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Variability of Leaf Phosphorus among Sugarcane Genotypes Grown on Everglades Histosols

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Abstract

The P content of drainage water of the Everglades Agricultural Area (EAA) of Florida must be reduced by at least 25% from a baseline mean calculated using 1978 through 1988 data. This minimum P reduction is one of several measures to sustain much of the unique habitat of remaining natural regions of the Everglades. The objectives of this study were to evaluate variability in leaf tissue P concentration among elite sugarcane (interspecific hybrids of *Saccharum* spp.) clones and to recommend sampling strategies to detect differences among clones. Leaf samples were collected four times per annual crop in the plant-cane and first-ratoon crops from three fields, representing low, medium, and high available soil P. Leaf P of sugarcane should be tested at several locations in at least two crop years, and at least once, but preferably twice, per crop. The clone with the most leaf P had 0.65 g P kg^{-1} leaf tissue more than the clone with the least. This difference among leaves allowed us to speculate that P removal may differ by about 8.5 kg ha^{-1} among commercial cultivars and that genetic improvement of this trait could be feasible. If further studies show that differences in leaf P concentration can reliably predict differences in total P removal from a sugarcane field, then classification of cultivars for leaf P concentration could make available to EAA sugarcane farmers another best management practice (BMP) to reduce P content of their drainage waters.

THE EAA is a 280 000 ha agricultural basin of Histosols (organic soils) in southern Florida. About 144 000 ha of sugarcane (interspecific hybrids of *Saccharum* spp.) are grown in the EAA (Glaz, 1995). To protect the natural Everglades, legislation mandated that the P content of water discharged from the EAA must be reduced by at least 25% from the baseline mean calculated using 1978 through 1988 data (Whalen and Whalen, 1994). In addition, about 16 000 ha in the EAA will be converted from agriculture to specially designed artificial wetlands to serve as Storm Water Treatment Areas (STAs) (Stone and Legg, 1992). The EAA water will flow into these STAs, and by the Year 2002, must have P concentration reduced to no more than 50 μg L^{-1} before it is released from the STAs (Walker, 1996). Walker (1996) further explained that ongoing and future research may require even lower P concentrations because 50 μg L^{-1} is more than five times greater than natural marsh background levels in the Everglades. The legislation mandating these P reductions recognizes that hydrologic changes must also occur to achieve meaningful restoration goals.

Farmers in the EAA are using a comprehensive BMP program to meet P-reduction requirements (Stone and Legg, 1992; Whalen and Whalen, 1994). Stone and Legg (1992) estimated that BMP implementation cost farmers about \$153 ha^{-1} and annual operation and maintenance costs were about \$9 ha^{-1}. A potential low-cost BMP would be productive sugarcane cultivars that remove more P from a given soil or require less P fertilizer. Coale et al. (1994) explained that the management of P in drainage water of the EAA is largely the management of P mineralized by oxidation of the organic soils rather than the management of seasonal P fertilizers. Therefore, controlling oxidation of organic soils should be the central focus of BMP programs for the EAA. However, the use of cultivars that require less P fertilizer or remove more soil P could become an important component of a long-term BMP program aimed at minimizing P content of drainage water.

Differences in nutrient concentration or accumulation among genotypes have been reported for several crops. Fageria and Baligar (1993) reviewed the screening of genotypes for macronutrient and micronutrient deficiencies. Some examples where differences among genotypes were found for P concentration or accumulation are Baligar et al. (1990) for alfalfa (*Medicago sativa* L.), Fageria et al. (1988) for rice (*Oryza sativa* L.), Raboy and Dickinson (1993) for soybean [*Glycine max* (L.) Merr.], and Gourley et al. (1993) for white clover (*Trifolium repens* L). Gourley et al. (1993) mentioned several plant mechanisms that help explain differential P uptake.

Deren et al. (1993) reported Si variability among 52 sugarcane clones from a late stage of a Florida selection program, but did not find differences among elite, commercial-type clones in the final selection phase of the program. Andreis (1975) reported differences in Florida sugarcane cultivars of up to 7.5 kg P ha^{-1} removed from a field by a sugarcane crop that yielded 90 Mg ha^{-1}. Similarly, in a study aimed at finding cultivars that yield well under low P fertility in India, Sundara (1994) reported differences among clones for both P concentration and accumulation. Under high fertility, the range

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Abbreviations: BMP, best management practice; CP, Canal Point; EAA, Everglades Agricultural Area; P, phosphorus; P_a, acid-extractable (0.5 *M* acetic acid) P; P_w, water-extractable P; STAs, Storm Water Treatment Areas.

of P concentration for 20 clones was 0.69 to 0.95 g kg^{-1} and the accumulation range was 16.2 to 37.0 kg ha^{-1}.

The objectives of this study were to evaluate variability in leaf tissue P concentration among 11 elite sugarcane clones and one cultivar and to recommend sampling strategies to detect differences among clones. This preliminary study was used to determine the feasibility of pursuing a long-term effort aimed at selecting for and genetically modifying P accumulation as another feature of a genetics program whose central focus is to develop commercial sugarcane cultivars for Florida.

Materials and Methods

All sugarcane clones in this study had been selected as superior in the Canal Point (CP) selection program, based on sugar content, cane yield, disease resistance, and growth habit. The parents of nine clones were commercially grown CP cultivars or clones in advanced stages of testing. One clone was a widely grown commercial cultivar, CP 70-1133, and the parents of two clones, CP 89-2376 and CP 89-2377, were not known. Thus, 10 of the 12 clones were known to be closely related, and the two with unknown parents may also have been closely related to the others.

All clones were planted in randomized complete-block designs with four replications at three locations in the fall of 1992. Plots were four rows, 10.7 m long with 1.5 m between rows. The experiment was for two crop years, plant cane and first ratoon. Leaf samples were collected from the first leaf under the top visible dewlap. Van Dillewijn (1952) described the dewlap in sugarcane as the junction of the leaf blade and leaf sheath. Each plot sample contained 10 leaves, including midribs, one from each of 10 randomly selected plants. The four sampling dates in 1993 for the plant-cane crop were 19 to 20 May, 22 to 24 June, 21 to 22 July, and 24 to 25 August. The four sampling dates in 1994 for the first-ratoon crop were 27 to 28 June, 20 to 21 July, 9 and 18 August, and 26 and 30 September. Leaves were dried at 60°C and ground in a stainless steel mill to pass a 1-mm screen. Ground tissue (0.3 g per sample) was wet-acid digested (Lowther, 1980) and P concentration was determined by inductively coupled plasma spectroscopy in the University of Florida/IFAS Analytical Services Laboratory, Gainesville (Hanlon et al., 1994).

All three experiments were planted on Histosols representative of the EAA, classified as euic, hyperthermic Typic Medisaprists. The experiments at Knight Farm (Location 1) and Okeelanta Corp. (Location 2) were on Lauderhill mucks and the experiment at South Florida Industries (Location 3) was on a Pahokee muck. As described by McCollum et al. (1976), the only difference between Lauderhill and Pahokee mucks is depth of soil over limestone rock. Lauderhill muck is more shallow than Pahokee muck. Both soils are comprised primarily of decomposed sawgrass (*Cladium jamaicense* Crantz). Fields at all three locations were fertilized according to soil-test recommendations for sugarcane (Sanchez, 1990). At Location 1, 45 kg ha^{-1} P_2O_5 were applied after the first harvest, at Location 2, 67 kg ha^{-1} P_2O_5 were applied at planting and 45 kg ha^{-1} P_2O_5 were applied after the first harvest, and at Location 3 no P_2O_5 fertilizer was applied.

Soil samples were taken 5 May 1993, several months after fertilizer was applied before sampling began in the plant-cane crop. Soil samples were not taken in the ratoon crop. Soil samples were analyzed for water-extractable P (P_w) (Sanchez, 1990), acid-extractable (0.5 *M* acetic acid) P (P_a), and pH (Sanchez, 1990) at the University of Florida/IFAS Everglades Research and Education Center Soil Testing Laboratory, Belle Glade. The P_w is a measure of labile P and the P_a values are a measure of total P.

Table 1. Water-extractable P (P_w), acid-extractable P (P_a), and pH of soil samples in 1993 at three locations before sugarcane leaf sampling began.

Location	P_w	P_a	pH
	kg ha^{-1}		
1	7.7	111	6.7
2	4.5	97	7.4
3	12.2	18	5.2

In location × crop analyses of variance, clones were main plots and sampling dates were subplots. To find specific clones that contributed most to clone × sampling date or clone × crop interactions, stability-variance parameters were calculated (Shukla, 1972). Significant differences of the Shukla variances among individual clones were calculated according to Kang and Miller (1984). Unless otherwise stated, significant differences for statistical analyses were at $P = 0.05$.

Results and Discussion

The pH values for the three soils ranged from 5.2 to 7.4 (Table 1). The P_w values indicated that for fields cropped continually with sugarcane, the three locations fit the classifications of low (Location 2), moderate (Location 1), and high (Location 3) available soil P (Sanchez, 1990). The P_a values differed widely among locations. The mean leaf P concentrations of most location × crop combinations (Table 2) related reasonably well with the soil analyses. One differing result was the low P concentration of the ratoon leaves at Location 3, the location with the highest available soil P. A probable explanation for this low value is the low P_a measured at Location 3. Perhaps by the ratoon crop, much of the available P measured as P_w during the plant-cane crop had diminished and not been replaced due to the low P_a.

Substantial variability for leaf P concentration existed among the 12 elite sugarcane clones (Table 2). The mean leaf P of CP 89-1717 was greater than the leaf P of all other clones, and the mean leaf P of CP 89-2377 was less than all other clones. Several other levels of significant differences occurred among these 12 clones. The wide range of variability for leaf P in these elite clones indicates that wide variability may also exist among current commercial sugarcane cultivars because they are most likely genetically similar to the clones in this experiment. A long-term goal is to use choice of sugarcane cultivar as a BMP to reduce P content of drainage water while maintaining optimal production. Genetic enhancement of sugarcane's ability to absorb soil P would support that long-term goal. The range of variability for leaf P measured in this study indicates that attempts at such genetic enhancement could be successful.

Clones and sampling dates accounted for most of the variation in each location × crop analysis of variance (Table 3). In all six analyses of variance, both clone and sampling date were highly significant. The clone × sampling date interaction was significant only in the first-ratoon crop at Location 2. Consistently significant clone × sampling date interactions would mean that relative differences among clones for leaf P would depend on sampling date and sampling at several dates would be necessary. Even though this interaction was significant in only one of six analyses, we analyzed it thoroughly due to its importance in devising a sampling

Table 2. Mean leaf P concentration at four sample dates for 12 sugarcane clones sampled at 3 locations in the plant-cane (PC) and first-ratoon (R1) crops.

Clone	Location 1		Location 2		Location 3		Mean	Stability†
	PC	R1	PC	R1	PC	R1		
	g P kg^{-1} plant tissue							
CP 89-1717	2.21	1.87	2.15	1.88	2.52	1.55	2.03	0.06**
CP 89-1632	2.17	1.86	1.95	1.74	2.22	1.52	1.91	0.04**
CP 89-1331	1.98	1.80	2.05	1.79	2.14	1.48	1.87	0.02*
CP 89-1643	1.77	1.75	1.88	1.70	2.09	1.43	1.77	0.02*
CP 89-2143	1.90	1.57	1.99	1.48	2.04	1.36	1.72	0.05**
CP 89-1945	1.82	1.60	1.81	1.52	2.02	1.43	1.70	0.00
CP 89-1268	1.69	1.58	1.71	1.47	2.02	1.36	1.64	0.01
CP 89-1756	1.75	1.52	1.71	1.49	1.95	1.35	1.63	0.00
CP 89-1325	1.71	1.51	1.65	1.43	1.89	1.35	1.59	0.01
CP 70-1133	1.58	1.46	1.70	1.50	1.92	1.31	1.58	0.01
CP 89-2376	1.63	1.39	1.58	1.35	1.87	1.32	1.52	0.01
CP 89-2377	1.32	1.28	1.44	1.27	1.77	1.21	1.38	0.05**
Mean	1.79	1.60	1.80	1.55	2.04	1.39	1.70	0.02
LSD(0.05)	0.17	0.17	0.18	0.13	0.12	0.17	0.05	

*,** Significant at the 0.05 and 0.01 probability levels, respectively.
† Shukla stability-variance parameter.
‡ LSD (0.05) for location means = 0.05 g P kg^{-1}.

strategy. The Shukla stability-variance parameter was used to find each clone's contribution to the significant interaction across the four sampling dates for the first-ratoon crop at Location 2 (data not shown). Only 4 of the 12 clones were identified as unstable by the Shukla values. Of these four, two had high P concentrations at three out of four dates and higher than average P concentrations at the other date. Two clones had low P concentrations at three dates and P concentrations slightly higher than the mean at the other date. Thus, the significant clone × sampling date interaction in the ratoon crop at Location 2 was due mostly to mild changes in degree rather than changes in rank. We concluded that this single interaction alone did not justify recommending the testing of P leaf concentration at more than one sampling date per crop.

The CP sugarcane cultivar selection program has limited plant material and resources available throughout its selection phases. Therefore, it would be difficult to increase replication for the purpose of testing P concentration. One reason for using more than one sampling date would be to assure sufficient samples to detect real differences among clones. Significance of F values for clones was determined for each of the 24 location × crop × sampling date analyses (data not shown). Twenty of the 24 analyses had a highly significant F value for clones. The four that were not significant had $P > F$ of 0.17, 0.14, 0.45, and 0.81. Two of the nonsignificant F values were determined for sampling date 4, and one each at sampling dates 1 and 3. Using at least two sampling dates per annual crop, rather than increasing replication, would be a reasonable means for enhancing the detection of significant differences among clones. Even though the previous discussion regarding a single clone × sampling date interaction did not justify use of more than one sampling date, logistical concerns that limit the number of replications led us to recommend testing P concentration of leaves at least twice per annual crop to assure good probability of detecting real differences among clones.

In analyses of variance for the combined plant-cane and first-ratoon crops at each location, the interaction of clone × crop had $P > F$ of 0.02 at Location 1, 0.06 at Location 2, and <0.01 at Location 3. These interactions meant that differences among clones for leaf P concentration may have depended on which annual crop was sampled. Based on these consistently significant interactions, we recommend testing P concentrations of sugarcane leaves for at least two crop years, for example in the plant-cane crop and one ratoon crop. Growers in Florida usually harvest a plant-cane and one to three

Table 3. Analyses of variance, by location and crop (plant cane and first ratoon) for P content measured as g P kg^{-1} leaf of 12 sugarcane clones sampled at four dates per location per crop.

Location	Crop†	Source of variation	df	Sum of squares	F
				(g kg^{-1})^2	
1	PC	Rep	3	0.04	0.3
1	PC	Clone (C)	11	10.80	26.3**
1	PC	Error a	33	1.23	
1	PC	Date (D)	3	6.79	49.3**
1	PC	C × D	33	1.93	1.3
1	PC	Error	108	4.95	
1	R1	Rep	3	0.08	0.7
1	R1	Clone	11	6.31	14.3**
1	R1	Error a	33	1.32	
1	R1	Date	3	2.66	19.4**
1	R1	C × D	33	0.86	0.6
1	R1	Error	108	4.91	
2	PC	Rep	3	0.10	0.7
2	PC	Clone	11	7.67	15.7**
2	PC	Error a	33	1.47	
2	PC	Date	3	1.87	16.5**
2	PC	C × D	33	1.64	1.3
2	PC	Error	108	4.07	
2	R1	Rep	3	0.27	3.5*
2	R1	Clone	11	6.01	21.5**
2	R1	Error a	33	0.84	
2	R1	Date	3	1.91	24.6**
2	R1	C × D	33	1.49	1.7*
2	R1	Error	108	2.79	
3	PC	Rep	3	1.45	21.5**
3	PC	Clone	11	6.76	27.4**
3	PC	Error a	33	0.74	
3	PC	Date	3	27.52	289.1**
3	PC	C × D	33	1.42	1.4
3	PC	Error	108	3.43	
3	R1	Rep	3	0.03	0.2
3	R1	Clone	11	1.61	3.4**
3	R1	Error a	33	1.42	
3	R1	Date	3	4.33	53.5**
3	R1	C × D	33	1.11	1.2
3	R1	Error	108	2.92	

*,** Significant at the 0.05 and 0.01 probability levels, respectively.
† PC = Plant-cane crop and R1 = First-ratoon crop.

more annual-ratoon crops before replanting a sugarcane field.

The clone × location interaction was significant in the analyses of variance performed for all dates and clones at each crop ($P > F = 0.01$ for plant cane and 0.04 for first ratoon). Thus, in addition to including crops, results of tests for leaf P concentration among clones should include several locations. The Shukla stability-variance parameter is a useful tool for showing which clones do not have consistent leaf P concentrations across crops or locations. Table 2 shows the leaf P concentrations of the 12 clones tested in this study with their corresponding Shukla stability-variance parameters calculated using the three locations × two crops as six environments.

The Shukla variances indicate that 6 of the 12 clones caused the significant clone by location or crop interactions. In the cases of the two extremes, the significant Shukla variances denoted differences in degree rather than rank. CP 89-1717 had high leaf P at all six location × crop combinations and CP 89-2377 had low leaf P concentrations. However, CP 89-1717 had more leaf P in plant cane at Location 3, whereas CP 89-2377 had less leaf P in plant cane at Location 1. CP 89-1632 was similar to CP 89-1717 in three of the location × crop analyses. However, CP 89-1632 was particularly different at Location 2 where its leaf P concentration was less than that of CP 89-1717 in both crops. CP 89-2143 was also strongly affected by location and crop. In plant cane at Location 2, no other clone had significantly more leaf P than CP 89-2143. At least one other clone had significantly more leaf P than CP 89-2143 in the five other location × crop combinations. Also, leaf P concentration of CP 89-2143 was greater than or equal to the mean in all plant-cane analyses and less than the mean in all ratoon analyses.

CP 70-1133, a popular commercial cultivar for several years (Glaz, 1995), had stable and low leaf P. This is an indication that CP 70-1133 might not have been a good choice for reducing P content of drainage water. However, further studies elucidating the role of leaf P concentration to predict total P removal by sugarcane is needed to support this conclusion. If further research shows that high leaf P concentration can be used to find clones that remove more P from the soil, then CP 89-1717, CP 89-1632, and CP 89-1331 would be the clones from this study of most interest to geneticists.

To speculate on the benefits of this research, an estimate is needed that relates leaf P to whole plant P. The average P concentration of leaf tissue in this study was 1.70 g P kg^{-1} and the range was 0.65 g P kg^{-1} (Table 2). Coale et al. (1993) reported 1.40 g P kg^{-1} for the total aerial dry matter of an EAA sugarcane crop. The whole crop P concentration reported by Coale et al. (1993) was 82% that of the leaf P concentration we measured. Reducing our range proportionally would yield a range of 0.53 g P kg^{-1} plant tissue. Using the 0.53 g P kg^{-1} range of 0.53 g P kg^{-1} and assuming an equal dry matter yield of 25.37 Mg ha^{-1} (actual yields for CP 89-1717 and 25.33 Mg ha^{-1} for CP 89-2377) would have accumulated 13.4 kg P ha^{-1} yr^{-1} more than the clone with the least P concentration (CP 89-2377). Coale et al. (1993) also calculated that 63% of the P accumulated by a sugarcane crop was removed from the field as millable sugarcane. Therefore, there would have been a difference between CP 89-1717 and CP 89-2377 of 8.5 kg P ha^{-1} removed from the field.

This study indicated that further research needs to be conducted to determine the effectiveness of choice of sugarcane cultivar as a BMP to reduce P in EAA drainage water. We showed that differences in leaf P concentration among clones can be reasonably determined on Florida Histosols with low, medium, and high P availability. The success at determining differences and the degree of variability among clones for leaf P concentration indicated that genetic programs to select for variable levels of leaf P concentration among sugarcane clones could be successful. Some studies, as reviewed by Jarrell and Beverly (1981), caution against our approach due to a dilution in concentration caused by increases in dry matter. The next logical step is to determine if leaf P concentration in sugarcane can reliably predict differences among sugarcane cultivars for total P removal.

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