CSIRO PUBLISHING

Crop & Pasture Science, 2020, **71**, 401–409
https://doi.org/10.1071/CP19324

Proximal sensing of *Urochloa* grasses increases selection accuracy

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Abstract. In the American tropics, livestock production is highly restricted by forage availability. In addition, the breeding and development of new forage varieties with outstanding yield and high nutritional quality is often limited by a lack of resources and poor technology. Non-destructive, high-throughput phenotyping offers a rapid and economical means of evaluating large numbers of genotypes. In this study, visual assessments, digital colour images, and spectral reflectance data were collected from 200 *Urochloa* hybrids in a field setting. Partial least-squares regression (PLSR) was applied to relate visual assessments, digital image analysis and spectral data to shoot dry weight, crude protein and chlorophyll concentrations. Visual evaluations of biomass and greenness were collected in 68 min, digital colour imaging data in 40 min, and hyperspectral canopy data in 80 min. Root-mean-squared errors of prediction for PLSR estimations of shoot dry weight, crude protein and chlorophyll were lowest for digital image analysis followed by hyperspectral analysis and visual assessments. This study showed that digital colour image and spectral analysis techniques have the potential to improve precision and reduce time for tropical forage grass phenotyping.

Additional keywords: *Brachiaria*, phenotyping, plant breeding, tropical forage grasses.

Received 7 August 2019, accepted 10 February 2020, published online 18 April 2020

Introduction

Livestock productivity depends on forage availability and quality. Grasses from the genus *Urochloa* (syn. of *Brachiaria*) have been widely planted in the tropics as forage for grazing ruminant livestock and are considered the most important forages in the American tropics (Miles *et al*. 2004). The International Center for Tropical Agriculture (CIAT) in Colombia conducts a *Urochloa* breeding program aimed at developing hybrids with outstanding performance of superior forage productivity and nutritional quality on infertile, acidic soils. The hybrid development process is time-consuming and expensive. In a regular, 3-year breeding cycle, >7000 hybrids are produced by open pollination, but <2% of these are retained for full evaluation. Approximately half of the population is discarded on the basis of reproductive mode, with sexual genotypes discarded and apomictic hybrids kept; another major proportion is then discarded based on visual estimations of biomass production; and only ~100 hybrids are finally evaluated for tolerance to different biotic and abiotic stresses (V Castiblanco, pers. comm.). The evaluation of genotypes is restricted mainly by insufficient economic resources and technology for rapid screening.

Forage grasses exhibiting high biomass production and nutritional quality are key to the productivity of grazing animals (Herrero *et al.* 2013). Therefore, shoot biomass production and quality parameters (i.e. crude protein, CP) are among the most important traits for improvement in any forage grass breeding program. However, owing to the destructive nature of these measurements and the insufficient economic resources, evaluation of these parameters is postponed to the final stages of the breeding program characterised by a reduced number of genotypes. Instead of using analytical measurements of forage quality and destructive biomass harvests, periodic visual evaluations of plant performance (i.e. plant biomass and greenness) over time are traditionally used in *Urochloa* breeding programs to select superior plants at initial stages of the breeding scheme (Miles *et al*. 2004; Miles 2007). These visual evaluations are laborious and may not be sufficiently accurate especially in breeding populations characterised by high genetic diversity and substantial genotype × environment interaction (Walter *et al*. 2012).

The use of new technologies for in-field, non-destructive, high-throughput phenotyping (HTP), including digital image

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analysis and proximal hyperspectral sensing, offers the possibility for precise evaluation of a larger number of genotypes than feasible in traditional ways, achieved at low cost and implemented in a short period (Montes *et al*. 2007; White *et al*. 2012; Andrade-Sanchez *et al*. 2014). Proximal hyperspectral sensing provides continuous information along the visual and near-infrared electromagnetic spectrum. This information often relates to plant traits and has been successfully studied in grasses as a means of estimating quality parameters (Skidmore *et al*. 2010; Pullanagari *et al*. 2012; Thulin *et al*. 2012; Ferner *et al*. 2015; Safari *et al*. 2016), diversity (Lopatin *et al*. 2017), and nutrient content (Fava *et al*. 2009; Knox *et al*. 2012; Ramoelo *et al*. 2013; Adjorlolo *et al*. 2015; Foster *et al*. 2017). Likewise, plant image analysis for phenotyping purposes is based on image segmentation to separate the soil background and the plant for further quantification of regions of interest (Tucker 1979; Woebbecke *et al*. 1995; Camargo 2004; Hunt *et al*. 2005). Digital image analysis has also been used for quantifying vegetation indices related to plant growth, greenness and nutritional status (Meyer and Camargo 2008; Hunt *et al*. 2013). Very few reports of hyperspectral (Numata *et al*. 2008) or image analysis (Jiménez *et al*. 2017) of *Urochloa* grasses exist in the literature.

No study combining hyperspectral information and image analyses, and comparing them with conventional phenotyping methods, is available. Moreover, hyperspectral data have not been used to evaluate target traits in *Urochloa* breeding programs. In this study, in-field visual evaluations, proximal hyperspectral data, and digital imaging data were collected over canopies of *Urochloa* hybrids. Partial least-squares regression (PLSR) was used to relate hyperspectral information to field measurements, and machine learning (i.e. Naive Bayes multiclass) was used to extract vegetation indices from overhead canopy images. The objectives of the study were: (*i*) to develop PLSR models for predicting CP, forage dry weight (DW) and chlorophyll content; (*ii*) to extract plant traits from digital image analysis that can relate to CP percentage, forage DW and chlorophyll concentration; and (*iii*) to demonstrate the superiority of HTP techniques over conventional visual evaluation of traits. CP, forage DW and chlorophyll were chosen as target traits in this study because they are key parameters determining both plant and cattle productivity. The development of HTP methodologies to evaluate tropical forages will increase the number of hybrids evaluated per selection cycle, thus permitting more intense selection and, hence, genetic gain. Identification of new hybrids with outstanding performance (i.e. higher biomass, greener and high CP) will result in more productive pastures with concomitant increases in milk and meat production in livestock systems in tropical savannahs.

Materials and methods

Field experiment

Field data were obtained in August 2016 at the International Center for Tropical Agriculture (CIAT) in Cali, Colombia (3°29′N, 76°21′W; altitude 965 m). Four thousand *Urochloa* hybrids generated from crosses between the CIAT's *Urochloa* breeding program population SX12 and *U. decumbens* cv. Basilisk (CIAT 606) were initially planted in an Andisol soil in an augmented block design and spaced at 1.5 m by 1.5 m. These plants were visually evaluated four times (data not shown) for persistence, vigour and greenness after sequential cuttings every 3 months for 1 year. After that period, 200 hybrids were randomly selected for further visual and HTP analyses. These 200 hybrids, rather than the entire population, were selected for economic and practical reasons. Visual evaluations of biomass and greenness and imaging and spectra collection were performed after 3 months of regrowth following cutting (see information below). Plant heights ranged from 20 to 50 cm and shoot architecture varied with both decumbent and erect growth.

Visual evaluation

Plant biomass was assessed on a 9-point visual scale: 9, high shoot biomass with many tillers and leaves; 1, stunted growth with fewer tillers and leaves. Plant greenness was visually evaluated on a 5-point visual scale: 5, intense dark green in all leaves of the plant; 1, yellow-pale colour in all leaves of the plant. This visual evaluation was conducted over 68 min, and HTP measurements were made 1 week later (Table 1).

Imaging collection and analysis

Individual digital colour images for each of the 200 hybrids were taken at 1.2 m above the soil surface by using a commercial digital 13-megapixel camera (Coolpix P6000; Nikon, Tokyo) fixed to a buggy tractor. Digital images were saved in 4224 × 3168 pixel JPG format. The canopy cover and six vegetation indices including the normalised green-red difference index, excess green index, excess red index, excess green minus excess red, green ratio and green leaf index were created using the formulae indicated in Table 2. The canopy cover was extracted by dividing the total number of pixels representing the plant by the total number of pixels in each image. The vegetation indices were extracted using Naive Bayes multiclass. Briefly, the distribution of colours in a set of digital colour images (training set) was used to estimate the probability density function for each of the different regions of interest (i.e. plant and background). Once the regions of interest were defined in the training set, the machine-learning process was applied to all images to classify and separate regions of interest accurately. Therefore, every pixel in an image was classified into the previously defined plant and background classes. Every pixel characterising the plant (but not the background) was then decomposed into red (R), green (G) and blue (B) channels. These channels were then normalised as follows:

$$r = \frac{R}{R+G+B}; g = \frac{G}{R+G+B}; b = \frac{B}{R+G+B}$$

Normalisation makes the variations of light intensities uniform across the spectral distribution; thus, the individual colour components (i.e. r, g, b) are independent from the overall brightness of the image (Cheng *et al*. 2001). Normalised channels were further used for the quantification of the vegetation indices (Table 2). Image analysis code was

Table 1. Phenotyping techniques used in the present study, time required for evaluation of 200 *Urochloa* plants under the conditions of the study, applications, advantages and disadvantages

Phenotyping techniques	Time to evaluate	Applications	Advantages	Disadvantages
Visual evaluation	68 min	Visual observations of different plant characteristics	Easy operation, low cost; evaluations can be done under diverse conditions and environments	Evaluation of low number of genotypes; evaluation is subject to human bias and fatigue
Image analysis	40 min	Quantification of canopy cover and vegetation indices in the visible electromagnetic spectrum	Easy operation, low cost, greater number of plants evaluated, determination of several vegetation and water indices	Changes in ambient light conditions limit calculation of vegetation indices; data analysis is moderately complex
Hyperspectral analysis	80 min	Canopy reflectance information in visible and near-infrared regions of the electromagnetic spectrum; information can be used to predict biochemical composition of plants	Moderately easy operation, greater number of plants evaluated, determination of nutritional and biochemical composition of leaf or canopy	Low solar radiation or cloudy days limit analysis; sensor and white reference calibration is frequently needed; data analysis is complex

Table 2. Canopy cover and vegetation indices calculated from digital images of 200 *Urochloa* hybrids

Vegetation indices were extracted by using a Naive Bayes multiclass machine-learning approach. Indices were then incorporated into a partial least-squares regression model to predict crude protein concentration, forage dry weight biomass and chlorophyll concentration. No normalisation was performed for the canopy cover quantification. Nc, Total number of pixels representing the canopy; Nt, total number of pixels in the picture; r, g and b denote the normalised pixel values of each channel on the RGB colour mode

Plant trait	Name	Formula	Reference
CC	Canopy cover	Nc/Nt	–
NGRDI	Normalised green red difference index	(g – r)/(g + r)	Hunt *et al.* 2005
ExG	Excess green index	2g – r – b	Woebbecke *et al.* 1995
ExR	Excess red index	1.3r – g	Meyer *et al.* 1998
ExGR	Excess green minus excess red	ExG – ExR	Camargo 2004
GR	Green ratio	g/(r + g + b)	Tucker 1979
GLI	Green leaf index	(2g – r – b)/(2g + r + b)	Louhaichi *et al.* 2001

written in Java and run in ImageJ software (National Institutes of Health, Bethesda, MD, USA). Images were collected early in the morning in order to avoid beam solar radiation interferences. Digital images contained the whole plant in addition to the 23-cm-diameter field of view (as indicated below for hyperspectral measurements; see Supplementary Materials fig. S1, available at the journal's website). The collection process took 40 min (Table 1).

Spectral collection and analysis

Hyperspectral field data collections were performed on clear days at full sun exposure around 11:00 by positioning a hand-held field spectroradiometer (Fieldspec 2; Malvern Panalytical, Malvern, UK) directly above the plant canopy. The instrument was used with no fore-optics, which provided a 25° full-conical-angle field of view. In order to avoid soil background noise, the bare optical input was positioned 50 cm from the top of the plant canopy to yield a 23-cm-diameter field of view. The instrument collected information in 750 narrow wavebands from 325 to 1075 nm in 1-nm intervals. Spectral collection involved one scan or 10 scans per plant, and 50 plants were evaluated daily over ~20 min. Differences in the collection protocols were tested to evaluate which was most effective. Different spectral collection processes (1 scan or 10 scans) did not yield significant differences in the root-mean-squared error of prediction (RMSEP) for the different traits evaluated (table S1). Radiometric collections over a 99% Spectralon panel (Labsphere, North Sutton, NH, USA) were used to describe incoming solar irradiance throughout the data-collection process. The radiometric collections over the calibration panel were made before starting and after every five canopy scans, or when slight changes in solar irradiance due to cloud cover occurred. The values of the Spectralon panel radiance were used to compute the canopy reflectance of the plants in each wavelength over the time of spectra collection. Subsequently, 401 bands from 500 to 900 nm were used for analysis. Based on visual inspection of reflectance spectra, these bands were typically less noisy than bands at the bounds of detector sensitivity. The spectral collection process was run over 80 min (Table 1).

Laboratory sample collections

Plants were immediately harvested after collection of spectra. Aboveground tissue was removed by cutting the area defined by a 23-cm-diameter plastic circle co-located with the spectral data-collection area. Tissues were packed in plastic bags and stored on ice in a cooler in the field and then transported to the laboratory. Extraction of chlorophyll was performed by adding fresh tissue (100 mg) to 80% (v/v) cold methanol, and the mixture was homogenised by using a pestle in a mortar until the plant residue was clear and the solution was uniform. This solution was then filtered and absorbance was determined with a spectrophotometer (Synergy HT; BioTek, Winooski, VA, USA). Total chlorophyll concentration was calculated according to Lichtenthaler and Wellburn (1983). Forage DW was measured on an electronic balance (PB602S; METTLER TOLEDO, Columbus, OH, USA) after oven-drying the samples for 3 days at 60°C. Nitrogen (N) concentrations in the dry tissue were determined by using an automated N-carbon analyser (Sercon, Crewe, UK). *Urochloa* and common bean (*Phaseolus vulgaris* L.) leaves were used as reference tissues for confirmation of the reliability of the analyses. The CP concentration was calculated by multiplying N concentration by 6.25, because protein is assumed to contain 16% N on average.

Statistical analyses

Visual evaluations and digital image analysis, spectral reflectance and plant trait data were incorporated into a PLSR algorithm (Mevik and Wehrens 2007) within R (The R Foundation: http://www.r-project.org). Models were developed to predict each plant trait (i.e. CP, forage DW and chlorophyll content) and to compare the precision for prediction of each of the different methods of phenotyping. PLSR was used in preference to conventional least-squares analysis in order to reduce collinearity effects. Thorp *et al.* (2011) provided the details on the PLSR methodology used in the present study. Briefly, if $\mathbf{Y}$ is an $n \times 1$ vector of responses (i.e. CP, forage DW or chlorophyll content) and $\mathbf{X}$ is an n-observation by p-variable matrix of predictors (a set of visual evaluations, digital image analyses, or spectral reflectance data), PLSR aims to decompose $\mathbf{X}$ into a set of A orthogonal scores such that the covariance with corresponding $\mathbf{Y}$ scores is maximised. The X-weight and Y-loading vectors that result from the decomposition are used to estimate the vector of regression coefficients, $\boldsymbol{\beta}_{\mathbf{PLS}}$, such that:

$$\mathbf{Y} = \times\, \boldsymbol{\beta}_{\mathbf{PLS}} + \varepsilon$$

where $\boldsymbol{\varepsilon}$ is an $n \times 1$ vector of error terms.

Leave-one-out cross-validation was used to test model predictions for independent data. Results were reported for PLSR models with the number of factors that minimised the RMSE of cross-validation. Pearson's correlation coefficients were calculated for the different traits extracted from digital colour images taken from *Urochloa* hybrids.

Results

In this study, visual evaluations of biomass and greenness, digital colour imaging and hyperspectral data were collected on 200 *Urochloa* hybrids in 68, 40 or 80 min, respectively (Table 1). A high degree of variability was found for the characteristics forage DW, CP percentage and chlorophyll concentration among the 200 *Urochloa* hybrids evaluated (Table 3).

Visual assessments

The PLSR for measured traits of forage DW, CP and chlorophyll based on visual evaluations of biomass and greenness performed with RMSEP of 8.47 g plant^{-1}, 1.76% and 0.60 mg g^{-1} fresh weight (FW), respectively (Fig. 1).

Spectral data and digital image phenotyping

The PLSR models developed from digital image analysis estimated forage DW, CP and chlorophyll with RMSEP of

Table 3. Phenotypic variation on plant traits measured in 200 *Urochloa* hybrids

CV, Coefficient of variation

Trait	Min.	Max.	Mean	CV (%)
Forage dry weight (g plant^{-1})	6.74	64.1	30.22	34.81
Crude protein (%)	6.76	21.58	11.23	19.68
Chlorophyll (mg g^{-1} fresh weight)	0.87	6.41	2.88	24.31

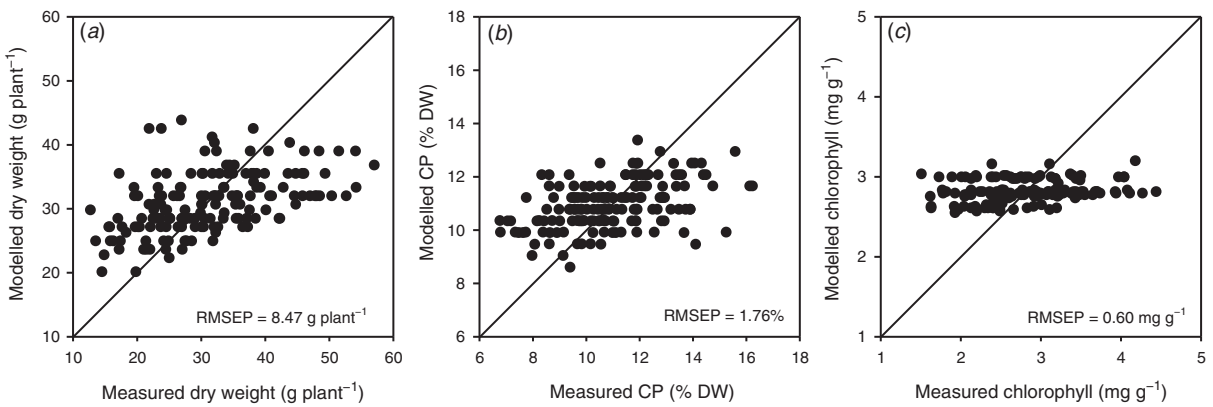

Fig. 1. Modelled versus measured forage dry weight (*a*), modelled versus measured crude protein percentage (*b*) and modelled versus measured chlorophyll concentration (*c*) when fitting partial least-squares regression models to relate each biophysical characteristic to visual evaluations of biomass and greenness of 200 *Urochloa* hybrids.

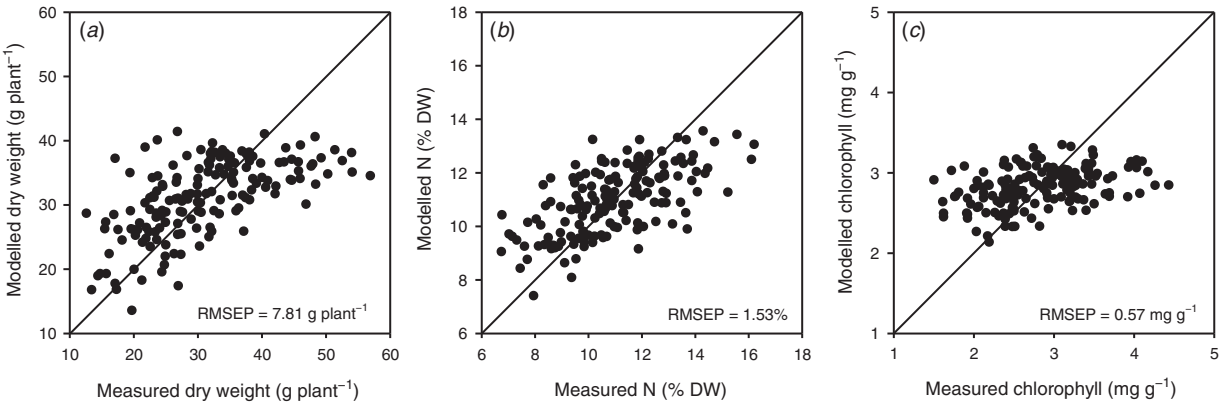

Fig. 2. Modelled versus measured forage dry weight (*a*), modelled versus measured crude protein percentage (*b*) and modelled versus measured chlorophyll concentration (*c*) when fitting partial least-squares regression models to relate each biophysical characteristic to digital image analysis of 200 *Urochloa* hybrids.

7.81 g plant^{-1}, 1.53% and 0.57 mg g^{-1} FW, respectively (Fig. 2). Differences in the correlation coefficients among traits extracted from image analysis indicated that including different indices into the model added independent information to build stronger PLSR models (Fig. 2). The contribution of each trait extracted from digital image analysis to the overall prediction of each destructively measured trait is shown in Table 4. The green leaf index had a stronger positive influence on the PLSR model for predicting forage DW. Excess green minus excess red had a stronger positive influence on the PLSR model for predicting both CP and chlorophyll concentrations.

The fitted PLSR models developed from 401 wavebands of canopy spectral reflectance estimated forage DW, CP and chlorophyll with a RMSEP of 7.90 g plant^{-1}, 1.63% and 0.55 mg g^{-1} FW, respectively (Fig. 3). The contribution of each spectral waveband to the overall prediction of each destructively measured trait is present in Fig. 4. In the PLSR model for forage DW, local extremes in regression coefficients

Table 4. Regression coefficients of the fitted partial least-squares regression models of seven traits extracted from digital image analysis

CC, Canopy cover; NGRDI, normalised green red difference index; ExG, excess green index; ExR, excess red index; ExGR, excess green minus excess red; GR, green ratio; GLI, green leaf index. Positive and negative coefficients indicate positive and negative influence on the prediction model, respectively

Traits	Forage dry weight (g plant^{-1})	Crude protein (% DW)	Chlorophyll (mg g^{-1})
CC	3.7606	–0.2311	–0.0167
NGRDI	9.9486	0.0860	–0.0353
ExG	–14.3163	0.0776	0.0731
ExR	–32.1262	–0.3911	–0.0776
ExGR	3.7245	0.2659	0.1033
GR	–34.7709	–0.3116	–0.0363
GLI	80.8715	–0.3111	–0.0358

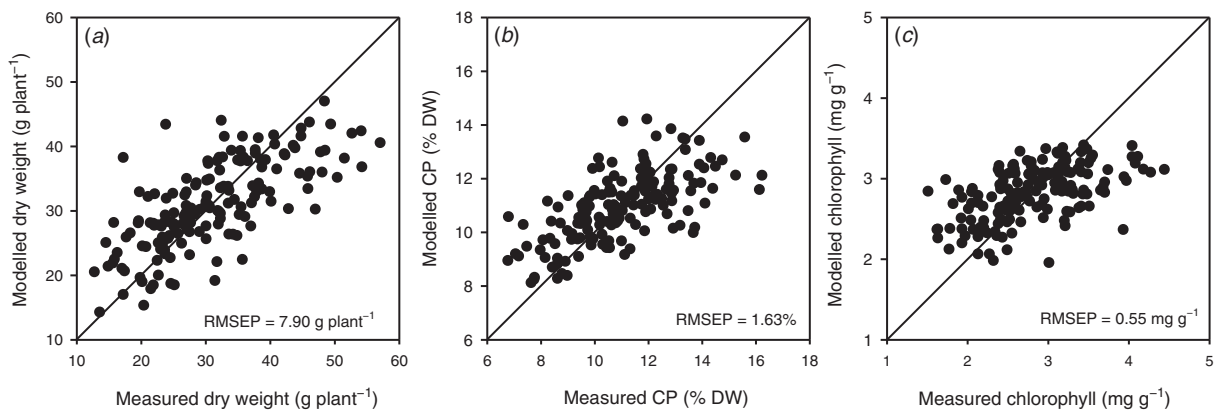

Fig. 3. Modelled versus measured forage dry weight (*a*), modelled versus measured crude protein percentage (*b*) and modelled versus measured chlorophyll concentration (*c*) when fitting partial least-squares regression models to relate each biophysical characteristic to canopy spectral reflectance of 200 *Urochloa* hybrids.

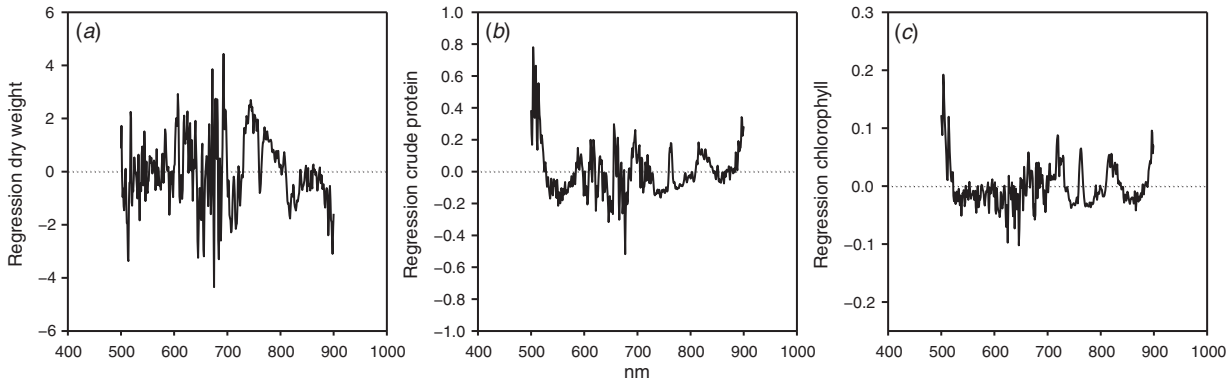

Fig. 4. Regression coefficients of the fitted partial least-squares regression models for forage dry weight (*a*), crude protein percentage (*b*) and chlorophyll concentration (*c*). The regression coefficients represent the contribution of each spectral waveband to the overall prediction of each destructively measured trait.

were found at 701 and 674 nm, corresponding to red light near the inflection band and red light, respectively (Fig. 4*a*). Strong positive contribution to forage DW estimation was made with NIR (700–750), and a strong negative contribution with red light (674–640). In the PLSR models for CP and chlorophyll, regression-coefficient plots showed a strong positive contribution for trait estimation in the visible green light (Fig. 4*b*, *c*). The PLSR models for CP contrasted wavebands in the visible spectrum, with positive contribution from wavebands ~503 nm and negative contributions from 678 nm. Similarly, regression coefficients for total chlorophyll indicated a strong positive contribution in the visible spectrum at ~504 nm and negative contribution throughout the visible wavebands, especially at 625 and 643 nm (Fig. 4*c*). This is logical considering that visible light absorption is increased with additional leaf chlorophyll.

Discussion

The results from this study demonstrate that the current visual-assessment methodology at initial steps of the breeding cycle in the CIAT *Urochloa* breeding program can be improved by using non-destructive HTP techniques. Colour imaging, hyperspectral analysis and PLSR models are more precise and faster than visual evaluations, thus increasing the number of plants evaluated in the tropical forage-breeding program.

Visual evaluations of plant growth and greenness (characteristics associated with N content, and therefore CP and chlorophyll concentration in leaves) have traditionally been used to discard *Urochloa* hybrids at initial stages of plant phenotyping. The visual evaluation of an entire breeding population (i.e. 7000 hybrids) is a slow, costly and tedious process, and is often biased by subjectivity and human fatigue, especially when phenotypic variation of such traits is high (Table 3). In this study, the estimation of forage DW, and CP and chlorophyll concentrations was more precisely and consistently estimated by HTP techniques. Forage DW and CP predictions were more accurate when digital image analysis was used, followed by spectral analysis and visual evaluations. Chlorophyll was better estimated by the analysis of 401 spectral wavebands, followed by colour image analysis and finally visual evaluations. The time required to run non-destructive HTP evaluations was considerably shorter for colour image analysis than visual evaluations, by 28 min per 200 plants, but longer in hyperspectral than in visual evaluations, by 12 min per 200 plants (Table 1).

The moderate trends in the relationship between *Urochloa* canopy imaging and reflectance and measured DW, CP and chlorophyll may indicate that the method is not appropriate for very precise estimations of these traits. However, for breeding purposes where a large percentage of hybrids are discarded without detailed evaluation owing to scarce resources, a difference of 7.90 g plant^{-1} in the forage DW or 1.63% in the CP concentration of plants may be acceptable during initial stages of plant breeding. The moderate trend between *Urochloa* canopy analysis and measured traits in this study can be explained by dissimilarities in the canopy architecture of the *Urochloa* genotypes (Numata *et al*. 2008), as well as different growth patterns during recovery from cutting. Further evaluation of breeding populations with contrasting canopy architectures will improve the accuracy of the PLSR model to predict the targeted traits. Nonetheless, by combining both digital image and hyperspectral analysis techniques, higher precision accuracy for forage DW, CP and chlorophyll contents can be achieved.

The vegetation indices (see Table 2) extracted from colour images of 200 *Urochloa* hybrids were originally developed to separate green plants from the background by extracting green and red colours from digital images. These indices have been related to different plant characteristics including biomass, chlorophyll content and nutritional status (Tucker 1979; Woebbecke *et al*. 1995; Camargo 2004; Hunt *et al*. 2005, 2013; Meyer and Camargo 2008; Lee and Lee 2013; Wang *et al*. 2013). In the present study, digital image analysis performed better than hyperspectral scanning analysis for estimating forage DW and CP (Figs 2, 3). Nonetheless, the use of spectral analysis over grasses becomes more important when this technique is used to detect either nutritional or anti-nutritional compounds (i.e. metabolisable energy, digestibility, fibre) that are better estimated with the near-infrared regions of the electromagnetic spectra (Curran 1989; Pullanagari *et al*.

2012; Ferner *et al*. 2015). In this sense, digital colour image analysis and hyperspectral analysis are complementary because, by using both techniques, a diverse set of plant traits can be accurately predicted, and by adding extra factors to the prediction model, higher prediction accuracy can be achieved (cf. Numata *et al*. 2008). Future efforts will use data-mining to fine-tune the spectral bands included in the PLSR model (Thorp *et al*. 2017), which can reduce model error and improve model-fit statistics. Although testing multiple methods of analysis was not the intention of this study, future research could test other techniques (e.g. artificial neural networks) for relating HTP measurements to plant traits.

The regression coefficients for the PLSR for forage DW and chlorophyll concentration obtained in this study highlight that the key wavelengths for the prediction of these traits occur in the green, red, red-edge and NIR regions of the electromagnetic spectrum (Fig. 4). Previous hyperspectral studies have highlighted those regions as being highly representative for dry mass and chlorophyll content in plants (Lichtenthaler *et al*. 1996; Thenkabail *et al*. 2000; Mutanga and Skidmore 2004; Fava *et al*. 2009; Thorp *et al*. 2011; Adjorlolo *et al*. 2015; Dou *et al*. 2018). Although some similarities were found for some individual wavebands among the different traits, the general regression coefficients differed among the traits, thus demonstrating that the reflectance data in a given waveband contributed differently towards the estimation of a given trait. Given the logistical burden to collect and analyse hyperspectral scans, the identification of informative key bands associated with each evaluated trait can improve the HTP process (Thorp *et al*. 2017). Results from this study will help guide the selection of optimal bands in the construction of multispectral sensors tailored to predict specific traits of interest in tropical forage breeding programs.

The PLSR models for predicting forage DW, CP and chlorophyll can be now used to evaluate the next generation of hybrids from the same *Urochloa* gene pool (i.e. *U. ruziziensis–U. brizantha–U. decumbens*). The accuracy of these prediction models relies on collection protocols similar to those explained in the *Materials and methods* and evaluations on plants with growth characteristics comparable to the hybrids evaluated here (i.e. about 3 months after regrowth). The prediction accuracy will likely be reduced on larger plants with higher biomass (Hill 2004) and a greater proportion of senescent leaves (Asner 1998). The development of more precise PLSR models to predict variables of interest in a breeding program requires ongoing effort. The collection of ground data every year, in addition to making improvements to standardise collection protocols and incorporate wider range of genotypes, will result in more accurate and robust models. Larger datasets will increase estimation precision.

Conclusions

In this study, 200 *Urochloa* hybrids were monitored in 40 min by digital imaging and 80 min by spectral analysis (Table 1). At this pace, >1000 *Urochloa* hybrids could be evaluated in <7 h. This means that forage biomass and quality in a high number of genotypes would be reliably evaluated with minimal increased acquisition costs relative to destructive harvest. This demonstrates the superiority of HTP techniques over conventional visual evaluation of traits. The PLSR models for predicting CP, forage DW, and chlorophyll contents developed in this study support the evaluation of higher numbers of genotypes at initial stages of the breeding program. The greater the numbers of plants evaluated reliably every year in the *Urochloa* breeding program, the greater the genetic gain will be. Therefore, the use of image analysis and hyperspectral monitoring over *Urochloa* hybrid canopies will benefit the ongoing breeding program. The application of this HTP method could be of great help in rural remote areas lacking facilities to perform destructive harvest and plant chemical analysis. Research is under way to improve the utility of proximal sensing by considering a greater range of canopy architectural configurations and evaluating the potential to assess nutritional quality, including characteristics such as metabolisable energy, fibre, digestibility, lignin and cellulose fractions in *Urochloa* grasses.

Conflicts of interest

The authors have no conflicts of interest to declare.

Acknowledgements

We thank Dr John W. Miles for helpful suggestions and comments on an early version of this manuscript. We thank Miller Escobar, Alfer Valencia and Carlos Gomez for assistance during harvest process. JCJ is grateful to the USDA Foreign Agricultural Service, Norman Borlaug Fellowship Program for a training fellowship. JCJ thanks the CIAT's Young Scientist Award 2016 Program for travel assistance. This work was partially undertaken as part of the CGIAR Research Program on Livestock. We thank all donors that globally support our work through their contributions to the CGIAR system.

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Handling Editor: Christian Huyghe