DISCRIMINATION OF SUGARCANE VARIETIES WITH PIGMENT PROFILES AND HIGH RESOLUTION, HYPERSPECTRAL LEAF REFLECTANCE DATA

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ABSTRACT

This study reports our evaluation of high resolution, hyperspectral leaf reflectance and pigment measurements as a potential tool to aid in the identification and delineation of commercial sugarcane varieties (interspecific hybrids of *Saccharum* spp.), noble canes (*Saccharum officinarum* L.) and wild canes (*Saccharum spontaneum* L.). Seven varieties of sugarcane were selected from the USDA-ARS, Sugarcane Research Laboratory (SRL) breeding program for reflectance analysis, including: five commercial cultivars, one noble cane, and one wild cane. Hyperspectral reflectance data (350 - 800 nm) at 0.4 nm intervals were collected from the third youngest fully open leaf from nine replicates using a dual input fiber optic spectroradiometer under natural light conditions from ≈1200 - 1600 h. Reflectance was measured ≈10 cm from the leaf tip. After reflectance measurements were completed, a 0.5 cm disc was bored from the same leaf for plant pigments analysis. The discs were extracted with 100% acetone and analyzed by HPLC. Reflectance data were averaged into 5 and 20 nm intervals and then, with plant pigment data, were subjected to analysis of variance and multivariate mean separation techniques. Differences in reflectance were observed for each variety, with the seven cultivars having approximately threefold difference in reflectance values. Several single, wavelengths ranging from 560 - 720 nm could be used to discriminate between selected varieties, with varieties being significantly different from each other in 76% of the cases. The degree of discrimination could be increased to 86% using vegetative indices. Multivariate analysis resulted in a 95 - 100% correct classification for all varieties with leaf reflectance data and from 76 - 81% correct classification with plant pigment data.

INTRODUCTION

Sugarcane is cultivated in ninety countries world-wide with a total production of 1.3 billion Mt on 20 million hectares in 2006 (Food and Agriculture Organization of the United Nations 2008). The U.S. is currently ranked 10th worldwide in total sugarcane production and 13th in total land in production with 27 million Mt produced on 363,450 hectares (Food and Agriculture Organization of the United Nations 2008). Sugarcane is cultivated as a perennial crop in tropical regions with an average crop cultivated for 12 - 24 mo. After each harvest, a subsequent ratoon crop emerges from the remaining underground buds to begin another production year. Typically three to four ratoon crops are produced from each planting, after which the crop is re-planted. In Louisiana, sugarcane is cultivated over a growing season ranging from 8 - 10 mo with the original plant-cane crop being harvested in December and the subsequent ratoon crops in either October or November.

Although traditionally cultivated solely for sugar production new technologies may further expand the use of sugarcane for the production of ethanol, bio-based products, and bio-energy (Beeharry 2001). These potential new uses have led to an expansion of an already active sugarcane selective breeding programs to find varieties that are better suited for these non-sugar uses as well as continuing efforts to increase sucrose and tonnage levels. Selective breeding efforts by the USDA-ARS in cooperation with the Louisiana Agriculture Experiment Station (LAES) and the American Sugarcane League (ASCL) of the U.S.A have led to an increase in sucrose content of Louisiana varieties from an initial level of 9% to its current level of 15% (Breaux 1984). Other benefits of the selective breeding efforts include increased disease and pest resistance, cold tolerance and improved harvestability. In a typical sugarcane seedling selection cycle 150,000 - 200,000 plants are evaluated through the combined efforts of the USDA-ARS, LAES and the ASCL. Many of these seedlings are the progeny of crosses made between commercial (current or historical) cultivars, noble canes and wild canes. Although many of these crosses are successfully carried out, some of the progeny are the result of "selfing" or self-fertilization. It is important to be able to discriminate these selfs from true hybrids to be certain the true genome of the parents is expressed in the given progeny. The selfed progeny will only contain the genome of one parent and thus offer no genetic improvement or hybrid vigor. In addition, it is particularly important to detect selfs of wild canes as the *S. spontaneum* are classified as noxious weeds and can not be planted in the field. Current methods for identification of specific cultivars are limited to genomic analyses and visual discrimination. Although only small samples are typically required for genomic analyses, the analytical equipment needed and expertise are considerable and genomic assessment of intron variability can lead to further complications in the discrimination process. Visual discrimination is possible with trained staff, but results between personnel and locations are often inconsistent due to plants of different age and localized effects of light, temperature, and moisture.

Several researchers have investigated the use of remote sensing as a potential method of identifying sugarcane varieties. Schmidt et al. (2000) investigated the use of a digital multi-spectral video camera to identify sugarcane varieties and to determine various crop conditions, including moisture stress, crop age, nutritional status, ripener response, and yield potential. The sensor system employed utilized four wavebands, 450, 550, 650, and 750 nm. The authors indicated that crop moisture stress and crop age could be detected and that it was possible to identify cane fields where ripener had been applied. The authors also reported that it was possible to discriminate between sugarcane varieties, although a significant degree of within variety variability was noted. Gers (2003) attempted to utilize Landsat 7 Enhanced Thematic Mapper Plus (ETM+) imagery to determine sugarcane phenology and to discriminate between different varieties. The author was successful in grouping data into four growth classes; pre-emergence, tillering, tiller senescence, and tiller stabilization/crop maturation. It was not possible, however to discriminate between varieties. Galvão at al. (2005) utilized EO-1 Hyperion data and multiple discriminant analysis in an attempt to discriminate between commercial sugarcane varieties in Brazil. The authors were able to discriminate one variety, SP80-1842, using a single near-infrared band threshold, however, the remaining four varieties possessed similar reflectance values thereby complicating the analysis. The authors utilized multiple discriminate analyses to separate these varieties and achieved an overall classification accuracy of 87.5%.

The use of hyperspectral remote sensing techniques, with high spectral resolutions, in combination with plant pigment analysis may significantly improve the ability to discriminate between sugarcane varieties compared with the aforementioned methods. A non-destructive method to assess the carotenoid content in leaves has been described (Gitelson et al. 2002). With this work, the carotenoid pool is estimated by measuring leaf reflectance from 400 - 800 nm using a spectral resolution of 2 nm. The method uses the 510 nm wavelength as the reflectance best correlated with carotenoid content and less correlated with chlorophylls over a wide range of pigment concentrations. Carotenoids serve several important functions in plants including energy transfer to photosystem complexes as well as dissipation of energy through resonance thereby preventing formation of oxygen radicals (Demmig-Adams et al. 1996). Although all carotenoids fluoresce within the 430 - 490 nm range, knowledge of individual pigment pools, including those associated with the violaxanthin cycle, would refine our understanding of stress responses to light and disease and may also serve to discriminate between species, cultivars, and commercial varieties of sugarcane. The objective of this study was to determine if plant pigment pools as estimated by direct measurement and from high resolution, hyperspectral leaf reflectance measurements could be used to discriminate between commercial sugarcane cultivars, noble canes, and wild canes.

MATERIALS AND METHODS

Sugarcane breeding material was maintained in greenhouses at the USDA-ARS, Sugarcane Research Laboratory (SRL) in Houma, LA. Individual culms or eye-pieces were planted in soil-less media in October of 2003 and grown using natural photoperiod and light. Plants were fertilized with a complete controlled-release fertilizer (Osmocote™, Scotts-Sierra Horticultural Products Co., Marysville, OH, USA) and were hand watered to maintain optimum growth conditions. Seven varieties of sugarcane were selected from the SRL breeding program for analysis, including: five commercial varieties (interspecific hybrids of *Saccharum* spp.), one noble cane, (*Saccharum officinarum* L.) and one wild cane (*Saccharum spontaneum* L.). Individual characteristics of each variety are included in Table 1 along with varietal references.

Table 1. Production characteristics of seven sugarcane varieties

Variety	TRS (kg/Mg)	Cane (Mg/ha)	Sugar (kg/ha)	Varietal Reference
LCP85-384	138[a]	64.3	8870	Milligan et al. (1994)
HoCP96-540	136	61.2	8350	Tew et al. (2004a)
L97-128	144	62.3	8943	Gravois and Bischoff (2007)
TUCCP77-042	124	73.0	9052	Mariotti et al. (1991)
Ho95-988	133[b]	67.2	8911	Tew et al. (2004b)
MPTH97-216	73[c]	-	-	Sugimoto et al. (2002)
LA-Purple	-	-	-	Artschwager and Brandes (1958)

[a] First-ratoon yields from 2003 outfield trials (Orgeron et al. 2003).

[b] First-ratoon yield from 2001 outfield trials (Orgeron et al. 2001).

[c] TRS from USDA-ARS, Sugarcane Research Laboratory selection trials.

Hyperspectral reflectance data were collected using a dual input Ocean Optics SD-2000 fiber optic spectroradiometer (Dunedin, FL, USA). This device has a 2048 channel detector and a theoretical wavelength range of 200 - 1100 nm at 0.4 nm intervals. In this experiment the wavelength range utilized was 350 - 850 nm. The system consisted of a master channel connected to a 25° field-of-view optical fiber that was used to collect upwelling radiation from the leaf and a slave channel connected to an optical fiber and cosine diffuser (which yields a hemispherical field-of-view) to collect downwelling incident solar radiation. The spectroradiometer was controlled using software from the Center for Advanced Land Management Information Technologies (CALMIT) at the University of Nebraska. Reflectance readings were collected using the CALMIT Data Acquisition Program (CDAP) from all varieties on 11 March 2004. Nine replicate measures were made on individually potted sugarcane plants from the third youngest fully open leaf under natural light conditions from ≈1200 - 1600 h. Reflectance data were collected ≈3 cm above each leaf and 10 cm from the leaf tip. Each reflectance measure was the mean of eight scans. A white Spectralon reference target (Labsphere, Inc., North Sutton, NH) was used to calibrate the spectroradiometer.

After reflectance measurements were completed the same leaf was sampled for plant pigment analysis by boring a 0.5-cm disc; the disc did not contain mid-rib tissue. Leaf discs were immediately frozen (-80°C) until analyses. Samples were ground using a mortar and pestle in 2-ml of 100% acetone, and extracted in the dark at 4°C for 4 h. Extracts were then filtered and analyzed for chlorophylls and carotenoids using high performance liquid chromatography (Zimba et al. 2001). Filtered extracts from each leaf were directly injected in an Agilent Technologies model HP1100 equipped with one Hewlett-Packard ODS-Hypersil C_{18} column (200 x 4.6 mm, 4µm, Palo Alto, CA, USA) and two Vydac 201TP C_{18} columns (250 x 4.6 mm, 5 µm; Grace, Deerfield, IL, USA) in series. Samples were analyzed by diode array detection at 436 nm. Pigments were identified and quantified using authentic commercial standards (VKI, Hørsholm, Denmark).

Reflectance data were first combined into 5 and 20 nm wavelength intervals to simplify analysis. The 5 nm data were used to evaluate the discrimination potential of individual wavelengths and also three vegetation indices. The first index evaluated was the simple ratio index (SR) of Huemmrich and Goward (1992). This index was calculated as the ratio of the near-infrared band (ρ_{NIR}) over the red band (ρ_{red}), where ρ_{NIR} and ρ_{red} were defined as the average reflectance from 730 - 805nm and 580 - 680 nm, respectively. The second index evaluated was the normalized difference vegetation index (NDVI) of Rouse et al. (1973) where NDVI = $(\rho_{NIR}-\rho_{red})/(\rho_{NIR}+\rho_{red})$. Two different estimates of the red band and three of the near-infrared band were utilized with ρ_{NIR} equal to the average reflectance from 730 - 805 nm and then at the individual wavelengths of 725 and 810 nm. The red band ρ_{red} was estimated as the average reflectance from 580 - 680 nm and at the single wavelength of 630 nm. Finally, the wide dynamic range vegetation index (WDRVI) of Gitelson (2004) was utilized where WDRVI = $(a^* \rho_{NIR}-\rho_{red})/(a^* \rho_{NIR}+\rho_{red})$ with ρ_{NIR} and ρ_{red} estimated as the reflectance at 810 and 630 nm, respectively and with the weighting coefficient (a) varying from 0.05 - 0.2. The index is similar to the NDVI but includes the weighting coefficient to linearize the relation between the index and the vegetation fraction. Multivariate procedures including canonical discrimination and discriminant analysis with cross-validation (SAS 2004) were used on the 20 nm data to investigate the relation between variety and reflectance. Pigment content was analyzed by oneway analysis of variance and mean separation by the least significant difference procedure (Tukey LSD procedure) and by canonical discrimination analysis and discriminant analysis with cross-validation.

RESULTS

Reflectance Data

Distinct differences for each variety were evident, with the seven varieties having ≈threefold difference in reflectance values (Figure 1). Changes in the slope of reflectance were evident in the 370 - 420, 510 - 550 and 580 - 620 nm ranges (Figure 1). Clear differences were observed between varieties in reflectance data with the varieties falling roughly into three to four groups (Table 2). Several single, wavelengths ranging from 560 - 720 nm could be used to discriminate between selected varieties, with varieties being significantly different from each other in 76% of the cases (Table 2). Cultivars LCP 85-384 and TUCCP 77-042 were significantly different from the remaining varieties in all cases and were significantly different from each other at 560 and 600 nm. L 97-128 was significantly different from all varieties at 560 nm and L 97-128 and HOCP 96-540 were different from the remaining varieties (but not each other) at five wavelengths ranging from 620 - 700 nm. The wild cane MPTH 97-216 could be discriminated from all varieties except HO 95-988 at 720 nm. The remaining varieties could not be individually discriminated utilizing single wavelengths.

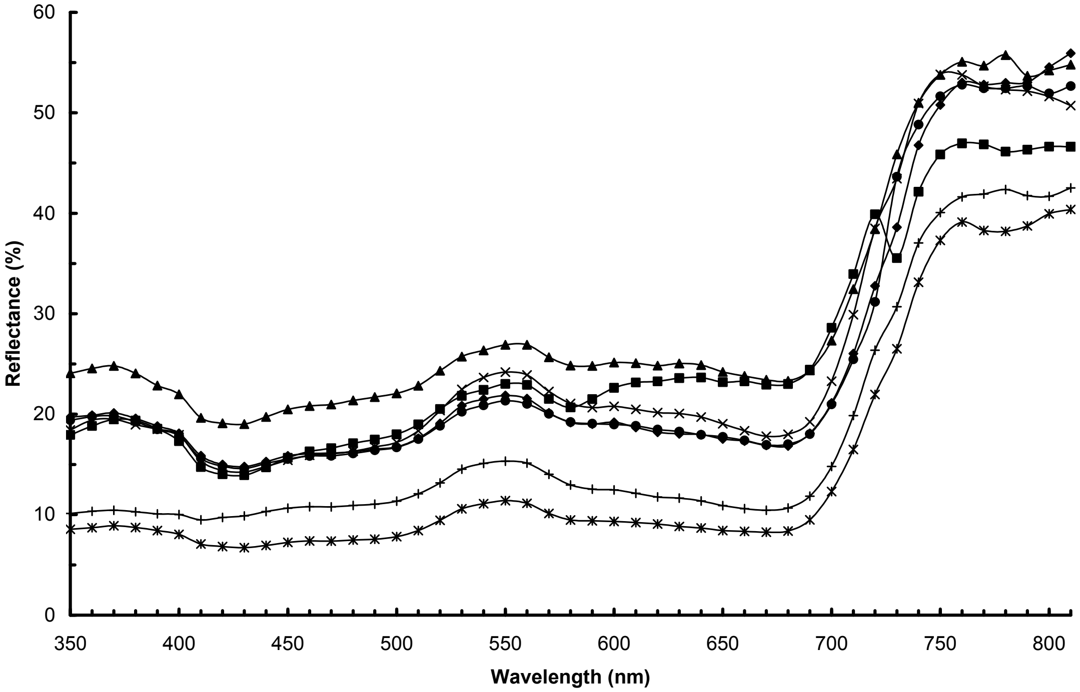

Figure 1. Sugarcane leaf reflectance data for LCP 85-384 (✳), HOCP 96-540 (■), L97-128 (▲), MPTH 97-216 (●), HO95-988 (♦), TUCCP 77-042 (+), LA Purple (X) from March 11, 2004 sampling date.

Table 2. Percentage reflectance and vegetation index means for seven sugarcane varieties

Wavelength or Index[a]	LCP 85-384	TUCCP 77-042	MPTH 97-216	Ho 95-988	LA Purple	L 97-128	HoCP 96-540	Discr. %[b]
560 nm	11.1e	15.1d	21.0c	21.5bc	23.9b	26.9a	22.9bc	76
600 nm	9.3e	12.5d	19.0c	19.2c	20.8bc	25.1a	22.6ab	76
620 nm	9.1c	11.8c	18.5b	18.2b	20.2b	24.8a	23.3a	76
640 nm	8.7c	11.4c	18.0b	18.0b	19.7b	24.9a	23.6a	76
660 nm	8.3c	10.6c	17.4b	17.3b	18.4b	23.8a	23.3a	76
680 nm	8.4c	10.7c	17.0b	16.8b	18.0b	23.3a	23.0a	76
700 nm	12.3c	14.8c	21.0b	21.1b	23.3b	27.3a	28.6a	76
720 nm	22.0c	26.4c	31.2b	32.8b	38.5a	38.4a	39.9a	76
SR	4.2a	3.4b	2.9c	2.8c	2.7c	2.2d	2.0d	81
NDVI	0.61a	0.54b	0.47c	0.47c	0.45c	0.37d	0.32d	81
WDVI	-0.05a	-0.17b	-0.27cd	-0.25c	-0.32d	-0.39e	-0.43e	86

Means within a row with the same letter are not significantly different (Tukey LSD, P = 0.05, n = 9).

[a] SR = Simple Ratio, NDVI = Normalized Difference Vegetative Index, WDRVI = Wide Dynamic Range Vegetative Index.

[b] Discrimination (%) between varieties.

In an attempt to improve the overall varietal classification success rate the SR, NDVI and WDRVI vegetation indices were evaluated. The overall success rate was improved to 81% with the SR and NDVI indices and to 86% with the WDRVI index (Table 2). Using the SR and NDVI indices the varieties could be grouped into four classes with LCP 85-384 and TUCCP 77-042 in their own respective groups, MPTH97-216, Ho 95-988 and LA Purple in a third group and L 97-128 and HoCP 96-540 in the final group. A similar result was obtained with the WDRVI index, with the exception that HO 95-988 and LA Purple could be discriminated from each other (Table 2). One final observation is that LCP 85-384 had significantly higher values for all of the vegetation indices as compared to the other varieties (Table 2). This variety was grown on up to 88% of the acreage in Louisiana and was known as a superior tonnage and sugar producer (Legendre and Gravois 2003). The higher NDVI values for this variety could be related to greater biomass accumulation.

The final techniques employed to discriminate between varieties were multivariate methods, including canonical discriminant analysis (PROC CANDISC) and discriminant analysis (PROC DISCRIM). In canonical discriminant analysis, linear combinations of either the reflectance or pigment measurements were derived that described the between-class (i.e. variety) variation (SAS 2004). These combinations (canonical variables) were also independent, or orthogonal, indicating that their individual contribution to the discrimination process did not overlap (Fernandez 2002). In the discriminate analysis, linear discriminate functions derived from the reflectance or pigment data were generated to classify the individual observations into the known groups (varieties). The number of correctly classified observations was determined by both re-substitution and cross-validation in an attempt to minimize sample bias (SAS 2004).

In the cross-validation procedure each observation in the data set is sequentially removed and the discriminant function re-determined. This newly determined function is used to classify the observation left out of the analysis. In the re-substitution procedure all observations are used to determine the discriminant function and to classify each observation.

Canonical discriminant analysis of the reflectance spectra showed that the varieties could be successfully separated with canonical axis 1 accounting for 49% of the separation and canonical axis 2 adding ≈20% more to the model (Figure 2). The clouds of canonical coefficients for each variety did not overlap and were well separated, with wavelengths 370 - 430, 470 - 510, and 590 - 630 nm accounting for most of the separation. Discriminant analyses of the reflectance spectra showed that all varieties were correctly classified in 100% of the cases examined when re-substitution techniques were utilized. When the more stringent, cross-validation procedure was utilized the varieties were correctly classified in 95% of the cases examined.

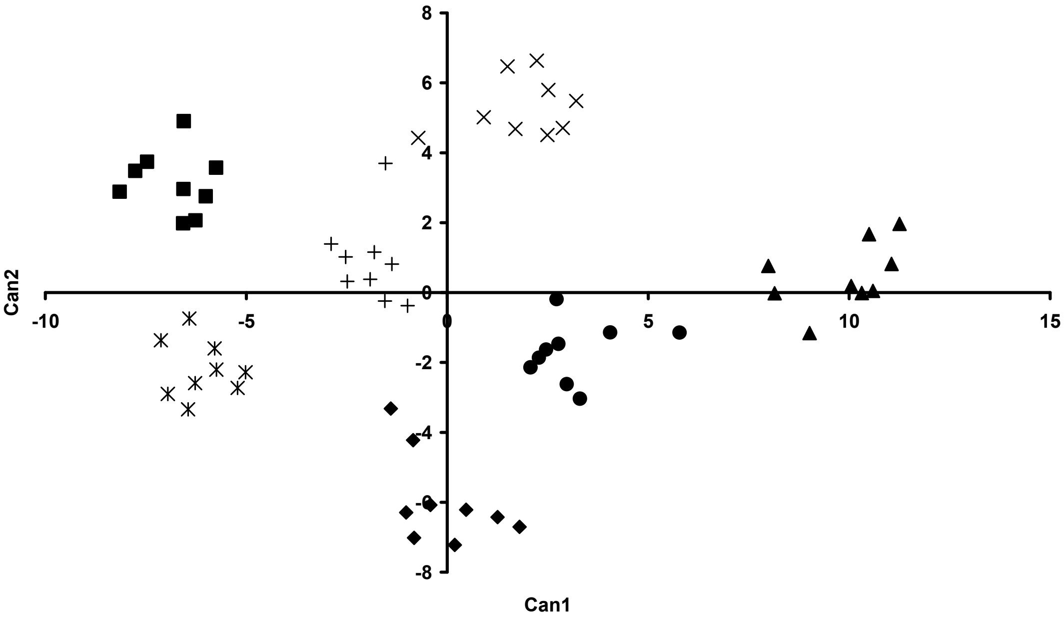

Figure 2. Canonical coefficients for pigment samples collected from LCP 85-384 (✳), HOCP 96-540 (■), L97-128 (▲), MPTH 97-216 (●), HO95-988 (◆), TUCCP 77-042 (+), LA Purple (x) on 11 March 2004 sampling date.

Pigment Data

As expected all varieties had typical green plant pigments including violaxanthin cycle intermediates (neoxanthin, violaxanthin, antheraxanthin) as well as lutein, β-carotene, and chlorophylls *a* and *b* (Table 3). It is also clear after examination of the plant pigment data that one perhaps unexpected result of the Louisiana recurrent selection program is an increase in overall pigment content in recently released commercial varieties. Ho 95-988 and L 97-128 had significantly higher total chlorophyll and total carotenoids as compared to all other varieties (Table 3). The wild cane MPTH 97-216 had a significantly lower total carotenoid content as compared to all other varieties. In general, the recently released commercial varieties (Ho 95-988, L 97-128, HoCP 96-540) had the highest pigment levels, followed by older commercial varieties (LCP 85-384, TUCCP 77-042), the noble cane (LA Purple) and the wild cane (MPTH 97-216) (Table 3). Only Ho 95-988 was clearly separated from all other varieties using canonical discrimination analysis (Figure 3). There was overlap in the clouds of canonical coefficients. When pigment canonical means were evaluated the separation was more distinct (data not shown). Canonical axis 1 accounted for over 54% of the separation between varieties and addition of axis 2 increased the resolution by 24% (Figure 3). Lutein concentration was the most important variable included in canonical axis 1 and violaxanthin concentration was the most important variable in canonical axis 2. Discriminant analysis of the pigment data resulted in an overall classification accuracy of 84% with re-substitution and 76% with cross-validation.

Table 3. Mean concentrations (ng mm^{-2}) of plant pigments extracted from seven sugarcane varieties from March 11, 2004 sample date

	Plant pigments[a]								
Variety	Neo	Viola	Anth	Lutein	Chl B	Chl A	Bcar	Chl Tot	Car Tot
Ho 95-988	2.72a	4.02a	1.13ab	9.71a	35.0a	184.0a	15.32a	219.0a	32.9a
L 97-128	2.44ab	2.89b	1.23a	8.42ab	30.2ab	145.6b	11.31b	175.7b	26.3b
HoCP 96-540	2.18bc	2.70bc	0.81c	7.80bc	25.4bc	130.7b	11.38b	156.1c	24.9bc
TUCCP 77-042	1.93cd	3.08b	0.78c	6.77cd	23.3cd	112.6cd	9.58bc	135.8cd	22.1c
LCP 85-384	1.91cd	3.06b	0.84bc	6.87cd	23.0cd	117.6cd	9.98b	140.6cde	22.7c
LA Purple	1.92cd	2.17cd	0.82c	7.61bcd	24.2cd	123.1bcd	10.16b	147.2de	22.7c
MPTH 97-216	1.63d	1.96d	0.65c	6.23d	20.4d	101.7d	7.74c	122.1e	18.2d

Means within a column with the same letter are not significantly different (Tukey LSD, *P* = 0.05, n = 9).

[a] Neo = neoxanthin, Viola = violaxanthin, Anth = antheraxanthin, Chl B = chlorophyll b, Chl A = chlorophyll a, Bcar = β-carotene, Chl Tot = total chlorophyll (a + b) and total carotenoids in leaf disks.

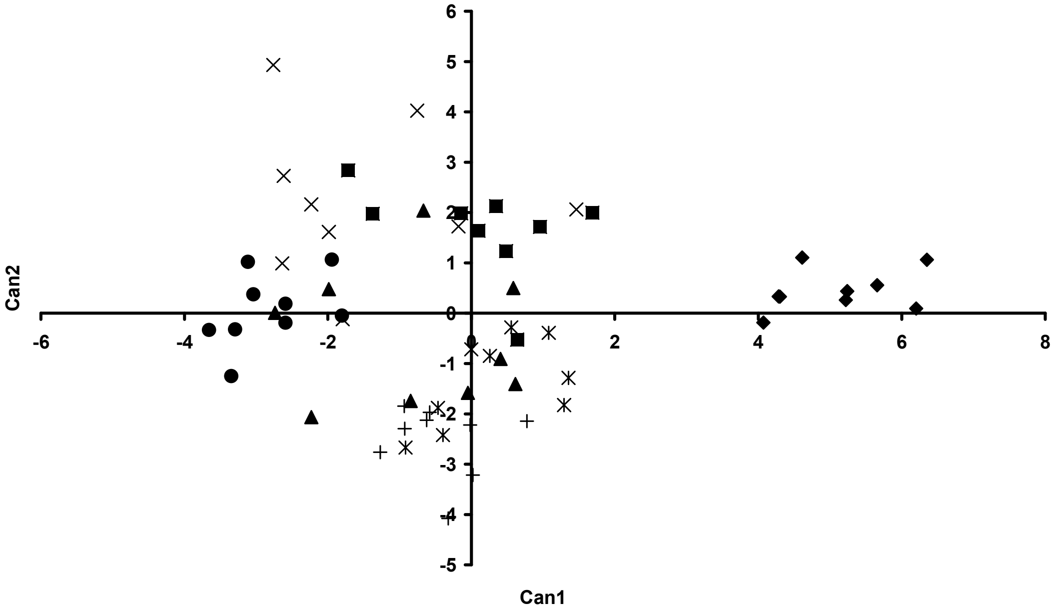

Figure 3. Canonical coefficients for pigment samples collected from LCP 85-384 (*), HOCP 96-540 (■), L97-128 (▲), MPTH 97-216 (●), HO95-988 (◆), TUCCP 77-042 (+), LA Purple (x) on 11 March 2004 sampling date.

DISCUSSION

Remote sensing equipment has become increasingly user-friendly and reasonably priced. Analytical methods for assessing carotenoid and plant pigments have also greatly improved in the past ten years; the notable improvement is in qualitative and quantitative analysis of pigments rather than qualitative identification only (Kirk 1994; Ritchie and Zimba 2005).

The ability to discriminate between cultivars of newly propagated crosses while plants are still in the seedling stage would provide a method for screening successful hybridization efforts. Methodology to do so rapidly is valuable in reducing costs associated with maintaining large F1 pools while genetic analyses occur. Non-destructive techniques to assess varieties may provide an opportunity to compare these results with more detailed genomic or production yield studies in real time, or target specific crosses demonstrating desired traits. As reflectance is inversely related to pigment composition (in the visible region of the spectrum), the coupling of these techniques should yield inverse canonical discriminant analyses maps of group centroids for the varieties. All varieties had inverse relationships between reflectance and pigment concentration (Figures 2 and 3). This is particularly evident if canonical means are evaluated (data not shown). Classification matrices by discriminant analyses yielded 95 - 100% correct classification for the reflectance data and 76 - 81% for the pigment data alone. This is a particularly encouraging observation for the *S. spontaneum* species classifications. Using discriminant analysis of either reflectance or pigment data the *S. spontaneum* MPTH 97-216 was correctly classified in 100% of the cases examined. There were two false positive MPTH 97-216 classifications (L 97-128 and LA Purple) using reflectance or pigments. If these results were used to screen for *S. spontaneum* species in a sugarcane crossing scenario, a "selfed" *S. spontaneum* would have been identified and not introduced to the field where it is classified as a noxious weed.

The varietal differences in pigment content could not always be reconciled with reflectance data and may result from differing specular reflectance, species specific differences in packaging effects of pigments, or differing fluorescence quantum yield of the chlorophyll molecules in different cultivars (Kirk 1994; Lillesand and Kiefer 1994; Falkowski and Raven 1997). Chlorophyll specific absorption coefficients are known to vary by fourfold (Bricaaud et al. 1995) and as these parameters are not typically measured, it is important to use several robust methods for assessing strain differences.

Carotenoid pools provided a clear reflectance and pigment basis for identification of specific cultivars. Specifically the lutein and violaxanthin pool size was responsible for much of separation of varieties. Ultra-violet absorbance spectra for violaxanthin have very high absorbance at 450 - 470 nm relative to other carotenoids (Jeffrey et al. 1997), and this may account for these observed differences. As previously stated, reflectance results in these regions were heavily weighted in discriminant functions. In this study plants were raised under uniform conditions in a greenhouse setting and therefore light, heat, nutrient deficiency, and water stress would not differ between replicates of each variety. Application of these methods to the field would require knowledge of specific growth conditions to identify these fields for optimal classification results. Our preliminary field work and previous mathematical estimates of the importance of this carotenoid pool (Gitelson et al. 2002) may offer a promising method for assessing plant health.

CONCLUSIONS

In this study we attempted to determine if plant pigment pools, as estimated by direct measurement and from high resolution, hyperspectral leaf reflectance measurements, could be used to discriminate between commercial sugarcane cultivars, noble cane, and wild canes. The combined leaf reflectance and pigment analysis clearly demonstrated that this was possible. Clear differences in reflectance were observed for each variety, with the seven cultivars having ≈threefold difference in reflectance values. Changes in the slope of reflectance were evident in the 370 - 420, 510 - 550 and 580 - 620 nm ranges. Several single, wavelengths ranging from 560 - 720 nm could be used to discriminate between selected varieties, with varieties being significantly different from each other in 76% of the cases. The degree of discrimination could be increased to 86% using vegetative indices. Multivariate analysis of leaf reflectance data resulted in a 95 - 100% correct classification for all varieties with reflectance data and a 76 - 81% correct classification with pigments. In all cases the *S. spontaneum* species MPTH 97-216 was classified correctly and could be discriminated from commercial and noble canes. Future research will include a significant expansion of the varietal reflectance survey to determine if the observed trends remain in a larger population. Preliminary data from on-going experiments indicate that these techniques can be used to effectively discriminate varieties under field conditions. Experiments will also be conducted to determine if high resolution, hyperspectral leaf reflectance measurements can be used as an indicator of sugarcane stress.

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