Korean J. Food & Nutr. Vol. 23. No. 4, 582～587 (2010)

THE KOREAN JOURNAL OF
한국식품영양학회지
FOOD AND NUTRITION

Determination of Seed Fatty Acids Using Near-Infrared Reflectance Spectroscopy(NIR) in Mung Bean(*Vigna radiata*) Germplasm

†Young-Yi Lee, Jung-Bong Kim*, Sok-Young Lee, Min-Hee Kim, Jung-Won Lee, Ho-Sun Lee, Ho-Cheol Ko, Do-Yoon Hyun, Jae-Gyun Gwag, Chung-Kon Kim and Yong-Beom Lee**

Agrobiodiversity Center, NAAS, RDA, Suwon 441-717, Korea
**Dept. of Agro-Food Resources, NAAS, RDA, Suwon 441-857, Korea*
***Dept. of Environmental Horticulture, University of Seoul, Seoul 130-743, Korea*

녹두 유전자원 지방산 함량 대량평가를 위한 근적외선분광법의 적용

†이영이 · 김정봉* · 이석영 · 김민희 · 이정원 · 이호선 · 고호철 · 현도윤 · 곽재균 · 김정곤 · 이용범**

농촌진흥청 국립농업과학원 농업유전자원센터, *농촌진흥청 국립농업과학원 농식품자원부
**서울시립대학교 환경원예학과

Abstract

본 연구에서는 녹두 유전자원의 지방산 함량을 신속 대량 검정하는 기술을 개발하여 유전자원 활용 및 육종 촉진에 기여하고자 하였다. 유전자원 평가에 적합한 신속하고 비파괴적인 지방산 함량 평가기술을 개발하기 위해 공시자원 1,125점의 녹두 종자를 종실상태와 분쇄한 분말상태로 근적외선분광분석기(NIR)를 이용하여 1,104～2,494 ㎚에서의 스펙트럼을 얻고 이들 중 스펙트럼이 중복되지 않는 원산지가 다양한 대표자원 106점을 선발하여 일반적인 방법으로 지방산 함량을 분석하고, 이 값과 NIR 스펙트럼 흡광도값 간의 상관분석을 위한 calibration set로 활용하였다. 그 결과 palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid 및 total fatty acid에 대한 NIR 흡광도와의 상관계수 R^2이 각각 0.74, 0.18, 0.12, 0.72, 0.48 및 0.78로 나타났고, 이들 중 R^2가 높은 검량식을 미지의 시료 10점으로 검증한 결과, palmitic, linoleic 및 total fatty acid에 대한 검증 상관계수 R^2이 0.96, 0.74, 0.81로 나타나, 다양한 녹두 유전자원의 지방산함량 신속 대량 예측에 유효하게 활용될 수 있는 것으로 나타났다. 한편, 공시된 녹두 유전자원 115점 중에서 자원번호 IT208075 자원은 저 지방산 자원(14.24 ㎎ g^{-1})으로 선발되었고, IT163279 자원은 고 지방산 자원(18.43 ㎎ g^{-1})으로 선발되어 향후 녹두작물의 성분육종에 유용할 것으로 생각된다.

Key words: fatty acids, mung bean, germplasm, near-infrared reflectance spectroscopy.

INTRODUCTION

Mung bean(*Vigna radiata*) is primarily grown in Asia, Africa, South and North America, and Australia principally for its protein-rich edible seeds. It has been reported as containing 45～62% of glucoside, 1% of fat and 20～28% of protein(Kye et al. 1989). Mung bean is similar in composition to other members of the legume family, with 24% protein, 1% fat, 63% carbohydrate and 16% dietary fiber (USDA 2001). It is commonly eaten as bean sprouts, bean cake, bean congee etc., and extruded mung bean starch is used for producing vermicelli. Approximately, 400,000 tons of vermicelli is produced in China per year, and Longkou vermicelli which is produced in Zhaoyuan of Shandong province of China is famous for its high quality, and

† Corresponding author: Young-Yi Lee, Agrobiodiversity Center, NAAS, RDA, Suwon 441-717, Korea. Tel: +82-31-299-1814, Fax: +82-31-294-6029, E-mail: youngyi@korea.kr

is exported to about 50 countries or regions. There are over 160 corporations in Zhaoyuan to produce more than 250,000 tons of vermicelli per year (Jiang & Ma 2008). Minor coarse grains such as mung bean serve as supplementary foods for humans and have functional traits that differ from major grain foods. Antioxidant, antitumor, and antimutagenic effects have been reported for some minor cereal crops including buckwheat, sorghum, millet, and Job's tears (Kwak et al. 2004), likely resulting from functional phytochemicals such as vitamins, phenolics, tocopherols, and phytosterols. These chemical traits could be targeted as principal breeding characters. Thus, characterization of coarse grain germplasms for these traits should be performed so that useful germplasms can be identified and recommended to breeders or users.

Rapid characterizations and evaluations of breeding-targeted characters are very important for conservation, management, and utilization of plant germplasm (Vines et al. 2005; Lee et al. 2009). The Rural Development Administration (RDA) genebank at the National Agrobiodiversity Center of Korea conserves 156,000 seed germplasms, more than 75% (118,045 accessions) of which are grain food crops (RDA 2009). For many years, rice and soybean germplasms have been the focus of food crop breeding in Korea, and the germplasms of these crops have been actively characterized and evaluated. However, other grain crop germplasms have been less investigated. The National Agrobiodiversity Center conserves germplasms of 23,070 accessions of coarse cereal crops, including mung beans.

Near-infrared reflectance spectroscopy (NIRs) is a rapid technique that can be used to measure many traits routinely tested in cereal breeding programs. This technique has several advantages over conventional laboratory methods. For some major components of rice grains such as starch, protein, amino acids and fats, NIRs calibration models are well developed. However, no NIRs models have been developed for other components such as phenolic and tocopherol compounds in more minor crop grains, including adzuki bean, mung bean, and foxtail millet, even though NIRs methodology has proved feasible in measuring the phenolic and flavonoid contents of other systems and crops. For example, phenolic compounds in red wine fermentation have been accurately predicted by NIRs (Cozzolino et al. 2004). Prediction models for total phenolics in eucalyptus leaves, a forage legume, green rooibos, and green tea leaves have also been reported.

In this study, mung bean (*Vigna radiata*) germplasm collections were characterized and evaluated via NIR. Our objective was to investigate whether fatty acids (FAs) of a coarse cereal grain could be predicted by NIRs. Successful prediction will contribute to more effective application of NIRs in coarse grain germplasm use and crop breeding programs.

MATERIALS & METHODS

1. Germplasm Seed Samples

1,125 accessions of mung bean (*Vigna radiata*) germplasm conserved at the National Agrobiodiversity Center RDA genebank were NIR-scanned as grains. Next, the samples were ground, NIR-scanned again as flour, and then analyzed for FAs using gas chromatography (GC).

2. Spectroscopic Analysis

To obtain NIR reflectance spectra, samples were scanned immediately after grinding in triplicate spectral analysis cells using a dispersive NIR Systems 6500 spectrometer (FOSS North America, Eden Prairie, MN, USA). The instrument was fitted with a spinning cup sampling device. Each cell was scanned 16 times, and the spectra were averaged and transformed to log (1/*R*). After visual inspection, the spectra of the triplicate subsamples were averaged.

3. Fatty Acid Analysis

Fatty acid methyl esters (FAMEs) were prepared using potassium hydroxide in dry methanol (2 N) and extracted with n-hexane, as described by American Oil Chemists' Society (AOCS) Official Method Ce 2-66 (AOAC 1990). FAMEs (1 $\mu\ell$) were analyzed using an HP 5890 series II gas chromatograph (GC; Hewlett-Packard, Wilmington, DE, USA) equipped with a flame ionization detector (FID) and an HP 7673A automatic injector (Agilent Technologies, Palo Alto, CA, USA). A fused silica DB23 capillary column (60 m × 0.25 ㎜ i.d., 0.25 ㎛ film thickness; J&W Scientific, Folsom, CA, USA) was used. Oven temperatures were programmed as follows: 170℃ for 3 min, increased to 220℃ at 3℃/min, and kept at 220℃ for 15 min. The injection and detector temperatures were 250℃ and 260℃, respectively. The carrier gas was nitrogen, and the flow rate was 1 ㎖ min^{-1}. The split ratio was 1/65. FAME identification was based on retention times compared with those of the standard FAME mixture. Results were expressed without correction as percentage of peak area. Fatty acid analysis of each sample of

each variety was performed in triplicate, and average values are reported.

4. Calibration Development

The NIRs reflectance model was developed using a commercial spectral analysis program (ISI40 NIRS 2 version 4.01 and WINISI software, FOSS North America). Preprocessing of the spectral data (1,104～2,494 ㎚) of 100 samples consisted of a normal multiplicative scatter. The data were centered on the mean spectrum and mean reference value using modified partial least-squares (PLS) regression. The PLS regression was modified so that reference values and reflectance data were scaled at each wavelength to a standard deviation of 1.0 before each PLS regression term. The preprocessing methods chosen for the model were the optimum to obtain minimum error following cross-validation (four cross-validation groups per germplasm collection). The optimum number of PLS regression terms for the calibration, also determined by cross-validation, was the number of factors yielding the minimum error between predicted and reference values (standard error of cross-validation, SECV). The modified PLS regression model was tested using independent validation samples (n=10). Statistics used to assess the model were the standard error of performance (SEP, not bias-corrected), coefficient of determination (R^2), and slope and intercept of the linear regression of NIRs predicted versus analyzed values.

RESULTS AND DISCUSSION

1. Spectra Collection Via NIRs Scanning

We obtained NIRs spectra of 1,125 accessions of mung bean grain. Of these, 100 accessions were selected by spectrum, origin, and seed coat color to be ground into flour. Fig. 1 compares the spectra of grain and flour foxtail millet germplasm. Because distinct small peaks were observed in raw spectra without mathematical treatment, these spectra were modified to examine the correlative wavelength and determine FAs via mathematical treatment of variable peaks and shapes. After this treatment, some absorption bands (e.g., the band at 1,974 ㎚) displayed clearer peaks. Subsequently, the selected 100 samples were ground and NIRs-scanned again before beginning conventional analysis of each chemical component. Fig. 1 (Upside) shows that the peaks in the spectra of flour samples were sharper and clearer than those of the unground grain samples (Fig. 1, Downside). Comparison and investigation of the two spectral patterns was

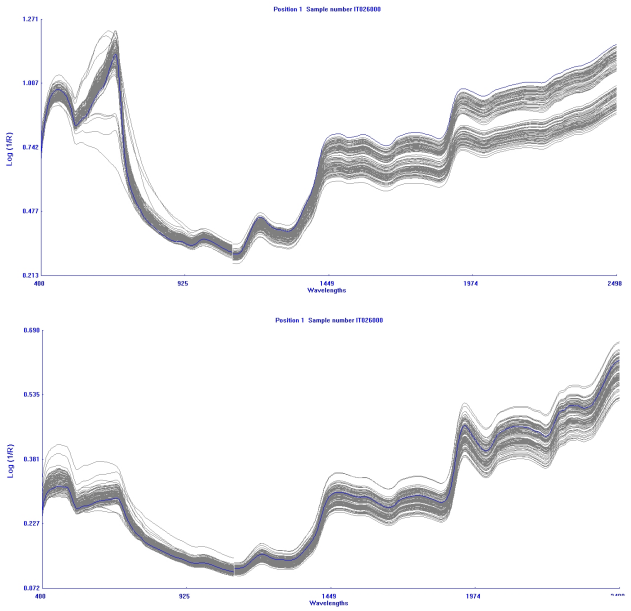

Fig. 1. Comparison of NIR spectra with grain and flour status of mung bean germplasm. Upside, spectra with grain status of 106 collections of germplasm; Downside, spectra with flour status of 106 collections of germplasm selected for conventional analysis of chemical components and NIR scan as flour status.

conducted using NIRs model regression. Zhang et al. (2008) reported that the spectra of dehulled rice grains were very similar to those of milled rice grains, but flour samples showed absorption more clearly than did grain samples.

2. Determination of Reference Values

Fig. 2 shows the FA contents of the mung bean samples. Total FAs averaged 15.87 ㎎ g^{-1}, with a range of 14.24～18.43 ㎎ g^{-1}. Among the mung bean collections, the IT208075 and IT163279 accessions showed especially low and high FA contents, respectively. Constituent acid content ranges were as follows: palmitic acid, 4.19～5.50; stearic acid, 0.79～1.53; oleic acid, 0.20～5.58; linoleic acid, 3.57～7.86; and linolenic acid, 1.33～4.08 ㎎ g^{-1}. Linolenic and linoleic acid values varied widely among the accessions, suggesting that the NIRs equation modeling values of the two acids would be significant, as collection diversity correlates positively with regression models.

3. NIRs Calibration for Modeling

The reference values of all of the samples were used in mathematical treatments designed to create prediction equations

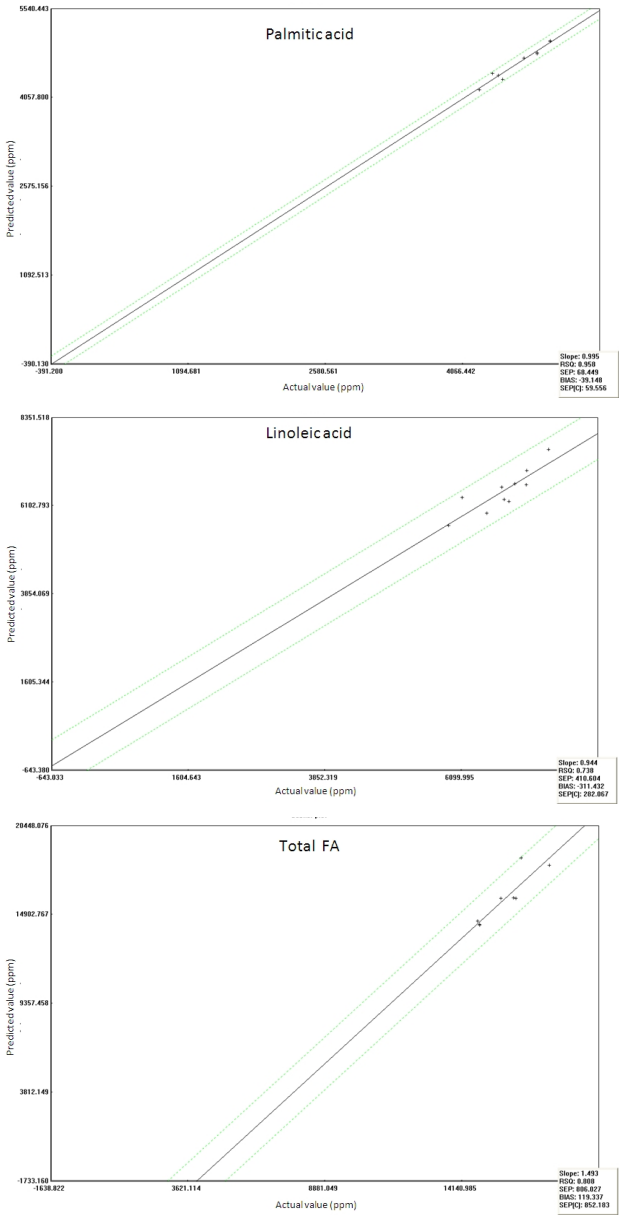

Fig. 2. Scatter plots of NIR predicted by developed equation versus actual-analyzed reference values for palmitic acid, linoleic acid and total FA (R^2=0.958, 0.738 and 0.808) in the external validation set (n=10) of mung bean germplasm.

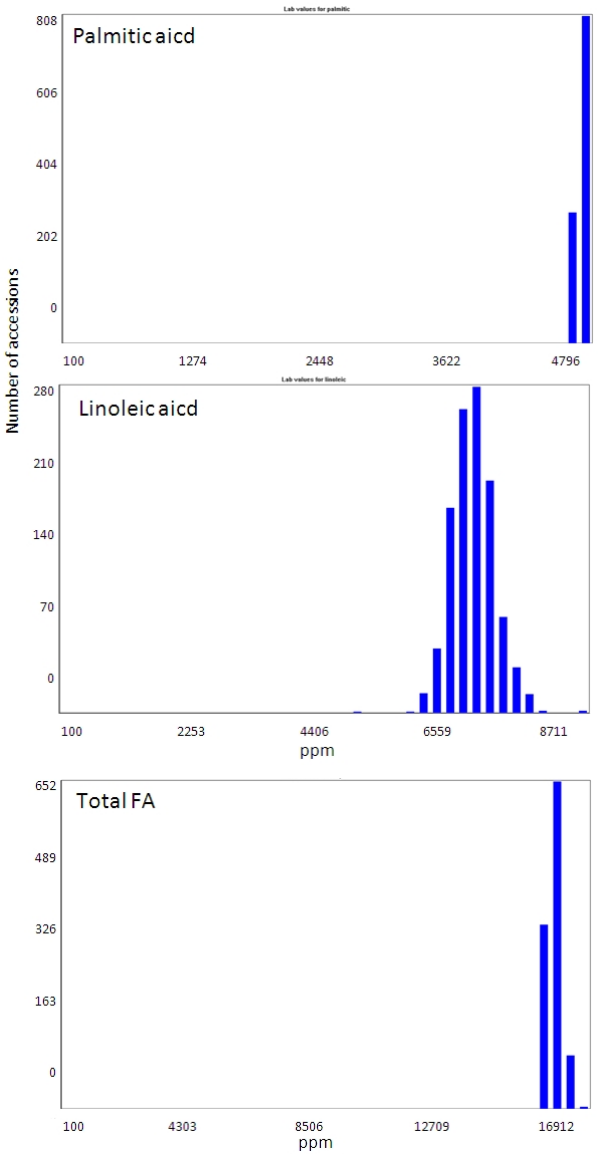

Fig. 3. Histogram for the distribution of whole 1,125 accessions according to NIR predicted value of palmitic acid, linoleic acid and total FA contents by the equation developed in this study.

using modified PLS. After using the equations to calculate predicted values, we conducted an external validation using an independent sample set (n=10) to ensure that the equations could be applied to the prediction of FAs. Fig. 2 shows the validation graphics, and Fig. 3 gives the predicted values.

Table 1 shows that the calibration statistics with grain condition and most reference values of FAs were little correlated each other. Table 2 shows that the calibration statistics with flour condition and reference values of palmitic acid, linoleic acid, and total FAs were better correlated (R^2=0.74, 0.72 and 0.78, respectively) than were other components. Because the R^2 values of the rest FAs were lower than those of other components, we hardly regard these acids as predictable by NIRs. Additionally, we used the SD/SECV ratio to evaluate the reliability of the calibration model (Moschner & Biskuper-Korell 2006). The SD/SECV values for all FAs were not above 3. These results suggest a necessity of supplementary study for significant correlation

Table 1. Equation statistics using regression model (MPLS) and scatter correction for NIR prediction of fatty acids in the calibration set (n=106) of mung bean grain samples

Constituent	N	Mean	Calibration		Cross-validation		Math treatment
			SEC	R^2	SECV	1-VR	
Palmitic acid	103	4,708.83	213.17	0.02	216.37	0.00	2,6,4,1
Stearic acid	97	1,040.15	110.95	0.04	113.92	−0.01	2,6,4,1
Oleic acid	102	436.91	115.37	0.08	128.35	−0.14	2,6,4,1
Linoleic acid	104	6,731.98	452.31	0.22	509.35	0.01	2,6,4,1
Linolenic acid	100	2,877.38	277.75	0.02	283.19	−0.01	2,6,4,1
Total FA	102	15,796.03	703.09	0.06	754.05	−0.09	2,6,4,1

N, number of samples used to develop the model; SEC, standard error of calibration; R^2, coefficient of determination of calibration; SECV, standard error of cross-validation; 1-VR, coefficient of determination for cross-validation.

Table 2. Equation statistics using regression model (MPLS) and scatter correction for NIR prediction of FAs in the calibration set (n=106) of mung bean flour samples

Constituent	N	Mean	Calibration		Cross-validation		Math treatment
			SEC	R^2	SECV	1-VR	
Palmitic acid	104	4,709.19	109.50	0.74	168.30	0.39	2,8,8,1
Stearic acid	100	1,041.87	102.04	0.18	108.45	0.07	2,8,8,1
Oleic acid	104	437.17	112.23	0.12	117.72	0.02	2,4,4,1
Linoleic acid	104	6,731.90	269.79	0.72	371.13	0.48	1,4,4,1
Linolenic acid	103	18.37	1.51	0.48	1.84	0.22	2,4,4,1
Total FA	103	15,835.30	353.10	0.78	548.52	0.47	2,4,4,1

N, number of samples used to develop the model; SEC, standard error of calibration; R^2, coefficient of determination of calibration; SECV, standard error of cross-validation; 1-VR, coefficient of determination for cross-validation.

between the reference values and NIRs estimated values. We also compared the effectiveness of NIRs scanning of grain versus flour samples. In all cases, the predictive capability of flour samples exceeded that of whole grain samples.

NIRs absorbance is greatly affected by OH groups. The moisture condition of all of the germplasm conserved at the National Agrobiodiversity Center was assumed to be the same, as all germplasm materials at the center are conserved in identical mid-term conservation conditions of 4℃. A future study of the seed moisture conditions of each accession would be warranted.

In conclusion, palmitic, linoleic, and total FA contents can be predicted with reliable accuracy using NIRs analysis of intact mung bean seeds. On the other hands, it was reported that the stearic, oleic and linolenic acid on rapeseed were predictable with 0.79, 0.98 and 0.90 of R^2 value, respectively(Kim et al. 2007). It means that a larger germplasm population covering a wide range of chemical values was required to accurately predict stearic, oleic and linolenic acid. In terms of SD/SECV values, a larger germplasm population covering a wide range of chemical values was required to accurately predict all FAs contents using NIRs analysis of intact mung bean seeds (Gotor et al. 2007). With a larger sample size and broader variation of the reference dataset, calibration models for determining FAs could be employed in a massive screening of breeding lines.

REFERENCES

Association of Official Analytical Chemists (AOAC). 1990. Official Methods of Analysis, 16th ed. Gaithersburg, MD

Cozzolino D, Kwiatkowski MJ, Parker M, Cynkar WU, Dambergs RG, Gishen M, Herderich MJ. 2004. Prediction of phenolic compounds in red wine fermentations by visible and near infrared spectroscopy. *Analytica Chimica Acta* 513:73-80

Gotor AA, Berger M, Farkas E, Labalette F, Centis S, Calmon A. 2007. Quantification of sunflower minor components by near infrared spectrometry(NIR). *Helia* 30:183-190

Jiang ZS, Ma C. 2008. Zhaoyuan of Shandong- vermicelli capital. *China Food Quality News* pp1

Kim KS, Park SH, Choung MG, Jang YS. 2007. Use of near-infrared spectroscopy for estimating fatty acid composition in intact seeds of rapeseed. *J Crop Sci Biotech* 10:15-20

Kwak, CS, Lim SJ, Kim SA, Park SC, Lee MS. 2004. Anti-oxidative and antimutagenic effects of Korean buckwheat, sorghum, millet and job's tears. *Korean Society of Food and Nutrition* 7:921-1077

Kye IS, Jun YS, Cheigh HS. 1989. Functional properties of mung bean protein isolates. *J Korean Soc Food Nutr* 18:300-306

Lee SY, Lee YY, Lee SK, Cho YH, Ma KH, Kang HK, Gwag JG, Park KH, Lee HS. 2009. Selecting high amylose lines of rice varieties using NIR spectroscopy at the RDA gene bank conserved. *Korean Journal of Breeding Science* 41:108

Moschner C, Biskupek-Korel B. 2006. Estimating the content of free fatty acids in high oleic sunflower by near-infrared spectroscopy. *Eur J Lipid Sci Technol* 108:606-613

Rural Development Administration (RDA). 2009. The status of plant genetic resource conservation

United State Department of Agriculture. 2001. Nutrient database for standard reference. pp.14

Vines, L, Kays SE, Koehler PE. 2005. Near-infrared reflectance model for the rapid prediction of total fat in cereal foods. *J Agric Food Chem* 53:1550-1555

Zhang, C, Shen Y, Chen J, Xiao P and Bao J. 2008. Nondestructive prediction of total phenolics, flavonoid contents, and antioxidant capacity of rice grain using near-infrared spectroscopy. *J Agric Food Chem* 56:8268-8272

접 수 : 2010년 11월 5일
최종수정 : 2010년 12월 1일
채 택 : 2010년 12월 19일