, which couldbe measured and used to differentiate main class of compounds in plants(Martens et al., 1985; Shenk and Westerhaus, 1991). The advantageous aspect of NIRS assay is explained as a non-destructive method. This means that sample tissue is prepared and treated without any artificial destruction or damage as well as use of any solvent or chemical. Samples are also applicable and measurable through NIRS under various types of sample conditions such as solid, solution, liquid, suspension, and powder. Sample containing moisture can be measured without drying procedure and moisture itself is regarded as a measurable item(Song et al., 2002). In addition, the assay was easily applied by beginners because of simple operation to acquire linear regression equation through regression analysis using chemometric computer software, low displacement of equipment, and low cost maintenance. Therefore, the assay is proven to be time-saving, and simple and economic.

The assay, since firstly introduced by Dr. Norris from USDA(Unites States Department of Agriculture), has been broadly developed in fields of agriculture and food. Much research has introduced to provide better understating of the potential or mechanics to measure certain constituents in plants, predict the responses of crop to fertilizers and to specific nutrient-containing soil, or to determine the quality of forage and seed(Fox et al., 1993; Norris et al., 1976; Shenk and Westerhaus, 1995). Moreover, soil profiles including moisture, organic matters, inorganic matters like C and N in soil, and minerals were evaluated using NIRS(Bowers and Hanks, 1965; Morra et al., 1991; Ben-Dor and Banin, 1994). In Korea, Zhang et al. (2004) firstly adopted NIRS technology to determine nitrogen contentsin fresh and dry leaves of apple tree.

NIRS has been also widely applied in studies of turfgrass. NIRS technique has been used to assess fungal infection levels in tall fescue(*Festuca arundinacea* Schreb.) (Hill et al., 1987; Roberts et al., 1988) and to predict thatch composition of creeping bentgrass(*Agrostis palustris* Huds.) (Couillard et al., 1994). Murphy(1993) stated that N concentrations predicted by NIRS have been positively correlated with TKN for perennial ryegrass(*Lolium perenne* L.) (r^2 = 0.93) and creeping bentgrass(r^2 = 0.89).

N status significantly affects growth and yield for crop plants. N fertility is highly related with visual quality and health condition in turfgrass so that physiological response of turfgrass to N fertilization is very crucial factor in turf management. Rodriguez and Miller(2000) suggested that N fertilization predicted by NIRS is more effective and economic in bermudagrass quality than time- or visual quality-based fertilization. As more studies, NIRS has been also used to schedule N fertilization on dwarf-type bermudagrasses(Ian and Grady, 2000), and to analyze Mono- and Polysaccharides in creeping bentgrass(Siddhartha et al., 2005).

The study was aimed to predict linear regression equations from certain nutrient components by collecting the measured values in 'CY2' creeping bentgrass clippings under seasonable management conditions using NIRS. NIRS for characterizing nutrient contents in turf leaves will be only accurate and precise method to evaluatethe prediction of the responses of turfgrass to fertility, nutrient uptake in soil, and turf quality, which further will establish more advanced fertilization program in turfgrass management.

MATERIALS AND METHODS

Preparation of turfgrass clippings

'CY2' Creeping bentgrass clipping was collected from the nursery of Turfgrass & Environment Research Institute(TERI), SAMSUNG EVERLAND andmaintained with a general management program from Anyang Benest GC management team. Turfgrass clippings for the study were collected from 22 April 2008 to 18 November using a walking greens mower with a catch basket depending on the growth rate. Clippings collected in the field placed in a plastic zip-closure bag then stored at -20℃. Frozen clippings were thawed at room temperature for over-night.

Laboratory Analysis(Wet method)

Laboratory values via chemical were analyzed from an authorized institute. The primary content of moisture was measured with the weight of clipping that dried at 60~70℃ in a drier for 24 hours then cooled for 2~3 hours at room temperature. Then clipping was grinded and homogenized with grinder(Ret mil) using 0.5 mm screen. The secondary content of moisture was measured with the weight of clipping that were dried at 135℃ with a high temperature-adjusted drier for 2 hours. The content of nitrogen was measured with kjeltec 2400(micro-kieldahi method) and the content of carbohydrates was measured with this equation

100 - (moisture-crude protein-crude fat - crude ash).

NIRs and software

A NIR spectrophotometer(NIRSystem, Model XDS, FOSS, Sweden) with a wavelength range of at least 400 to 2500nm was used for this study. The computer software WinISIⅢ Project Manager v1.50e and ISIscan®(Infrasoft International) was used for collecting, storing and analyzing spectral information. The spectrophotometer was pre-warmed for 1 hour for uniformed light source. A sample cup was filled with clippings and cardboard back was pushed into the holder to press the sample firmly against the window of a sample cup and loaded into NIRs spectrophotometer. Measurement of spectrum was repeated 3 times.

We used the algorithm CENTER for the calculation of principle components and Mahalanobis distance(H), a measure of the difference between a sample and the mean value of a population for the multivariant situation. This calculated information was used for the description of spectral boundaries and detection of outliers(Shenk and Westerhaus, 1995). Principle component analysis(PCA) was used to rank the spectra of all samples according to their Mahalanobis distances from the mean spectra of the file. Global "H"(GH) values are the H values which were standardized by dividing them by their average values. Spectra in the file were reordered from smallest to largest GH values. Any sample which had a GH value less than 3.0 was retained for further analysis by wet methods. Predictive partial least squares regression(PLSR) is generally used as a regression method to formulate an equation for NIRS quantitative analysis(Chung and Kim, 2000). In this study, for NIRS equation we used Modified PLS(MPLS) which is roughly analogous to PCA. MPLS typically uses information from a much larger array of wavelengths than stepwise regression(Martens and Naes, 1989).

To improve the accuracy of calibrated equation, the predicted value for each sample in the calibration set is used for cross validation group set to 4 and the cross-validation was performed at the same time. Randomly selected 3/4 samples of all samples including samples which had high and low spectrum were used as celibates. And rest of the samples(1/4 samples) were used as validates. All these processes are repeated 4 times.

Critical outlier values for "T" and "GH" were set as 1.00, 3.00 where neighborhood "H"(NH) set as 0.60. The diverse calibrated equations were developed with different mathematical and statistical pretreatments of the spectral data. To optimize the equation results, combination of the first and the second derivative, gap values of 4 and 10, smooth values of 4 and 10, and standard normal variate(SNV) and detrend scatter correction were used. Optimum equations were identified as those with high coefficients of determination for calibration(r^2) and 1-VR(Variance Ratio), a low standard error of calibration(SEC), and a low standard error of cross validation(SECV). To show the derived equation, the measuring-line and the primary functional graph was used. The correlation between actual measurement and predicted values was verified with the slope of an equation and individual correlation coefficient, and then finally the accuracy of derived equation was checked.

RESULT AND DISCUSSION

Nutrient components in 'CY2' creeping bentgrass clippings were quickly estimated by the linear regression equation, as formulated by measuring diffuse reflectance at near-infrared radiation with wavelengths of 400-2500nm. Fig. 1 indicates NIR raw spectra described as Absorbance [log (1/R)].

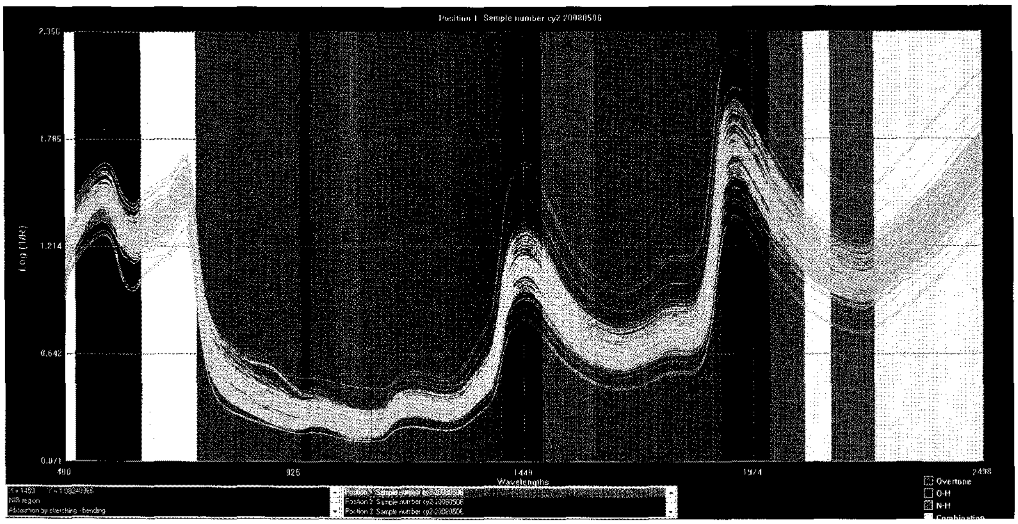

Fig. 1. Diagram of absorbance spectra[z] of creeping bentgrass clipping using NIRS.

[z]Absorbance were measured 3 times and expressed as a mean spectrum.

To accentuate certain absorbance spectra of accessing components in a sample, scatter correction and mathematical pre-treatment were applied to the raw spectra reflected at broad reflectance. That is, some distracted spectra regarded as nonlinear function distort the correlation between real data and absorbance spectra, which can be revised by Scatter correction.

Diffusion effect of the spectra from the reflected sample was corrected through Standard Normal Variant and Detrend. Pre-treatment of the spectra by calculation of SNV transformation scales each spectrum to have a standard deviation of 1.0 to help reduce particle size effects. Detrending removes the linear and quadratic curvature of each spectrum with the use of a second degree polynomial regression.

Differential calculation, generally used for the mathematical pre-treatment, emphasizes absorbance spectra and finally amplifies variation of the spectra. Namely, useless baseline was removed by differential calculus. Also, "Eliminate slope" and "particle size effects" were diminished. The result of mathematic pre-treatment of Standard Normal Variant, Detrend, and 2^{nd} derivative, is shown in Fig. 2. The spectra were considerably stabilized by correction with the pre-treatment.

Principal component analysis was conducted using the spectra obtained from 'CY2' creeping bentgrass clippings. Three dimensional scores graphics were used to inspect mutual positions of each datum in a population. A population was analyzed by calculating the distance of each datum from the principal element regarded as the center. Fig. 3 indicates distribution of samples within a population through 3-dimensional diagram.

Fig. 2. Pre-treated[z] spectra of creeping bentgrass 'CY2' clipping.

[z]Standard Normal Variant, Detrend and 2[nd] derivative were used as mathematic pre-treatment.

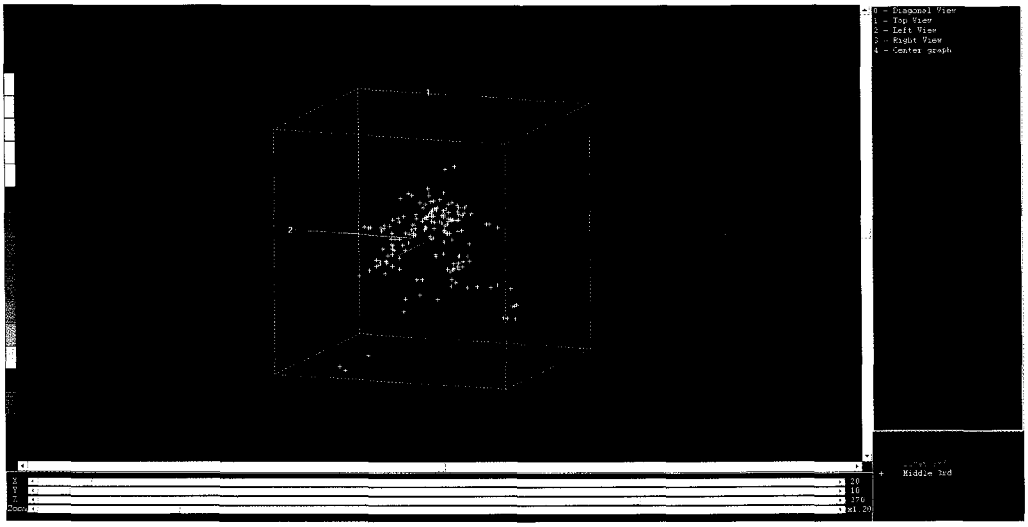

Fig. 3. Three dimensional diagram of fresh clipping of 'CY2' creeping bentgrass.

The principle elements in the collected population also can be analyzed through Histogram. The histogram of analyzed nutrient contents showed a similar pattern to closely approach the normal distribution(Fig. 4). In order to obtain more accurate equations, the more sample should be collected and analyzed.

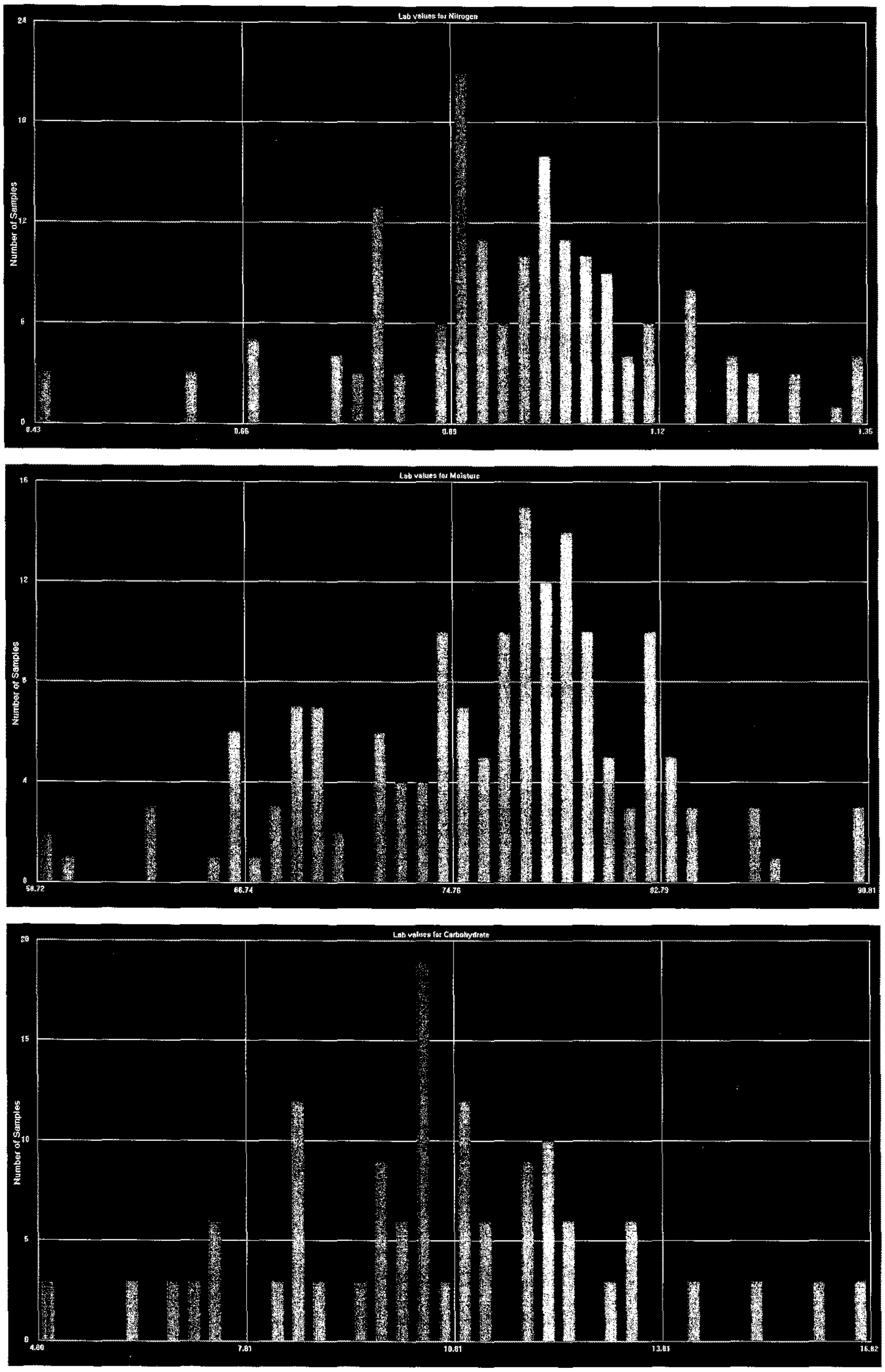

Fig. 4. The ranges of the total nitrogen contents(a), moisture contents(b), carbohydrate contents(c) in clipping of 'CY2' creeping bentgrass.

The equations for this study were prepared from the modified PLS(MPLS). MPLS, similar to principle component regression(PCR), is known to be an appropriate method to precisely describe the concentration variations of sample material by simultaneous utilization of both spectral data and the sample concentrations. The method is also used to analyze the very complex mixtures.

Then, the spectra observed were differentiated with the mathematical pre-treatment as the most common method to improve the accuracy of equations and to reduce the variables of regression analysis. To select the optimized conditions for each measuring item, mathematical pre-treatment of [1,4,4,1], [2,4,4,1], and [2,10,10,1] were used(Table 1). The analysis results were slightly different depending on the used mathematical pre-treatment. Contents of nitrogen and carbohydrate showed the highest r^2, 0.8873 and 0.9643, after mathematical pre-treatment of [2,4,4,1]. But, the content of moisture after mathematical pre-treatment of [2,10,10,1] showed the highest r^2, 0.9215. Based on these results, mathematical pre-treatment with the highest r^2 was adopted to acquire the equations.

Table 1. The Modified PLS analysis as different mathematical pre-treatments of NIR raw spectra for contents of total nitrogen, moisture and carbohydrate in fresh clipping of creeping bentgrass.

Pre-treatment	Constituent	No. of samples	Mean (%)	SEC[z]	RSQ[y]	SECV[x]	1-VR[w]
1-4-4-1	Nitrogen	158	0.9558	0.0524	0.8856	0.0662	0.8175
	Moisture	158	76.3286	1.6687	0.9156	2.0234	0.8754
	Carbohydrate	131	10.3644	0.4343	0.9642	0.5201	0.9491
2-4-4-1	Nitrogen	163	0.9593	0.0537	0.8873	0.073	0.7919
	Moisture	160	76.0924	1.7013	0.9148	2.5673	0.8051
	Carbohydrate	136	10.463	0.439	0.9643	0.6561	0.921
2-10-10-1	Nitrogen	162	0.9626	0.0572	0.8639	0.0672	0.8123
	Moisture	158	76.3999	1.6055	0.9215	2.0926	0.866
	Carbohydrate	134	10.4824	0.5081	0.9514	0.5985	0.9336

[z]SEC: standard error of calibration, [y]RSQ: coefficient of determination, [x]SECV: standard error of cross validation, [w]1-VR: 1-variance ratio

The equations acquired should be verified to improve the accuracy of equations. At first, cross-verification is accomplished while equations are prepared. The final verification was done by a monitoring program at the final step. Predicted values are compared with reference values using the monitor program. As a result, r^2 of nitrogen, moisture, and carbohydrate in 'CY2' creeping bentgrass was 0.840, 0.904, and 0.944, respectively. After outlier treatment, r^2 of nitrogen and moisture was 0.892 and 0.925, respectively as an increase by 0.052 and 0.021, individually after deletion of 4 outliers. And r^2 of carbohydrate was 0.971 as on increase by 0.027 after deletion of 5 outliers. All equations showed higher correlation(Table 2), as compared with other research studies. Murphy(1993) stated that N concentrations predicted by NIRS have been positively correlated with TKN for perennial ryegrass(*Lolium perenne* L.) (r^2 = 0.93) and creeping bentgrass(r^2 = 0.89). Miller and Dickens(1996) reported a high correlation(r^2 = 0.86) between laboratory TNC and NIRS predictions in 'Tifdwarf' and 'Tifway' bermudagrasses.

Table 2. The predictive statistics for comparing predicted vs. laboratory values of nitrogen, moisture and carbohydrate in a creeping bentgrass clipping using the optimum calibration equations.

Constituent	Outlier treatment	Mean Laboratory^z (%)	Mean NIRS^y (%)	No. of Sample	SEP(C)^x	SD laboratory^w	SD NIRS^v	GH^u	NH^t	R^{2s}	Slope^r
Nitrogen	Before	0.966	0.960	167	0.066	0.164	0.148	0.962	0	0.840	1.016
	After	0.959	0.959	163	0.053	0.160	0.149	0.964	0	0.892	1.016
Moisture	Before	76.162	76.274	163	1.871	5.964	5.368	0.968	0	0.904	1.056
	After	76.358	76.383	159	1.582	5.738	5.355	0.965	0	0.925	1.030
Carbohydrate	Before	10.614	10.542	140	0.599	2.457	2.253	0.835	0	0.944	1.059
	After	10.435	10.448	135	0.395	2.308	2.239	0.841	0	0.971	1.016

^{z}Mean laboratory values of validation samples; ^{y}Mean NIRS, mean of the predicted values for validation samples; ^{x}SEP(C), standard error of prediction corrected for bias; ^{w}SD laboratory, standard deviation of the laboratory values; ^{v}SD NIRS, standard deviation of the NIRS values; ^{u}GH, average global H values; ^{t}NH, average neighborhood H values; ^{s}R^2, coefficient of determination; ^{r}Slope, slope of the equation between laboratory and NIRS-predicted values. All of the validation sets were within the limits for average GH values

Fig. 5 showed the results after outlier treatment from the data in Table 2. The graphic was expressed as primary function of the correlation between laboratory value and NIRS value analyzed with WinISIⅢ software. Mathematical pre-treatment of raw spectra was conducted to improve the accuracy of predictive model. The equation line was explained by conversion of the individual spectrum to numerical value. Regression analysis with MPLS indicated as the optimized predictive data. The equation lines indicating the predicted values and reference values of nitrogen, moisture, and carbohydrate, were almost identical and all slopes were close to 1. Moreover, r^2 of nitrogen(0.892), moisture(0.925), and carbohydrate(0.971) showed higher correlation. It was assumed that this prediction model could be put into practical use for more turf research.

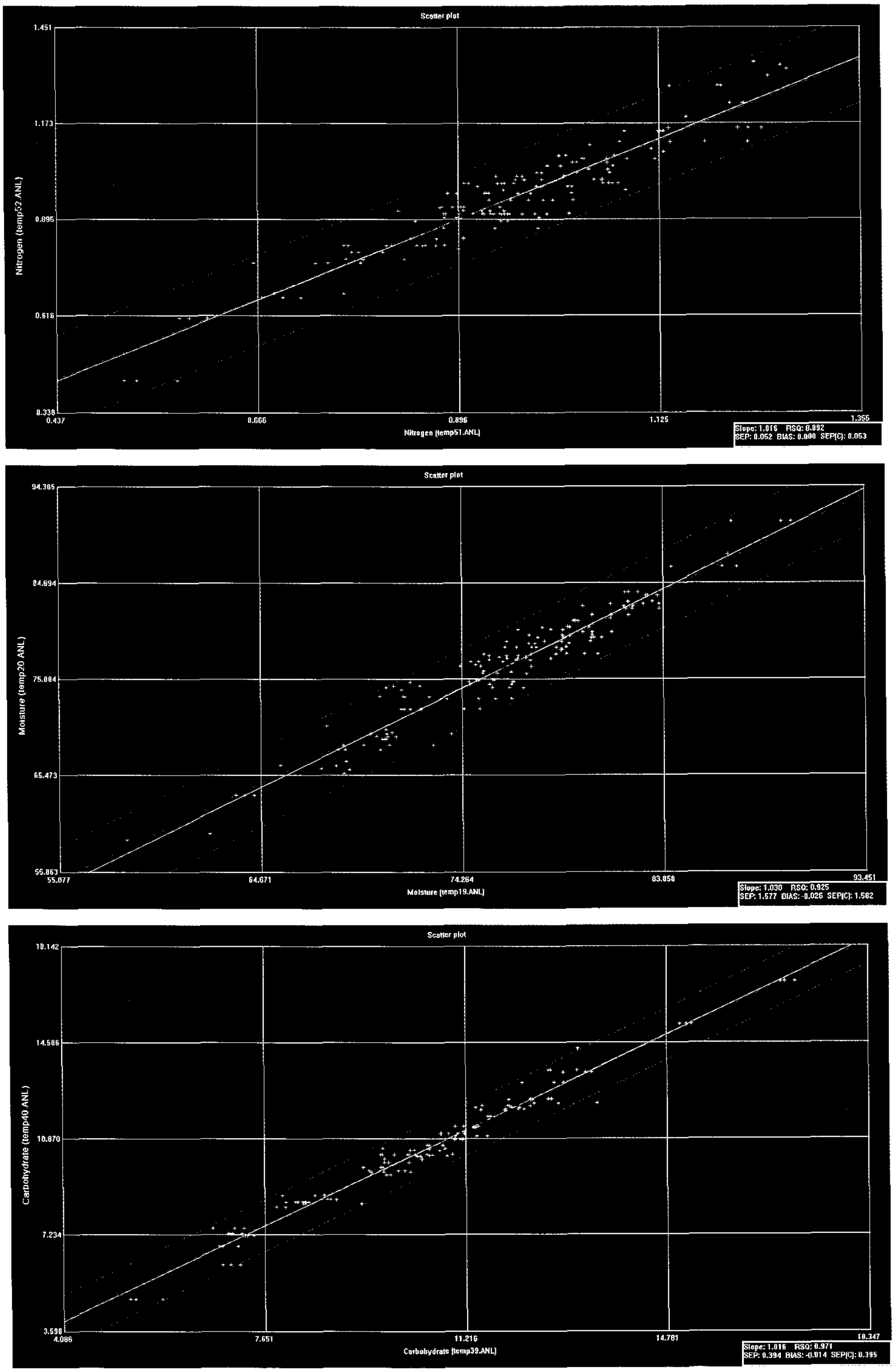

Fig. 5. Graphs showing the correlation coefficients of laboratory and NIRS-predicted values of nitrogen(a), moisture(b) and carbohydrate(c) in creeping bentgrass. The blue line means a NIRS value and the white line means a laboratory value.

국문 요약

Near Infrared Reflectance Spectroscopy(NIRS)는 짧은 시간 안에 식물의 다양한 영양소를 동시에 정확하고 빠르게 측정할 수 있다. 본 연구는 creeping bentgrass 'CY2' 엽의 여러 가지 기본 요소의 값을 예측하기 위해서 NIRS(근적외선 분광분석기)를 사용하여 측정하였다. 그 결과, 질소와 수분 그리고 탄수화물의 r^2은 각각 0.892, 0.925, 0.971이었다. 검량식에 대한 검증에서 r^2이 높은 상관관계를 나타냈으므로, 잔디에서 더 많은 연구를 위한 실용화 가능성을 확인 할 수 있었다.

주요어 : 질소분석, Bentgrass leaves, calibration, NIR

REFERENCES

1. Ben-Dor, E. and A. Banin. 1994. Visible and near-infrared analysis of arid and semiarid soils. Environ. 48:261-274.
2. Bower, S.A. and R.J. Hanks. 1965. Reflection of radiant energy from soils. Soil Science, 100(2):130~138.
3. Chung H.I. and H.J. Kim. 2000. Near-infrared spectroscopy: principles. Analytical Science & Technology. 13:1A-14A.
4. Couillard, A.A., A.J. Turgeon, J.S. Shenk and M.O. Westerhaus. 1994. Comparison of near-infrared reflectance spectroscopy and wet chemical analysis on thatch composition. HortScience 29:249.
5. Eckman, D.D., J.S. Shenk, P.J. Wangsness and M.O. Westerhaus. 1983. Prediction of sheep responses by near infrared reflectance spectroscopy. J. Dairy Sci. 66:1983–1987.
6. Fox, R.H., J.S. Shenk, W.P. Piekielek, M.O. Westerhaus, J.D. Toth and K.E. Macneal. 1993. Comparison of near-infrared spectroscopy and other soil nitrogen availability quick tests for corn. Agron. J. 85:1049–1053.
7. Halgerson, J.M., C.C. Sheaffer, O.B. Hesterman, T.S. Griffin, M.D. Stern and G.W. Randall. 1995. Prediction of ruminal protein degradability of forages using near infrared reflectance spectroscopy. Agron. J. 87:1227–1231.
8. Hattey, J.A., W.E. Sabbe, G.D. Basten and A.B. Blakeney. 1994. Nitrogen and starch analyses of cotton leaves using near infrared reflectance spectroscopy (NIRS). Commun. Soil Sci. Plant Anal. 25:1855–1863.
9. Hill, N.S., J.C. Petersen, R.A. Shelby, L.W. Dalrymple and F.E. Barton, II. 1987.

Quantification of *Acremonium coenophialum* in tall fescue seed using near infrared reflectance spectroscopy. Crop Sci. 27:1291–1295.

10. Ian, R.R. and L.M. Grady. 2000. Using near infrared reflectance spectroscopy to schedule nitrogen applications on dwarf-type Bermudagrasses. Agron. J. 92:423–427.
11. Marten, G.C., J.S. Shenk and F.E. Barton II (ed.). 1985. Near infrared reflectance spectroscopy(NIRS): Analysis of forage quality. Agric. Handb. No. 643. USDA, Washington, DC.
12. Martens, H. and T. Naes. 1989. Multivariate calibration, John Wiley&Sons, Chichester, England.
13. Miller, G.L. and R. Dickens. 1996. Bermudagrass carbohydrate levels as influenced by potassium fertilization and cultivar. Crop Sci. 36:1283–1289.
14. Moore, K.J., C.A. Roberts and J.O. Fritz. 1990. Indirect estimation of botanical composition of alfalfa–smooth bromegrass mixtures. Agron. J. 82:287–290.
15. Morra, J.M., M.H. Hall and L.L. Freeborn. 1991. Carbon and nitrogen analysis of soil fractions using near-infrared reflectance spectroscopy. Soil Sci.Soc. Am. J. 55:228-291.
16. Murphy, J.A. 1993. Near-infrared reflectance spectroscopy to quantify leaf nitrogen concentration in turfgrass. p.162. *In* 1993 Agronomy abstracts. ASA, Madison, WI.
17. Norris, K.H., R.F. Barnes, J.E. Moore and J.S. Shenk. 1976. Predicting forage quality by near infrared reflectance spectroscopy, Journal of Animal Science 43: 889-897.
18. Roberts, C.A., F.E. Barton, II and K.J. Moore. 1988. Estimation of *Acremonium coenophialum* mycelium in infected tall fescue. Agron. J. 80:737–740.
19. Rodriguez, I.R. and G.L. Miller. 2000. Using near infrared reflectance spectroscopy to schedule nitrogen applications on dwarf-type bermudagrasses. Agron. J. 92:423–427.
20. Roy, S., R.C. Anatheswaran, J.S. Shenk, M.O. Westerhaus and R.B. Beelman. 1993. Determination of moisture content of mushrooms by vis-NIR spectroscopy. J. Sci. Food Agric. 63:335–360.
21. Shepard, D.P., J.M. Dipaola, J.C. Burns and C.H. Peacock. 1990. Near infrared reflectance spectroscopy and its role in turf research. p. 182. *In* 1990 Agronomy abstracts. ASA, Madison, WI.
22. Song, H.S., K.T. Lee, S.M. Park and S.Y. Hwang. 2002. Utilization of near infrared spectroscopy for analysis of proximate composition and starch in Alaska Pollack surimi. S. Korean Fish. Soc. 35:321-326

23. Shenk, J.S. and M.O. Westerhaus. 1991. Population structuring of near infrared spectra and modified partia; least squres regression. Crop.Sci. 31:1548-1555.
24. Shenk, J.S. and M.O. Westerhaus. 1995. Forage analysis by near infrared spectroscopy. p. 111–120 *In* R.F Barnes et al. (ed.) Forages. Vol. II. The science of grassland agriculture. 5th ed. Iowa State University Press, Ames, IA.
25. Siddhartha N., W.F. Thomas and M.S. John. 2005. Analysis of mono- and polysaccharides in creeping bentgrass turf using near infrared reflectance spectroscopy. Crop Sci. 45:266–273
26. Zhang, G.C., S.H. Seo, Y.B. Kang, X.R. Han and W.C. Park. 2004. Determination of nitrogen in fresh and dry leaf of apple by near infrared technology. Korean. J. Soil Sci. Fert. 37(4), 259-265.