Determination of Tryptophan Content in Quality Protein Maize using Near-Infrared Reflectance Spectroscopy

Legesse Shiferaw, Girmay Tsegay, Gelila Asamenew
Ethiopian Institute of Agricultural Research, P.O.Box 2003,AdissAbeba, Ethiopia
Corresponding author e-mail:legeshiferaw@gmail.com

Abstract

With the development of QPM in recent years, which has approximately twice tryptophan (Trp) and lysine (Lys) concentrations compared to normal maize, it is believed that the problem of protein deficiency in maize would be considerably alleviated, if the concentration of both amino acids is regularly monitored in the breeding program as their biosynthesis is controlled by several factors. However, the conventional analytical method (wet chemistry) of determination of Trp and Lys concentration in maize grains would definitely requires chemical and reagents and take longer time. The objective of this study was, therefore, to evaluate the effectiveness and efficiency of Near Infrared Reflectance Spectroscopy (NIRS) method in determining tryptophan content of QPM, which would help to enhance the QPM breeding program by partially replacing more expensive and time-consuming wet chemistry method of analysis. Grain samples of two hundred sixty-eight maize genotypes were used to develop NIRS models for Tryptophan content. Standard error (SE) and coefficient of determination for the calibration were found to be 0.007 and 0.76, respectively. When the NIRS model was subjected to external validation with 40 S2 maize lines from QPM breeding population, the standard error of prediction (SEP) for validation and coefficient of determination between data generated by NIRS and the wet chemistry method were found to be 0.008 and 0.84, respectively.

Introduction

Maize is among the major cereals produced and consumed in Ethiopia. The crop grows in various agro-ecologies of the country and, thus, its breeding program has been focused on increasing stability and yield potential regardless of its nutritional quality under different abiotic and biotic stress conditions. However, in the past few years, attempt has been made in bio-fortification programs to increase the nutritional quality of maize for human and animal consumption (Montes *et al*., 2006). Quality protein maize (QPM) is a type of maize with pronounced amounts of two essential amino acids, lysine (Lys) and tryptophan (Trp), and provides increased nutritional value for protein-deficient populations who depend upon maize as a staple food (Aldo *et al*., 2011).

It has been reported that multiple genes control and modify the protein quality of QPM (Gibbon and Larkins, 2005) and, hence, Trp and/or Lys monitoring is required to ensure and maximize genetic gain in the breeding programs (Krivanek*et al*., 2007).

Most QPM breeding programs routinely monitor Trp and/or Lys concentrations in protein by colorimetric methods or high-performance liquid chromatography (HPLC) (Nurit*et al*., 2009). Although the colorimetric method is less complex than HPLC, it requires more than 20hr to complete analysis because of overnight digestion procedures (Nurit*et al*., 2009). These chemical analysis methods have limitations, including costs, time, and method robustness (Aldo *et al.*, 2011).

On the other hand, Near-Infrared Reflectance Spectroscopy (NIRS) is a technique that combines spectroscopy and mathematics to produce rapidly indirect and quantitative estimates of concentrations of OH-, NH-, CH- or SH-containing compounds. In comparison to wet chemistry procedures, NIRS requires none or simple sample preparation methods and is a rapid and relatively inexpensive technique that facilitates the analysis of several traits simultaneously(Melchinger *et al*.,1986), where spectral data are correlated with values of biochemical components obtained by standard methods. However, NIRS is an indirect method that requires development and validation of calibrations by analysis of a large number of samples covering the range of variability for each trait and with uniform distribution between extreme values (Shenk and Westerhaus, 1991).

Aldo *et al*., (2011) have developed NIRS calibrations for protein, lysine and tryptophan contents using 276, 756 and 424 maize samples, respectively, collected from mid altitudes and tropical environments of Africa and Mexico, and the results showed that NIRS data were accurate enough to screen QPM genotypes from normal maize population in the breeding program. Even though both lysine and tryptophan concentrations increase in QPM materials, only tryptophan is analyzed on a routine basis, as of both amino acid values are highly correlated (Vivek *et al*., 2008).

Breeding programs devoted in developing QPM materials are being implemented in Ethiopia, and these programs require robust, fast, and inexpensive laboratory methods to screen the materials from normal maize population. To this end, currently, the colorimetric method is widely used and no NIRS model developed yet for the determination of Trp content for screening of QPM materials in Ethiopia.

The objective of this work was therefore, to develop and validate NIRS models for Tryptophan content based on samples collected from low moisture stress, mid altitude and high land QPM breeding programs in the country.

Materials and Methods

Sample collection and preparation

Grain samples of 268 maize genotypes, consisting of 38 from low moisture stress area (Melkasa Agricultural Research Center). 137 from mid altitude (Bako Agricultural Research Center) and 63 from high land (Ambo Agricultural Research Center) areas were harvested in 2015 and 30 samples with high, medium and low tryptophan content were collected from Maize Nutrition Quality Laboratory (MNQL)

of CIMMYT in Mexico and used for the development of NIRS calibration. Besides, 40 samples were also selected for external validation of the developed calibration, making the total number of samples in the trial 308. Each sample was split in to two and each half was sent to MNQL for wet chemistry analysis, while the remaining halves were milled using cyclone mill (Tecatorcyclotec, Foss) with 1 mm sieve and stored in glass cup for NIRS analysis.

NIRS analysis

Two to three grams of each milled sample was scanned by NIRS monochromatic model FOSS 6500 (FOSS NIRSystems, Inc., Silver Spring, Denmark) using small ring cups (internal diameter of 35 mm and depth of 8 mm). Spectra were collected between 400 and 2500 nm, registering log of the absorbance values (1/R) at two nm intervals for each sample.

Wet chemistry analysis

Tryptophan content of the samples was determined at MNQL using colorimetric method as described by Nurit *et al.* (2009). Ten samples were included as an internal standard to verify the accuracy of the method.

Mathematical procedures for calibrations and validation

Calibrations were performed using a modified partial least squares (MPLS) regression of Win ISI (FOSS). Calibration statistics included the following parameters: standard deviation of the population (SD), coefficient of determination (R^2), standard error of calibration (SEC),and standard error of cross-validation (SECV). For validation, standard error of prediction (SEP) and coefficient of determination of validation (R^2v) (the fraction of the variance of the reference values explained by the variance of NIRS determinations) were calculated. In addition, the ratio of SD to SEP was determined, as the quality and robustness of a NIRS calibration can also be judged by the SEP and SD/ SEP; where SD/SEP ratio less than 2 indicates an unsuitable calibration (Montes *et al*., 2006).

Results and Discussion

Reference analysis and sample distribution

Results of the reference analysis showed that the standard error for laboratory value was found to be 0.004. Reference values for samples in the calibration set were distributed over a range of 0.02 – 0.12 %. This is so because both normal and QPM maize samples were included in the analysis, as QPM has more than 0.07% Trp (Aldo *et al*., 2011).

NIRS calibration development

Twenty calibrations (Cal) were developed using modified partial least squares (MPLS) regression, cross-validation techniques, different data pre-treatment and spectra transformation and of which those three with better calibration statistics were selected for independent validation.

NIRS calibration equations, developed on the basis of 242, 236 and 240 samples for Cal 1, Cal 2 and Cal 3, respectively, had high coefficients of determination for calibration (R^2c = 0.72 - 0.76) and slightly lower coefficients of determination for cross-validation (R^2cv = 0.65 - 0.69) (Table 1).

Table 1: Reference Values, NIRS Calibration, and Cross-Validation Statistics for Trp, in the Calibration Set of Maize

Calibrations	N	Range	Mean	SD	R^2c	SEC	R^2cv	SECV
Cal 1	242	0.02 - 0.10	0.060	0.015	0.76	0.007	0.68	0.0084
Cal 2	236	0.02 - 0.11	0.060	0.015	0.74	0.007	0.69	0.0087
Cal 3	240	0.02 - 0.12	0.061	0.015	0.72	0.008	0.65	0.0090

SD = standard deviation. R^2c = coefficient of determination in calibration. SEC = standard error of calibration. R^2 cv = coefficient of determination in cross-validation.SECV = standard error of cross-validation.

Besides, the difference between R^2c and R^2cv was also minor, indicating that the calibrations were homogeneous (Aldo *et al.,* 2011). The values of SEC and SECV were small and comparable with the standard error of the reference method that was 0.004. These results were in agreement with the result obtained by Aldo *et al.*(2011) at CIMMYT Maize Nutrition Quality Laboratory in Mexico.

External/independent validation

An independent validation of the calibrations was carried out for Cal 1, Cal 2 and Cal 3 using 40 samples from the high land and mid altitude QPM breeding programs. The coefficients of determination for independent validation (R^2 v) were larger than measured for the cross-validations (Table 2). Except for Cal 1, which was 1.9, the SD/SEP ratio for Cal 2 and Cal 3 were 2.25 and 2.3, respectively, indicating that the calibration is satisfactory as it falls within the range of 2 - 3. Hence, both SD/SEP ratio and R^2v value for Cal 2 show that reliable selection of maize genotypes through determination of tryptophan content is possible by NIRS method.

Table 2: External Validation Statistics of the NIRS Calibrations for Trp Contents in Maize

Calibration	N	Range	Mean	SD	R^2V	SEP	SD/SEP
Cal 1	40	0.033 - 0.098	0.062	0.019	0.789	0.010	1.9
Cal 2	40	0.033 - 0.098	0.062	0.018	0.830	0.008	2.3
Cal 3	40	0.033 - 0.098	0.062	0.018	0.790	0.008	2.3

SD = standard deviation. R^2 v = coefficient of determination in validation. SEP = standard error of prediction.

The ratio of SD to SEP for validation of the calibration was 2.3, which is in agreement with the result reported by Aldo *et al.* (2011). Samples used to develop the calibrations included a wide range of materials from low land, mid-altitude and high land areas to ensure broad applicability of the NIRS models. Therefore, these calibrations seem quite reliable and applicable for diverse maize genotypes grown under different agro-ecology.

Conclusion

Overall performances of the developed calibrations for determination of tryptophan content of maize grains indicated that NIRS method could be confidently used and provide a fast and simple option for screening of thousands of samples in each breeding cycle. Besides, it appears to be cheaper and cost effective, as extractions and chemical reactions are not required in the process as the case with wet chemistry method. However, it is recommended to verify the accuracy of extreme values by chemical analysis, especially for very advanced breeding materials.

References

Aldo R,G Luis, O Ezequiel, I Catalina, and P Natalia.2011. Near-Infrared Reflectance Spectroscopy (NIRS) for Protein, Tryptophan, and Lysine Evaluation in Quality Protein Maize (QPM) Breeding Programs. *J. Agric. Food Chem.,* 59, 10781– 10786.

Gibbon B and B Larkins.2005. Molecular genetics approaches to developing quality protein maize. *Trends Genet*. 21, 227–233.

Krivanek AF,H De Groote, N Gunaratna, A Diallo, and D Friesen.2007. Breeding and disseminating quality protein maize (QPM) for Africa. *Afr. J. Biotechnol*., 6. 312–324.

Montes J, H Utz, W Schipprack, B Kusterer, J Muminovic, C Paul, and AE Melchinger. 2006. Near-infrared spectroscopy on combine harvesters to measure maize grain dry matter content and quality parameters. *Plant Breed*. 125, 591–595.

Melchinger AE, G Schmidt, and H Geiger.1986. Evaluation of near infra-red reflectance spectroscopy for predicting grain and stover quality traits in Maize. *Plant Breed*. 97. 20–29.

Nurit E, A Tiessen, K Pixley,and N Palacios-Rojas.2009. A reliable and inexpensive colorimetric method for determining protein-bound tryptophan in maize kernels. *J.Agric. Food Chem* 57. 7233–7238.

Shenk J and M Westerhaus.1991. Population definition, selection, and calibration procedures for near infrared reflectance spectroscopy. *Crop Sci.* 31. 469–474.

Vivek B, A Krivanek, N Palacios-Rojas, S Twumasi-Afriyie, and A Diallo.2008. Breeding Quality Protein Maize (QPM): Protocols for Developing QPM Cultivars. Mexico, D.F.:CIMMYT. pp.105.