Seventh framework programme
Food, Agriculture and Fisheries, and Biotechnology

Specific International Co-operation Actions
Small or medium scale focused research project

Sweet Sorghum
an alternative energy Crop

SWEETFUEL / Grant Agreement n° 227422

WP1
Deliverable 1.2:

NIRS calibration for biomass composition

Composition of the consortium

CIRAD
ICRISAT
EMBRAPA
KWS
IFEU
UniBO
UCSC
ARC-GCI
UANL
WIP

Use of Near Infrared Spectroscopy (NIRS) for the prediction of chemical composition and degradability of sorghum.

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Chemical characterization of samples

In the framework of Sweetfuel projet, it is essential to characterize the properties of many sorghum lines.

- Chemical composition is assessed by measurement of a serie of biochemical parameters :
  1. Organic matter : OM=100-MM with MM= total mineral content
  2. Fibre content and characteristics
     Crude fibre (CF) as a classical parameter for the estimation of fibre content

     Fractionation of fibre according to Van Soest analysis : NDF (Neutral detergent fibre), ADF (Acid detergent fibre), ADL (acid detergent lignin).

     By calculation derived parameters can be obtained as

     HEM=NDF-ADF : estimation of hemicelluloses content
     CVS=ADF-ADL : estimation of cellulose content
     ADL represents lignin content

  3. Crude protein content
     The estimation of protein content is done by measurement of total nitrogen (N). Then CP is by convention calculated as CP=6.25*N, assuming that sorghum protein contains on average 16%N.

  4. Degradability
     An estimation of enzymatic susceptibility of samples is provided by digestion with proteolytic (Pespsine) and cellulolytic (Cellulase) enzymes. Calculations were made to determine in vitro degradation of dray matter (IVDDM) and of organic matter (IVDOM).

     The synthetic parameters obtained (IVDDM / IVDOM) are considered as indexes of degradability in relative value. It allows to rank samples from low to high degradation potential.

Materials and methods

NIRS analysis

All the samples were scanned on a monochromator NIR spectrophotometer (NIRS 6500, Foss NIRSystems, Silver Springs, MD, USA). Measurement was done in reflectance mode in small circular cups (diameter 50 mm) with quartz glass. Spectral data were collected every 2 nm from 400 to 2500 nm. Samples were scanned a second time after repacking and duplicate spectra were averaged.

Laboratory analysis

From the spectral database obtained, a subset was selected for reference chemical analyses. As CIRAD already possessed a preliminary Sorghum database, the selection of samples was performed

to ensure the best adaptation of the existing database with new samples. This was achieved by the SELECT procedure in WINISI software (WINISI III, Infrasoft international, Port Matilda, PA, USA).

Selected samples were analysed for the chemical parameters listed above : MM (OM), CF, NADF, ADF, ADL, CP, IVDDM, IVDOM.

NIRS calibration

Calibration was performed by partial least square regression, using the modified partial least squares regression (mPLS) of WINISI III software. Calibration procedure involved elimination of major prediction outliers, in order to avoid biases due to spectral or laboratory errors. For each analytical parameter, the optimization of mathematical pretreatments of spectra was performed by comparing the cross-validation error of models differing by detrending and normalization parameters, derivative order (none, 1st, 2nd) and spectral range.

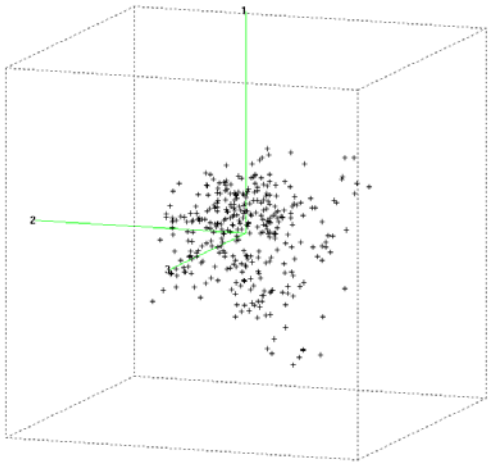
Calibration performance was described through coefficient of determination r^2 and standard error of calibration (SEC). A cross validation was performed on four groups and led to the calculation of the standard error of cross-validation (SECV). The performance to deviation ratio (RPDcv = SD/ SECV) was used as an indication of model quality.

Results

NIRS database

The global database obtained (existing database + updating samples) includes more that 350 samples with reference data. It is to our knowledge the widest existing sorghum stems database.

The figure below represents the first 3 principal components of PCA analysis of spectral database. It can be seen that the database is quite homogeneous, without apparent subgroups that could interfere with calibration process. No major outlier is detected.



Basic statistics of the laboratory values of the database are presented below:

	MM	CP	CF	NDF	ADF	ADL	INDDM	IVDOM
Mean:	5.06	4.24	37.14	67.25	41.21	6.34	39.71	37.56
Std.Deviation:	3.20	2.59	6.78	10.28	7.87	2.25	11.71	11.93
CV (%)	*63.3*	*61.2*	*18.3*	*15.3*	*19.1*	*35.42*	*29.5*	*31.8*
Minimum:	1.07	0.68	19.95	37.58	21.81	1.38	10.52	9.05
Maximum:	19.60	16.89	57.28	89.45	61.14	13.63	71.86	70.61

Variation is high for all parameters, which is important for the robustness of calibration equations and their adaptability to a wide range of materials in the future. Coefficients of variation are around 60% for MM and CP, 30% for ADL, IVDDM and IVDOM and 15-20% for CF, NDF and ADF.

NIRS calibration equations

The basic statistics of calibration equations are presented below:

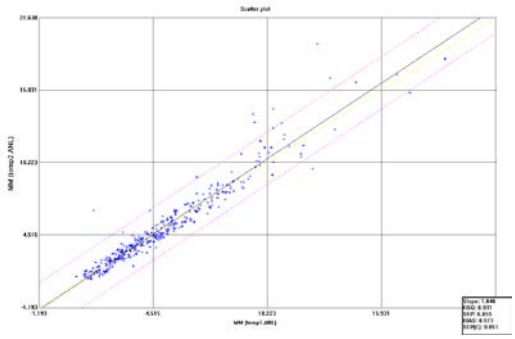
Constituent	SEC	RSQ	SECV	RPD
MM	0.47	0.96	0.65	3.84
CP	0.37	0.97	0.50	4.49
CF	1.11	0.97	1.29	5.21
NDF	1.39	0.98	1.66	6.05
ADF	1.15	0.98	1.34	5.86
ADL	0.55	0.94	0.64	3.41
IVDDM	1.60	0.98	1.94	5.78
IVDOM	1.79	0.98	2.04	5.60

SEC, Standard error of calibration
RSQ, coefficient of determination of calibration (R^2 between measured and predicted values)
SECV, standard error of cross-validation (estimation of prediction error)
RPD, ratio performance ti deviation =SD/SECV

The calibration equations obtained have a good precision, with comparable or better SECV values than classically obtained / published on such materials. RPD is a synthetic criterion of calibration quality: RPD values in the range 1-2 are very poor calibration, while RPD= 3-4 are good and values above 5 are very good.

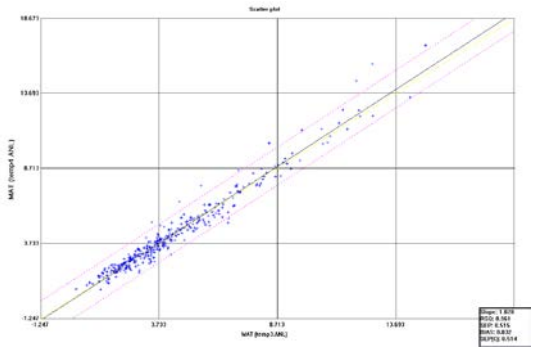
Calibration performance are illustrated and discussed below, representing the relationship between predicted and laboratory values. :

MM



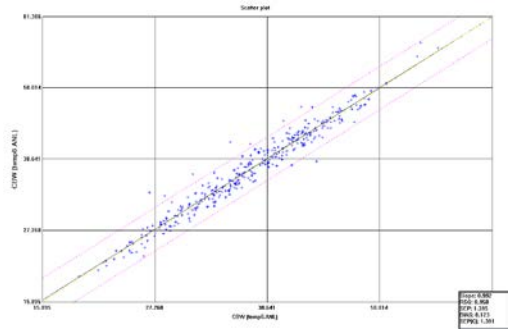
Calibration for minerals is classically poor since NIR mineral don't absorb NIR light. Calibration obtained here is good (RPD=3.8), with the exception of high MM values for which outlier values are obtained. Hypothesis is that high values are due to contamination of sorghum by sand / earth.

CP



Calibration obtained is quite precise, with a standard error of prediction estimated around 0.5%. However there are some errors in high values, which are very unusual in sorghum stems.

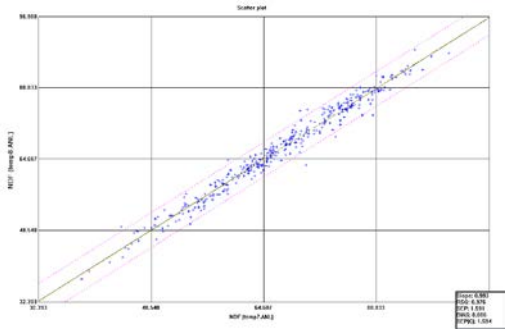
CF



CF calibration is very good, with RPD >5.

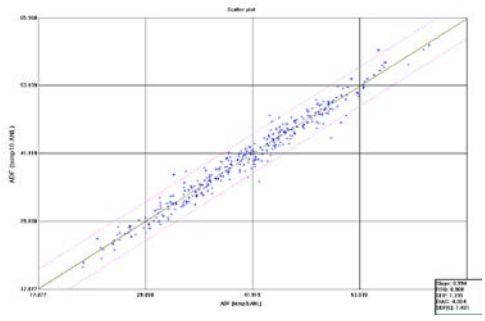
Some outliers have to be checked

NDF



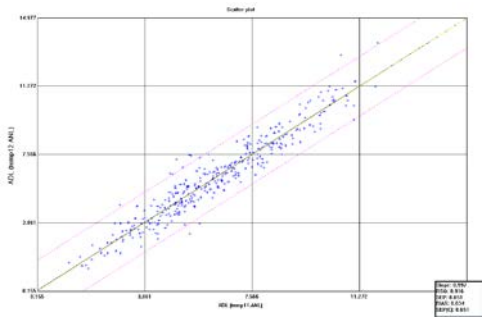
NDF calibration is very good. RPD value is above 6 and prediction error is below 2, which is below classical NIRS models for NDF (generally 2-2.5).

ADF



ADF calibration is comparable to CF calibration, which is logical since they represent more or less the same biochemical constituents (roughly : lingo-cellulose content)

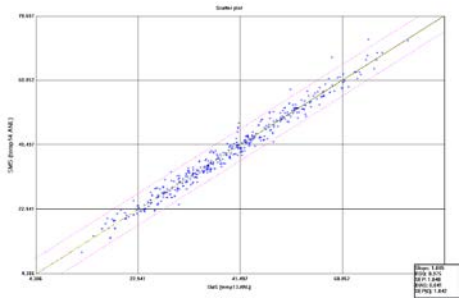
ADL

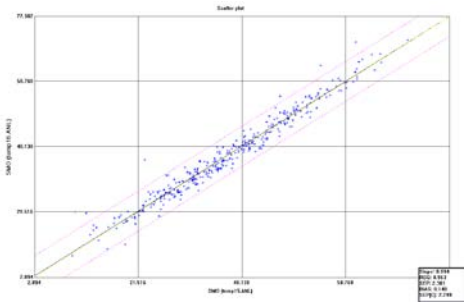


Calibration for ADL is less accurate than other fibre parameters. However prediction precision is satisfactory (error = 0.64). It is classical that ADL is less precisely predicted, primarily due to reference laboratory measurement which is less precise than other parameters.

IVDDM

IVDOM





IVDDM and IVDOM are very well predicted, particularly if we consider that they are not biochemical parameters but degradation properties, which should be harder to detect by NIRS. In the present case, the error (around 2%) is relatively low and the RPD value is high (close to 6 !) thanks to a high variability in the population studied.

Preliminary conclusion

The creation of calibration equation for SWEETFUEL project was successful. The equations are based on the improvement and adaptation of an already existing database, which allowed to reach a high number of samples, creating a unique database for sorghum stems.

The precision reached in the prediction of biochemical constituents of sorghum is high and allows a use in routine for prediction of characteristics of sorghum samples. Updating will be required every time there will be new series of samples, with new variation not yet included in the database. But the updating process will be possible with a very limited number of samples, and will therefore be quite simple to achieve.

This task produced a useful tool which can be available for samples originating from SWEETFUEL project.