Near-infrared reflectance spectroscopy as a tool for breeding *Andropogon gayanus* Kunth for forage quality

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Abstract: *The objective was to investigate the usefulness of Near Infrared Spectroscopy (NIRS) to predict bromatological traits concentrations of gamba grass (Andropogon gayanus Kunth). Random samples from different plant genetic backgrounds, climatic conditions over years, experimental sites, and harvests were used for spectral reading and reference analysis. Partial Least Square (PLS) regressions were used for developing the models with 239 samples for calibration and 119 for external validation. Crude Protein, Ash, and Dry Matter models had $R^2 = 0.91$, 0.91, and 0.90 and low standard errors of calibration (SEC) of 8.7, 3.8, and 5.5 g.kg^{-1}, respectively. Neutral and Acid Detergent Fibers, and Organic Matter had $R^2 = 0.85$, 0.88, and 0.89 and SEC valuesof10.2, 9.4, and 7.9 g.kg^{-1}, respectively. In vitro digestibility of dry matter had $R^2 = 0.79$ and SEC 28.0 g.kg^{-1}. Acid Detergent Lignin had a poor fit with $R^2 = 0.36$.A high correlation between NIRS predictions and wet lab data was observed. Models were adequate and accurate for predicting all bromatological traits but ADL. Significative differences among 51 half-sib families were detected for quality traits predicted through PLS models. NIRS can be effectively used in gamba grass breeding programs for selecting superior forage quality genotypes.*
Key words: *Gamba grass; NIRS prediction; bromatological composition; genotypic selection.*

I. Introduction

    Andropogon gayanus Kunth, known as gamba grass, is a cross-pollinated forage species originally from Africa, well adapted to tropical savannahs with different altitudes, climate and soils[20]. In the tropical savannah ecoregion of Brazil, known as Cerrados, gamba grass is mainly cultivated in marginal lands, rocky soils, and in livestock production systems with pasture diversification. Gamba grass has natural resistance to spittlebug, good growth on acid and low fertility soils and great ability to regrow at the end of the dry season, after the first rainfalls[36].

    Animal performance is largely affected by forage quality and 1% genetic increase in IVDMD has led to a 3.2% increase in average daily live-weight gains[13]. Selection for improved quality has been implemented in forage breeding programs worldwide[10,12,17,18,35,45]. Quantification of organic compounds from plant tissue is usually accomplished by reference methods, based on standard wet chemistry analytical routines, which are expensive, time-consuming, and complex to be used as effective tools for screening large number of samples, typical of forage breeding programs.

    NIRS is a chemometric technique when coupled with wet chemistry analytical data and the development of mathematical calibrations, can be used to predict organic compounds in plant tissues. NIRS

Abbreviations: *ADF, acid detergent fiber; ADL, acid detergent lignin (Lignin sa); Ash, mineral matter; CP, crude protein; DM, dry matter; IVDMD, in vitro dry matter digestibility; MSC, multiplicative scatter correction; N, nitrogen; NDF, neutral detergent fiber; NIRS, near-infrared reflectance spectroscopy; OM, organic matter; PLS, partial least square regression; r, coefficient of correlation; R, reflectance; R^2, coefficient of determination; RPD, ratio-performance deviation = SD/SEP; SEC, SECV, SEP standard errors of calibration, cross-validation, and performance, respectively, corrected for the bias; SG123, Savitzky Golay 1st derivative algorithm with 2nd order polynomial and 3 point smoothing; SNV, standard normal variate.*

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stands out as a low cost, precise, repeatable, and fast high-throughput alternative to predict quality and has been widely used in forage breeding programs for phenotyping[8,11] and screening genetic materials for protein, digestible carbohydrates, indigestible fibers contents, and gross calorific value, among other forage traits[2,3,4,10,13,14,17,18,33,35,45]. All those authors reported ADF, ADL, CP, DM, IVDMD, NDF, neutral detergent soluble fiber, and water-soluble carbohydrates are effectively and efficiently estimated for a number of sample types and different species. The use of NIRS as an effective alternative, nevertheless, requires the development and validation of accurate prediction models for each quality trait.

To our knowledge, no information is available on the development of NIRS calibration models for predicting forage quality of gamba grass populations. The development of NIRS calibrations for analysis of quality components may be a feasible solution for screening a large number of samples to select superior forage quality genotypes. The objective was to ascertain the viability of NIRS to predict CP, NDF, ADF, ADL, DM, OM, IVDMD, and Ash concentrations to be used as an effective tool to select superior quality gamba grass genotypes.

II. Material and Methods

Plant material: All samples were collected from three genetically broad-based gamba grass populations from field experiments of the Embrapa Cerrados forage-breeding program. The experiments were carried out in Planaltina, Federal District, Brazil (15°35'S, 47°42'W; 993 m a.s.l.), from January 2016 to April 2018. The climate at the experimental site is tropical savannah according to the Köppen-Geiger classification[30]. The experiments were planted in a clay soil (Rhodic Haplustox Oxisol) in average with $pH_{(H2O)}$ 5.3, organic matter concentration of $27g.kg^{-1}$, K concentration of 48 $mg.kg^{-1}$, Al concentration of 24 $mg.kg^{-1}$ and P concentration of 2.0 $mg.kg^{-1}$ (Mehlich-I) at 0-0.2 m soil depth.

Population 1 was Planaltina cultivar, from CIAT 621 access, a direct introduction from Africa[36]. Population 2 was an experimental population developed from selection for semi-erect type of plant, tillering vigor, and high leaf/colm ratio. Population 3consisted of 51 superior genotypes selected from the fourth generation of a mass selection procedure while developing Population 2. A random sample consisting of 120 half-sib families from populations 1 and 2 as well as 51 half-sib families from population 3 were used to set up three independent field experiments, previously designed to evaluate DM yield and forage quality characteristics. Parent plants from populations 1 and 2 and half-sibs from population 3 were sampled at 5-week intervals, three times in 2016 (April, May, and November) and three times in 2017 (January, February, and April). Half-sibs from population 3 were also sampled once in 2018 (April). All sampling were done during the rainy season, from November to April, but one on May 2016, that was sampled in the beginning of the dry season, in which the plants are in seed development stage and their forage quality greatly decreases.

A total of 3,026samples of about 400-g fresh weight each, composed of randomly selected colms, were harvested at 20-cm stubble from each parent plant and each half-sib row. Samples were dried for 72 h in a forced air oven at 55° C, ground through a 1-mm screen Wiley mill (A. H. Thomas Co., Boulder, CO), and stored in plastic containers for laboratory analyses.

Spectral readings: Right after grinding, spectral data were collected for all samples using a NIRS FOSS 5000 System II type 461006 (FOSS Analytical SA, DK 3400 Hilleroed, Denmark)with the ISIScan software v.2.85.3 (ISI Software, FOSS Analytical AB, Höganäs, Sweden). About 2-g homogenized samples were placed in 3.8 cm inner diameter ring cup cells, with a quartz window and closed with foam card board rings for the spectral readings. Scans were collected over a wavelength range of 1100 to 2500 nm with 2 nm resolution and 32 scans averaged for each sample. The spectral absorbance was recorded as the logarithm of the inverse of the reflectance (A = 1/R).

Wet Lab Reference Analysis: About 12% of the samples were randomly selected in each harvest, assayed for quality traits via reference wet lab methods and used as the full calibration set to develop the NIRS equations. A total of 358 samples were used to develop the calibration equations. The number of wet samples for IVDMD was 310 due to rumen fluid availability. Samples encompassed different climatic conditions over years, experimental sites, half-sib families, individual genotypes as well as harvests. Therefore, they probably incorporated a great deal of chemical and physical variations expected for the calibration equations.

DM content of the samples was determined by drying approximately 2 g of each sample in a forced-air oven at 105°C ± 2°C for at least 2 hours[7]. Sequential NDF, ADF, and ADL analysis from 0.5g initial sample weight placed in ANKOM F57 filter bags[23,24,25,26,27,39,40]was performed on the full calibration set. For NDF and ADF analysis, samples were digested in an Automatic Fiber Analyzer model ANKOM 2000 (ANKOM Tech. Corp., Fairport, NY, USA) using a neutral detergent solution pH 6.9-7.1,without α-amilase and sodium sulfite, and1 M H_2SO_4 acid detergent solution, sequentially, after drying and weighing procedures. ADL determination was performed by placing the ADF dry residue in a 12 M H_2SO_4 solution and incubated in a Tecnal *in vitro*

incubator system (TECNAL Scientific Equipments, Piracicaba, SP, Brazil) for 3 hours. Ashing was done by placing folded filter bags in crucibles and in muffle furnace at $500^{o}C$ for at least 5.5 hours. N concentration was determined by Kjeldahl method[6] with a Tecnal™ 0365 digestion-distillation system and CP was calculated as N x 6.25. True IVDMD was determined by 48-hour ruminal fermentation at 39.5 ^{o}C in a Tecnal™ *in vitro* incubator system using the procedure described by Tilley and Terry[37], with modification by Goering and Van Soest[19]. OM was estimated by subtracting Ash from DM. All results were expressed inclusive of residual ash, on g.kg^{-1} DM[38].

Calibration and validation of NIRS models: Pretreatment of raw spectral data was used to overcome problems associated with radiation scattering due to differences in particle sizes, to remove random noise, to heighten weak absorption bands, and to sharp waveband peaks to decrease overlapping. Tested pretreatments included: standard normal variate, maximum normalization, baseline, multiplicative scatter correction, the first derivative of Savitzky Golay algorithm, 2nd order polynomial, 3-point smoothing, the second derivative of Savitzky Golay algorithm, 3nd order polynomial, and 5-point smoothing, as well as the combinations among them. The major advantages of derivatives over original absorbance bands are a well defined zero baseline, as well as narrower bands, which makes it easier to regress reference data to bands that are less influenced by interference in the spectra[22]. The best pre-treatment for each trait was chosen based on the best cross-validation calibration. Outliers removed from the full calibration set were defined during the development of the cross-validation equations for each trait.

The development of the final calibration equations was done using an external validation set. Two thirds of all 358 wet lab samples, n = 239, were randomly selected and used as the calibration set. The remaining one third, n = 119, was used as external validation set, to evaluate the accuracy of the calibration models. The software package "The Unscrambler® X v.10.5.1" (CAMO Software AS, Oslo, Norway) was used for all chemometric pre-treatments and analysis.

PLS with cross-validation was initially used for developing all calibration models, with 10 sets of 20 samples each randomly removed from the full calibration set to be used as the validation set. In PLS, the optimal number of factors to explain the variability of the model is the one that carries the smallest residual variance. The cross-validation suitability of the models was evaluated based on the highest and similar R^2 between calibration and cross-validation, which measures the goodness of fit of the model, as well as the smallest and closest SEC and SECV, which measures the dispersion of the calibrations and cross-validation samples around the regression line, respectively. Some outlier samples were also removed from the validation set when the difference between predicted and reference values was three times higher than the original SECV. This first step was used to choose the best spectra transformation, the best cross-validation calibration, and to identify the outliers to be removed from the final fine-tuning calibration using an external test set.

PLS with an external test set was then used for developing the final calibration models. Effectiveness of the calibrations was evaluated based on R^2 that accounts for the proportion of explained variation by the model, as well as on SEC, that measures the dispersion of the calibration samples around the regression line. Accuracy of the calibration was evaluated through SEP, that measures the dispersion of the external set samples around the regression line, degree of closeness between the NIRS-predicted and the reference value statistics, as well as RPD ratio > 2.4 for successful calibration equations for screening[42].

The best calibration model for each trait was then chosen by the optimum combination of the following:(i) difference between SEC and SEP less than 5 g.kg $^{-1}$; (ii) calibration models with smallest SEC and largest R^2; (iii) validation with the lowest SEP and no more than 1.33 larger when compared to the SEC, small bias, large R^2, slope closer to 1, and high r between the predicted and the reference method values; and (iv) RPD greater than 2.4.

Effectiveness of the models: Could the models to be effectively used to discriminate forage quality among gamba grass half-sib families? To answer this question, the calibrated NIRS equations were used to predict NDF, ADF, CP, IVDMD, Ash, DM and OM concentrations of 918 samples from 51half-sib families, 6 harvests and 3 blocks from population 3field experiment. ANOVA for each trait was performed with data averaged across all 6 harvests to assess differences among half-sib families. Fisher's SD at 0.05level was then performed to define the least significant difference among half-sib families and to discriminate the best ones, only for the traits which were significantly different in ANOVA. Analyses were performed using SAS 9.0, SAS Institute Inc., Cary, NC, 2002.

III. Results
Spectral data: Figure 1 illustrates the maximum, the minimum and the mean NIRS spectral data for 358 gamba grass samples as the relationship between absorbance, given as log (1/R), and wavelength. The NIR raw data spectra had broad overlapping peaks (Fig. 1a) and spectra transformation was used for removing the

baseline shifting and sharpening the peaks (Fig.1bcd). In all three transformations, the overlapping peaks became much narrower, better revealing the main absorption bands, which are associated with organic components of the samples.
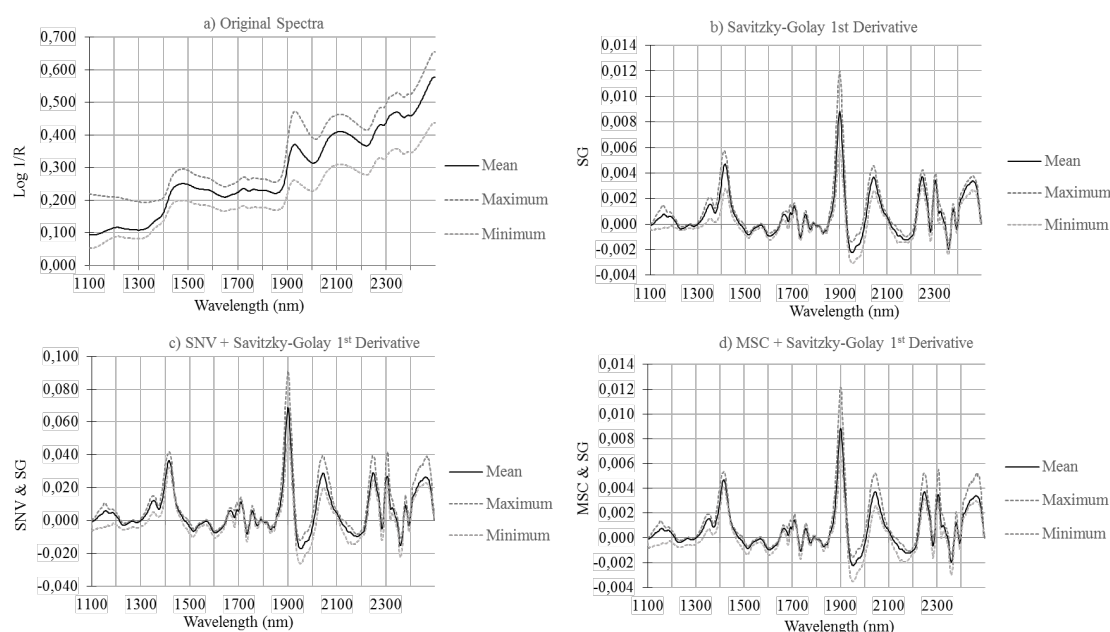

Figure 1. Spectral data synthesis from 358 gamba grass samples: original raw values (Fig. 1a) and transformed by Savitzky-Golay 1st derivative (Fig. 1b), by standard normal variance (SNV) plus 1st derivative (Fig. 1c), and by multiplicative scatter correction (MSC) plus 1st derivative (Fig. 1d).

Wet lab reference data: The reference values, as measured by means of wet chemistry analysis, consisted in broad ranges for NDF, ADF, ADL, CP, IVDMD, Ash, DM, and OM concentrations (Table 1). Similar results for gamba grass were reported by Silva et al., 2014. Range value of 170 g.kg^{-1} was observed for NDF, 196 g.kg^{-1} for ADF, 69 g.kg^{-1} for ADL, 145 g.kg^{-1} for CP, 351 g.kg^{-1} for IVDMD, 81 g.kg^{-1} for Ash, 77 g.kg^{-1} for DM, and 128 g.kg^{-1} for OM, reflecting a good trait variability among the samples.

Table 1. Concentrations of NDF, ADF, ADL, CP, Ash, DM and OM of 358 full calibration gamba grass samples based on wet lab reference methods.

	Mean	±SD	Range
	(g.kg^{-1})	
NDF	681.0	29.3	610.4 - 780.6
ADF	392.9	31.5	318.9 - 515.0
ADL	38.4	9.0	19.0 - 87.8
CP	98.2	28.9	21.2 - 165.8
IVDMD	538.6	66.1	348.8 - 699.8
Ash	66.2	13.0	35.4 - 116.8
DM	946.0	18.8	899.9 - 976.9
OM	879.8	23.8	803.2 - 931.4

Table 2 displays the concentrations of the quality traits analyzed by means of the reference methods for the 239 random selected samples used for the final calibration and the remaining 119 samples used for its validation. The values for range, mean, and standard deviation were quite similar for both calibration set and validation test set, expressing a good similarity between the two groups of samples.

Table 2. Concentrations of NDF, ADF, ADL, CP, Ash, DM, OM and IVDMD of 239 gamba grass samples used for the calibration model development and 119 used as a test set for the model validation, based on wet lab reference methods.

Trait	Calibration			Validation		
	Mean	±SD	Range	Mean	±SD	Range
			———— (g.kg-1) ————			
NDF	682.0	26.4	626.0 - 780.6	678.1	30.4	614.4 – 772.0
ADF	391.2	27.6	323.6 - 511.0	394.1	32.9	318.9 – 492.5
ADL	35.8	5.6	22.1 - 50.6	35.8	5.6	22.1 – 50.6
CP	97.5	29.0	21.2 - 153.6	97.5	27.4	24.9 – 165.8
IVDMD	539.4	61.2	360.7 - 670.9	539.3	57.6	403.6 – 634.0
Ash	65.6	12.4	35.4 - 102.8	66.0	11.7	41.1 – 97.3
DM	945.5	18.9	899.9 - 973.5	946.7	18.1	900.3 – 969.3
OM	879.6	23.6	809.5 - 926.3	879.5	23.3	803.2 – 922.0

NIRS calibration &validation: For all traits, 15 to 19 cross-validation models with different spectra math transformations were developed. Only the best math transformation and the best model fit for each quality trait, with the highest R^2 and the lowest and closest SEC and SECV for calibration and cross-validation were reported (Table 3). Bias, as a systematic difference between predicted and measured values, was not reported because was virtually insignificant for all traits, ranging from to -0.08 to 0.03 g.kg^{-1}. The number of outliers removed from calibrations was 8 for CP, 10 for ADF, Ash, DM and OM, 17 for NDF and 25 for IVDMD in 358 total samples.

Table 3. Calibration and cross-validation statistics developed for gamba grass quality traits from the full calibration set.

Trait	Math Treat.	Cal. & †Val	n	nF	Slope	Offset (g.kg^{-1})	r	R^2	SEC & SECV (g.kg^{-1})	n Out
NDF	MSC&SG123	Cal.	341	9	0.86	9.32	0.93	0.86	10.3	17
		†Val.			0.84	10.60	0.91	0.83	11.3	
ADF	SNV&SG123	Cal.	348	9	0.87	4.94	0.93	0.87	10.6	10
		†Val.			0.86	5.50	0.92	0.85	11.6	
ADL	MSC&SG123	Cal.	358	7	0.42	2.22	0.65	0.42	6.8	0
		†Val.			0.39	2.35	0.59	0.35	7.3	
CP	SG123	Cal.	350	7	0.94	0.62	0.97	0.94	7.2	8
		†Val.			0.93	0.69	0.96	0.93	7.7	
IVDMD	SG123	Cal.	285	8	0.79	11.20	0.89	0.79	27.3	25
		†Val.			0.77	12.26	0.87	0.75	30.1	
Ash	SNV&SG123	Cal.	348	11	0.91	0.56	0.96	0.91	3.5	10
		†Val.			0.89	0.69	0.94	0.89	4.1	
DM	SNV&SG123	Cal.	348	6	0.92	8.00	0.96	0.92	5.4	10
		†Val.			0.91	8.34	0.95	0.91	5.7	
OM	SG123	Cal.	348	7	0.90	9.14	0.95	0.90	7.6	10
		†Val.			0.89	9.97	0.94	0.88	8.0	

Cal. = calibration, †Val. = cross-validation, n = number of samples, nF = number of factors, n Out = number of outliers removed from calibration

The amount of explained variability of the models ranged from R^2 0.42 and SEC 6.8 g.kg^{-1} for ADL to R^2 0.94 and SEC 7.2 g.kg^{-1} for CP. DM and OM had R^2 values of 0.92 and 0.90, and SEC 5.4 g.kg^{-1} and 7.6g.kg^{-1}, respectively. Ash had a high R^2 0.91 and SEC 3.5 g.kg^{-1}. NDF and ADF had R^2 values of 0.86 and 0.87 with SEC 10.3 g.kg^{-1} and 10.6 g.kg^{-1}, respectively. IVDMD had R^2 value of 0.79 and SEC 27.3 g.kg^{-1}. Correlations (r) between the reference and predicted values were high, ranging from 0.89 for IVDMD to 0.97 for CP, and low for ADL with r = 0.65. The difference between the SEC and SECV were all below 3 g.kg^{-1} in a threshold value of 5 g.kg^{-1}, with 1.1 g.kg^{-1} for NDF, 1.0 g.kg^{-1} for ADF, 0.4 g.kg^{-1} for ADL, 0.5 g.kg^{-1} for CP, 2.8 g.kg^{-1} for IVDMD, 0.6 g.kg^{-1} for Ash, 0.3 g.kg^{-1} for DM, and 0.4 g.kg^{-1} for OM.

The final PLS calibration statistics using an external test set for validation, for all quality traits, are reported in Table 4. Traits CP, Ash, and DM had R^2 values of 0.91, 0.91, and 0.92, with very low SEC values of 8.7, 3.8, and 5.5 g.kg^{-1}, respectively, and RPD above 3. Traits NDF, ADF, and OM had R^2 values of 0.85, 0.88, and 0.89, low SEC 10.2, 9.4 and 7.9 g.kg^{-1}, respectively, and RPD values above 2.8. Yet, IVDMD had R^2 value of 0.79, SEC 28.0 g.kg^{-1}, and RPD value of 2.2. Finally, ADL had a fit with R^2 value of 0.36, SEC 7.3 and RPD 0.89. The differences between the SEC and SEP were 0.8 g.kg^{-1} for NDF, 2.4 g.kg^{-1} for ADF, 1.0 g.kg^{-1} for ADL, 0.5 g.kg^{-1} for CP, 1.8 g.kg^{-1} for IVDMD, 0.1 g.kg^{-1} for Ash, 0.2 g.kg^{-1} for DM, and 0.2 g.kg^{-1} for OM.

Table 4. Calibration and test set validation statistics developed for gamba grass quality traits from 239 sample-calibration set and 119 sample-validation set.

Trait	Cal. &Val.	n	nF	Slope	Offset (g.kg^{-1})	r	R^2	SEC & SEP (g.kg^{-1})	RPD SD/SEP
NDF	Cal.	228	9	0.85	10.17	0.92	0.85	10.2	2.80
	Val.	113		0.89	7.53	0.93	0.87	11.0	
ADF	Cal.	225	10	0.88	4.56	0.94	0.88	9.4	2.79
	Val.	113		0.84	6.33	0.93	0.87	11.8	
ADL	Cal.	239	5	0.36	2.46	0.60	0.36	7.3	0.89
	Val.	119		0.42	2.22	0.71	0.49	6.3	
CP	Cal.	233	5	0.91	0.88	0.95	0.91	8.7	3.36
	Val.	117		0.89	1.13	0.95	0.91	8.2	
IVDMD	Cal.	190	8	0.79	11.33	0.89	0.79	28.0	2.19
	Val.	95		0.77	12.31	0.89	0.79	26.2	
Ash	Cal.	232	10	0.91	0.60	0.95	0.91	3.8	3.11
	Val.	116		0.93	0.45	0.95	0.90	3.7	
DM	Cal.	232	6	0.91	8.04	0.96	0.92	5.5	3.45
	Val.	116		0.93	6.58	0.96	0.91	5.3	
OM	Cal.	232	6	0.89	9.91	0.94	0.89	7.9	2.85
	Val.	116		0.87	11.21	0.94	0.88	8.1	

Cal. = calibration, Val. = test set validation, n = number of samples, nF = number of factors

Table 5 summarizes the ANOVA for quality traits averaged over 6 harvests for 51 half-sib families from population 3. Statistically significant differences among families resulted for NDF, ADF, CP at 0.01 α-probability level and IVDMD at 0.05 α-probability level. The ranges, i.e., the differences between maximum and minimum were 21.9 g.kg^{-1} for NDF, 20.4 g.kg^{-1} for ADF, 14.3 g.kg^{-1} for CP, 40.9 g.kg^{-1} for IVDMD, 13.0 g.kg^{-1} for Ash, 7.6 g.kg^{-1} for DM, and 18.6 g.kg^{-1} for OM. The comparison among means via LSD was able to discriminate groups of families with higher quality characteristics.

Table 5. Mean square and descriptive statistics of quality traits for 51 half-sib families of gamba grass estimated from data averaged across six harvests from population 3 field experiment.

Source of Variation	df	NDF	ADF	CP	IVDMD	Ash	DM	OM
					Mean square			
Block	2	11.43	2.20**	0.47	0.51	0.65	1.03**	2.83**
HS Family	50	0.68**	0.49*	0.28**	2.49*	0.29	0.11	0.42

Error	100	0.41	0.31	0.14	1.64	0.27	0.17	0.29
					$g.kg^{-1}$			
Mean		670.8	377.3	96.2	560.1	57.5	949.6	891.8
LSD 0.05		10.4	8.9	5.9	20.7	8.4	6.7	11.5
Maximum		683.7	389.5	103.8	579.8	64.9	953.8	900.0
Minimum		661.8	369.1	89.5	538.9	51.9	946.2	881.4

*, ** Significant differences among half-sib families at the 0.05 and 0.01 α-probability levels, respectively.

IV. Discussion

NIR spectra of gamba grass samples showed distinct and overlapping peaks that are directly related to kind and concentration of organic compounds. The definition of the quality component for the absorbed radiation at a specific wavelength frequently oscillates according to sample material and its chemical composition[31].However, peaks at wavelengths around 1400 and 1900 nm are related to O-H, C-H and N-H bonds from water, different carbohydrates, and protein. Peaks at wavelengths between 2100 and 2200 nm are often related to N-H bonds from proteins, and between 2300 and 2400 nm are often related C-H from different carbohydrates, such as cellulose, hemicellulose and starch[9,44].

The range of absorbance from the maximum and the minimum lines suggested a great deal of variability for sample composition (Fig. 1a). The best pretreatments of spectra data were the first derivative alone or in combination with MSC or SNV, depending on the quality trait (Table 3). MSC followed by SG123 transformation improved the linear relationship between reference and spectral values for NDF and ADL, while SNV followed by SG123 improved linearity for ADF, Ash and DM. SG123 alone resulted in good linearity for CP, IVDMD, and OM. After applying the first derivative of Savitzky Golay, the MSC, and the SNV transformations (Fig. 1b, c, d), the spectral lines tended to lie closer, except in the positive and negative peaks, where their distances are clearly larger. The distance between the maximum and the minimum spectral lines is the range of samples variation and is directly associated with their chemical composition variability in a specific wavelength.

The reference values from the wet lab analysis also consisted in broad ranges for NDF, ADF, ADL, CP, IVDMD, Ash, DM, and OM concentrations in the full calibration set, as well as in the sub-sets for the final calibration. This wide variation in the quality trait concentrations was consistent with the variability of the samples, as expected, since they came from different plant genetic backgrounds, different climatic conditions over years, different experimental sites, as well as different harvest times (Tables 1 and 2).Both, the spectral data variability and the wet lab data variability are important not only to the calibration process, to represent the universe to be predicted by the NIRS models, but also to the breeding process, to accomplish forage quality gains from selection.

The best cross-validation model fit was for CP, followed by DM, Ash, and OM with R^2above 0.90 and very small SECs (Table 3). Yet, NDF and ADF had good fits with R^2 values above 0.86 and also very small SECs while IVDMD had a moderate fit withR^2 0.79 and small SEC. The worst fit was for ADL with R^2 0.42 and large SEC. Although mineral components in forages theoretically show extremely poor NIRS absorption bands[47], as well as weak calibrations as for corn[16], soybean[15], and meadow grass[41], Ash had a very good model fit in this study. It is not so unusual to get good calibrations from total Ash, the inorganic component of the sequential residue, as NIRS usually is directly related to OM. Windham[43] reported similar results and concluded that silica was a component with unusual spectral properties and provided useful information for NIRS calibration. According to Shenk et al. (2007), minerals forming organic complexes or chelates may be detected, but there are no spectral matches for minerals in the ionic or salt form.

Correlations between the reference and predicted values of all traits were strong, with r ranging from 0.89 to 0.97, but ADL with r 0.60. The difference between the SEC and SECV were all below 3 $g.kg^{-1}$ in a threshold value of 5 $g.kg^{-1}$, reflecting a very good accuracy of the cross-validation calibrations. The definition of the best mathematic treatment transformation for each trait, as well as the identification of outliers to be removed from the set (Table 3) were done at this stage and then, the final calibration with an external set was performed.

Calibrations with a external set performed very well for all traits but ADL (Table 4). Indeed, the statistics for calibrations and validations were quite similar when comparing Tables 3 and 4. Test set calibrations were pretty much analogous to the cross-validation calibrations, as expected, indicating a good adequacy of the kind and number of samples for all sets in the development of the models. The traits CP, Ash, and DM had the best fit with R^2 values above 0.90, with very small and almost no dissimilarity between SEC and SEP and RPD above 3.0. CP is long reported for having excellent calibrations[5,17,21,34]. The traits NDF, ADF, and OM had a very good fit with R^2 values above 0.80, small deviations between SEC and SEP, which ranged from 0.3 to 2.4

g.kg^{-1}, and RPD values above 2.8. IVDMD presented a moderate fit with R^2 0.79, a small deviation between SEC and SEP of 1.8 g.kg^{-1}, and RPD value of 2.2. The difference between the SEC and SEP were all below 2,5 g.kg^{-1} in a threshold value of 5 g.kg^{-1}, with 2.4 g.kg^{-1} for ADF, 1.8 for IVDMD, and all other lower than 1.0 g.kg^{-1}, reflecting a very good accuracy of the test set validation models. The above results indicate that NIRS is an effective approach to accurate predict gamba grass NDF, ADF, CP, IVDMD, Ash, DM, and OM for screening purposes. Other studies reported success in the use of the NIRS to estimate most of the quality components in tropical forages[28, 29] and temperate forage[4].

At last, ADL had the worst fit with R^2 0.36 and RPD 0.89, indicating a poor adequacy the model for any kind of prediction. However, PLS models were reported to be accurate for ADL prediction of some species, e.g., alfalfa[17], corn stover[46], switch grass and canary grass[1].

Gamba grass is a cross-pollinated species and the natural variability from the original population is high. The 51 half-sib families were derived from parent plants selected at the 5th cycle of mass selection for leaf/colm ratio, tillering vigor and semi-erect plant type. At this stage, the selected subpopulations less heterogeneous, with lower variability for the traits it was selected for, because of the selection pressure of 10% imposed from the 1st to the 3rd cycle, the 3.5% in the 4th cycle, and 10% in the 5th cycle of selection. The 51parent plants are more uniform because they were selected individual plants to be polycrossed to form the new generation to be selected from. As a result, there was even less variability among the 51 half-sib families (Table 5) when compared with the original populations (Table 1), which explains the lower magnitude range for all traits. Even though there were less variability and lower ranges, the ANOVA detected significant differences among the 51 families for NDF, ADF, CP and IVDMD. Also, LSD at 0.05 level allowed the discrimination of the best families for each significant trait. These results strengthen the effectiveness of NIRS as a tool to accurate predict NDF, ADF, CP, IVDMD, Ash, DM, and OM for screening gamba grass families or genotypes for superior forage quality.

V. Conclusions

The substantial forage quality variation among the samples from all 3 populations permitted the development of useful prediction models with great accuracy. Low and similar SEC and SEP values, higher magnitudes of R^2, as well as high correlations between spectral data and wet laboratory data indicated the methods were useful for predicting quality traits. Significant differences were found among 51 half-sib families for quality traits predicted through NIRS PLS models. The results endorse the use of NIRS as a tool for selecting the superior forage quality genetic materials. The final PLS models were adequate and sufficient accurate for predicting NDF, ADF, CP, IVDMD, Ash, OM, and DM concentrations. In contrast, ADL prediction models did not have enough accuracy and further studies should include more variability to improve the development of more robust and useful calibrations. NIRS resulted in a feasible tool to predict most of the gamba grass quality traits for phenotyping and screening purposes.

Acknowledgements

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