

Characterization of *Saccharum* Species Germplasm for Starch Content

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Abstract

The renewed interest in wild *Saccharum* species germplasm across sugarcane breeding programs has been necessitated by the need to widen the genetic base of breeding populations. Modern sugarcane cultivars were derived from inter-specific hybridization between *S. officinarum* and *S. spontaneum*. Very few genotypes were used in the initial hybridization event in 1900s resulting in narrow genetic diversity in modern sugarcane cultivars. Characterization of genotypes in the *Saccharum* collections would aid its utilization. Starch is considered as an impurity in sugarcane juice because it adversely affects the quantity and quality of sugar products, refining processes and is negatively associated with sucrose content. Therefore knowledge about the starch content of *S. spontaneum* and other related species would be of interest to breeders. The objective of this study was to characterize the United States of America (USA) sugarcane wild germplasm and related species for starch content. The juice samples used in this study were collected at the United State Department of Agriculture, Agricultural Research Services (USDA-ARS) Sugarcane Research Station at Houma, Louisiana; Canal Point and Miami, Florida germplasm collections. There were highly significant ($P < 0.001$) differences in starch content among species and genotypes within species. There was a non-discrete distribution for starch within the species providing an opportunity for identifying low starch genotypes for use in germplasm enhancement programs. *S. spontaneum* genotypes produced the greatest variability for starch providing an opportunity for identifying low starch genotypes for use as parents. Cultivated species such as *S. officinarum* and *S. robustum* produced low starch indicating that low starch offered advantages for sucrose production. The results of this study would be more useful for parent selection within germplasm for introgression breeding.

Keywords: sugarcane, starch, introgression, *S. spontaneum*, *S. officinarum*, *S. robustum*

1. Introduction

In recent years, there has been renewed interest in wild *Saccharum* species germplasm across sugarcane breeding programs. The wild germplasm is being utilized to widen the genetic base of breeding populations and to tap into genes that exist in the wild species. Modern sugarcane cultivars were derived from inter-specific hybridization between two major *Saccharum* species, namely *S. officinarum* and *S. spontaneum*, in the early 1900s (Price, 1963). During initial sugarcane interspecific hybridization, *S. officinarum* was the primary source of genes for sucrose accumulation, whereas *S. spontaneum* contributed genes for high biomass, general adaptability and ratooning ability but also brought unfavorable attributes related to sugar quality (Roach, 1986). Very few *Saccharum* species clones have been used in the sugarcane breeding (Berding & Roach, 1987), thereby resulting in narrow genetic diversity in modern sugarcane cultivars. Recently, there has been renewed effort to widen the genetic base using more wild *Saccharum* species in the germplasm introgression programs. The use of exotic germplasm for the improvement of sugarcane is an excellent example of the contributions that wild relatives of plants have made towards the genetic improvement of economically important crop species (Martin, 1996).

Efforts to broaden the genetic base while introducing novel genes to cultivated sugarcane varieties have continued to place priority on *S. spontaneum*. In Louisiana, resistance to mosaic virus was successfully transferred to BC₄ progenies in cultivar x *S. spontaneum* crosses culminating in the release of LCP85-384 (Milligan et al., 1994). Despite this success, only a limited number of new *S. spontaneum* clones are represented

in the genetic background of Louisiana cultivars. For example, all current commercially recommended varieties in Louisiana, LCP85-384, HoCP85-845, L97-128, and HoCP96-540 share the same single *S. spontaneum* clone, US56-15-8, in their pedigree. Although the issue of genetic diversity is being addressed, incentives that could encourage more diverse use of the *S. spontaneum* germplasm available in the collection are warranted. Characterization of clones in the collection for starch content could serve this purpose.

Sucrose yield is one of the most important traits in a commercial sugarcane breeding programs. Sucrose yield is both a function of sucrose accumulation in the plant and the ability to extract the sucrose during milling. Juice quality can affect the amount of the extracted sucrose during milling and processing. Starch is considered an impurity in sugarcane juice because it adversely affects the quantity and quality of sugar products and refining processes (Eggleston et al., 2006). In Louisiana, starch content is currently not considered as a parameter when deciding which *S. spontaneum* clones to use for germplasm enhancement, whereas F_1 (commercial x *S. spontaneum*) progenies are severely penalized for low sucrose yield. Starch content in the *S. spontaneum* parent may inadvertently influence sucrose yield in the F_1 and subsequent generations, and may be responsible for the slow progress in improving sucrose content during germplasm enhancement. Knowledge about the starch content of *S. spontaneum* might be of interest to breeders seeking to use this germplasm for variety improvement in their breeding programme. *S. spontaneum* clones with low starch content, when used as parents, may minimize the unfavourable juice quality among the progenies.

The two landmark events in the genetic development of sugarcane were the discovery of sexual reproduction in the late 19th century and the beneficial effects of interspecific hybridization (Berding & Roach, 1987). The benefits of interspecific hybridization renewed sugarcane breeders' interest in the use of diverse germplasm and ended the error of collecting *S. officinarum* clones as the exclusive germplasm for commercial sugarcane production. This was further enhanced by the discovery of artificial induction of flowering in sugarcane in subtropical areas (Brett, 1948; Brett, 1951; Brett, 1954; Brett & Harding, 1974; Brett et al., 1975; Moore & Nuss, 1987). However, germplasm introgression remained limited to a few *S. spontaneum* clones (Arceneaux, 1967; Mangelsdorf, 1983; Price, 1967; Roach, 1972).

An understanding of the traits among germplasm and their interrelationships is important to their exploitation (Stalker, 1980). One reason for the limited use of wild germplasm was the lack of characterization of the clones in the sugarcane germplasm collections around the world (Tai & Miller, 2002). Germplasm that has been well characterized will receive more attention in maintenance than uncharacterized germplasm (Berding and Roach, 1987) and is likely to be used in germplasm enhancements (Allison, 1984; Zhou et al., 2007). Berding and Roach (1987) concluded that germplasm characterization was an important bridge linking collection and utilization, yet these authors acknowledged that characterization has been largely ignored. Without characterization, interspecific hybridization is difficult to justify. Introgression of new germplasm is important to improve the commercial breeding populations. Also, introgression with wild germplasm is constrained by many difficulties and requires a lot of effort. Clear objectives for introgression will be enhanced by the availability of characterized germplasm. However, germplasm characterization would help identify the need for further germplasm collection to fill in the gaps and would also help reduce identification errors of clones in the collection. Characterization will allow breeders to determine the trait variability within and among species and thus guide them to the best sources of desired traits. Guidelines to characterize agricultural traits (Daniels, 1972), disease resistance (Hutchinson & Daniels, 1972) and botany (Skinner, 1972) have been documented. However, there is little evidence of characterization at present (Berding & Roach, 1987).

The *S. spontaneum* germplasm has been utilized to increase stalk numbers, yield (Roach, 1986), cold tolerance (Brandes & Martz, 1939; Duncelman & Breaux, 1969) and mosaic resistance (Abbott & Todd, 1963; Duncelman & Breaux, 1970). However, one of the disadvantages of *S. spontaneum* is low sucrose and high starch content. Starch and sucrose are storage carbohydrates in sugarcane (Artschwager & Brandes, 1958). Starch (α -1 $\rightarrow$ 4-glucan), is a sugarcane juice impurity that adversely affects both factory and refinery processes and the quantity and quality of sugar products (Eggleston et al., 2006). Unfortunately, starch in sugarcane delivered for milling and processing has risen markedly in recent times because of the increased harvesting of green (unburnt) sugarcane as well as new varieties from introgression efforts (Godshall et al., 2000). Current methods for removing starch in the mills are expensive (Eggleston et al., 2003). Breeding low starch clones provides a cheaper method for reducing starch delivered to the mills. By using low starch parents, low starch progenies could be made available for selection for other agronomic and quality traits and this approach would likely lead to low starch genotypes without directly selecting for starch in the breeding program.

The important species of the *Saccharum* complex are *S. officinarum*, *S. Barberi*, *S. sinense*, *S. spontaneum*, *S. robustum* and *S. edule*. Other germplasm of immediate interest to sugarcane breeders include *Erianthus*,

Ripidium, *Sclerostachya*, *Narenga* and *Miscanthus* (Daniels et al., 1975; Daniels & Roach, 1987; Mukherjee, 1957). The objective of this study was to characterize the United States of America (USA) sugarcane wild germplasm and related species for starch content using collections maintained at Canal Point and Miami in Florida, and Houma in Louisiana.

2. Materials and Methods

The juice samples used in this study were collected at the United State Department of Agriculture, Agricultural Research Services (USDA-ARS) Sugarcane Research at Houma, Louisiana; Canal Point and Miami, Florida sugarcane germplasm collections (Table 1). At USDA-ARS Houma, the samples were collected in October 2005 from 51 *S. spontaneum* clones growing in cans and one *S. officinarum* control. Another set of samples were collected from USDA-Houma from *Saccharum* and related species (*S. barberi*, *S. bengalense*, *S. officinarum*, *S. robustum*, *S. sinense*, *S. spontaneum*, *Erianthus*, and *Miscanthus*) in October 2005. In December 2007, juice was collected from *Saccharum* species (*S. officinarum*, *S. robustum*, *S. spontaneum*) and 6 hybrid varieties maintained at USDA-ARS, Houma, Louisiana, USA.

In Florida, all the samples were collected in December, 2007 from the Canal Point and Miami collections. Juice samples were collected from four *Saccharum* species (*S. barberi*, *S. officinarum*, *S. sinense*, *S. spontaneum*) and 14 hybrid varieties growing at the Canal Point nursery. Another set of samples were collected from 20 *S. spontaneum* clones growing in cans at Canal Point. From the Miami collection, samples were taken from five species (*S. barberi*, *S. edule*, *S. officinarum*, *S. robustum*, and *S. sinense*).

Table 1. The species and dates of juice sampling at Houma in Louisiana, Canal Point and Miami in Florida, USA

Population	Species (number of clones)	Date of Sampling
Houma - Germplasm 1	<i>S. spontaneum</i> (51), <i>S. officinarum</i> (1)	October 2005
Houma - Germplasm 2	<i>S. barberi</i> (13), <i>S. bengalense</i> (1), <i>S. officinarum</i> (9), <i>S. robustum</i> (11), <i>S. sinense</i> (8), <i>S. spontaneum</i> (5), <i>Erianthus</i> (1), <i>Miscanthus</i> (1) <i>S. barberi</i> (7), <i>S. officinarum</i> (1),	October 2005
Canal Point - Nursery	<i>S. sinense</i> (36), <i>S. spontaneum</i> (4), <i>Saccharum</i> Hybrid (14)	December 2007
Canal Point - Cans	<i>S. spontaneum</i> (20) <i>S. barberi</i> (3), <i>S. edule</i> (1),	December 2007
Miami	<i>S. officinarum</i> (15), <i>S. robustum</i> (9), <i>S. sinense</i> (6)	December 2007
Houma - Racks	<i>S. officinarum</i> (5), <i>S. robustum</i> (4),	December 2007
Replicated	<i>S. spontaneum</i> (51), <i>Saccharum</i> Hybrid (6)	

2.1 Sampling Methods, Juice Extraction and Starch Analysis

At crop maturity, five stalks were randomly cut from each plot, leaves were removed and the stalks were bundled and labeled. The samples were shredded and juice was extracted in the laboratory. The brix and other quality variables were immediately measured in the laboratory. Twelve ml of juice was pipetted into 15 ml tube and heated on a dry block heater for 10 minutes at 90 °C. The heating was done to denature the natural amylase enzyme in the juice and stop further starch degradation. The juice was immediately cooled and stored in a -80 °C freezer until starch analysis. Starch in juice was analyzed using the Sugar Processing Research Institute (SPRI) method (Godshall et al., 2004) used in the factory as modified by Eggleston et al. (2006). Each juice sample was divided into three sub-samples during analysis and each sub-sample was analyzed separately.

To determine starch, three ml of juice was transferred to three test tubes (labeled A, B, and C) and covered with aluminum foil. The test tube was placed in a boiling water bath for 10 minutes to gelatinize starch. After boiling, the juice was allowed to cool on ice. To each of the sample test tubes, the following chemicals were pipetted in that order: 1.2 ml 2N acetic acid, 0.25 ml 10 % KI, and 2.5 ml KIO₃. A blank tube was prepared where the 3 ml

juice was replaced by distilled water. The contents of the test-tubes were mixed by inverting three times. The test tubes were centrifuged to settle the solid material at the base of the test tubes. The absorbance of the samples was measured at 600 nm on the Shimadzu spectrophotometer. The final absorbance was calculated as the sample absorbance minus blank absorbance. The µg starch was determined directly from the calibration curve.

2.2 Data Analysis

The data was analyzed using SAS Institute (2008) statistical procedures. Graphs were generated using Microsoft Excel. The data from germplasm 1, germplasm 2 at Houma and Canal Point nursery (Table 1) was analyzed using the statistical linear mixed model:

$$Y_{ijk} = S_i + V(S)_{j(i)} + \text{Sample}(V(S))_{k(j(i))}, \quad \text{Equation 1}$$

where Y_{ijk} is the starch content of the k th sub-sample from the j th variety within the i th species, S_i is the fixed effect from the i th species, $V(S)_{j(i)}$ is the fixed effect from the j th variety nested within the i th species, $\text{Sample}(V(S))_{k(j(i))}$ is the random effect of the k th sub-sample nested within the j th variety which is in turn nested within the i th species and was the residual error and was also the experimental error for the species and variety within species effect.

The samples collected from the *S. spontaneum* clones growing in cans at Canal Point were analyzed using the statistical linear mixed model:

$$Y_{ij} = V_i + \text{Sample}(V)_{j(i)}, \quad \text{Equation 2}$$

Where Y_{ij} is the starch content of the j th sub-sample of the i th variety, V_i is the effect of the i th variety, $\text{sample}(V)_{j(i)}$ is the random effect of the j th sub-sample nested within the i th variety and was the residual error as well as the experimental error of the variety fixed effect.

The samples collected from the replicated species growing in pots at USDA Houma were analyzed using the statistical linear mixed model:

$$Y_{ijk} = R_i + S_j + RS_{ij} + V(S)_{k(j)} + RV(S)_{ik(j)} + \text{Sample}(RV(S))_{m(ik(j))}, \quad \text{Equation 3}$$

where Y_{ijk} is the starch content measured from the m th sub-sample nested of the k th variety, j th species and i th replication, R_i is the random effect of the i th replication, S_j is the fixed effect of the j th species, RS_{ij} is the random effect of the interaction of the i th replication by j th species and was the experimental error for the species effects, $V(S)_{k(j)}$ is the fixed effect of the k th variety nested within the j th species, $RV(S)_{ik(j)}$ is the random effect of the interaction of the i th replication by the k th variety nested within the j th species and was the experimental error for the effect of the k th variety nested within the j th species, $\text{sample}(RV(S))_{m(ik(j))}$ is the random effect of the m th sub-sample nested within the interaction of the i th replication by k th variety which is in turn was nested within the j th species and was the residual error.

3. Results

3.1 *Saccharum Spontaneum* at Houma

Fifty-one *S. spontaneum* clones were analyzed for starch with one *S. officinarum* clone as a control (Table 2). The *S. spontaneum* clones produced significantly ($P < 0.001$) more starch (75%) than *S. officinarum*. There were highly significant differences ($P < 0.0001$) for starch content among *S. spontaneum* clones. The frequency distribution of starch content among the *S. spontaneum* clones produced a non-discrete distribution (Figure 1). The clones could also be classified into low, medium and high starch content (Figure 1, Table 3). The low starch clones produced 41 to 106% starch content of the control, medium 121 to 195%, and high 205 to 364%. From a total of 51 clones, 10 (20%) were low starch content, 26 (51%) medium starch content and 15 (29%) were high starch content. There were eight *S. spontaneum* clones that produced less starch content than *S. officinarum* control.

Table 2. The mean, standard deviation (Std Dev) and % of control for starch content (ppm/^oBrix) for the *S. spontaneum* and *S. officinarum* control sampled at USDA, ARS Sugarcane Research Station at Houma (Zhou et al., 2008)

Species	Number of clones	Mean	Std Dev	% of <i>S. officinarum</i>
<i>S. officinarum</i>	1	2144.14	86.20	
<i>S. spontaneum</i>	51	3755.45	1504.24	175 ($P < 0.0001$)

Table 3. The mean, standard deviation (Std Dev) and mean % of control for starch content (ppm/°Brix) for 51 *S. spontaneum* and *S. officinarum* control

Clone	Mean	Std Dev	% of control
LA-Stripe (control)	2144.14	86.20	100
COIMBATORE	3187.20	53.77	149
DJATIROTO	4460.69	143.42	208
GUANGXI86-5	2088.26	110.77	97
GUANGXI87-21	3232.88	131.33	151
GUANGXI87-22	2602.08	90.16	121
IMP9068	3528.80	65.26	165
IMP9089	1836.17	139.54	86
IND81-080(9775)	1911.95	94.35	89
IND81-142(9819)	3759.09	83.45	175
IND81-144(9821)	3391.91	55.97	158
IND81-161(9834)	4560.30	126.78	213
IND81-165	3367.99	104.87	157
IND82-257A	4406.18	18.20	205
IND82-311	7126.05	0.00	332
MPTH97-107	5347.42	391.94	249
MPTH97-200	3597.98	391.08	168
MPTH97-204	7472.47	95.99	349
MPTH97-209	6629.05	991.95	309
MPTH97-213	2093.38	91.64	98
MPTH97-216	5593.66	157.19	261
MPTH97-218	1653.97	69.35	77
MPTH97-233	3973.14	205.91	185
MPTH97-3	869.37	114.88	41
MPTH98-388	3414.08	931.68	159
PCA-NOR84-2A	5272.83	193.86	246
PCAV84-12A	4835.69	290.45	226
PCAV84-12B	2646.90	162.90	123
PCAV84-12C	3376.99	13.96	157
PIN84-1B	3289.35	123.79	153
PQ84-3	3713.47	86.49	173
S66-084A	3945.03	242.05	184
S66-084B	2242.97	132.53	105
S66-121A	4590.63	9.54	214
SES-234B	2273.11	404.55	106
SES-323A	7805.25	460.99	364
SES006	3062.55	57.94	143
SES084-58	3543.43	183.92	165
SES114	2674.71	110.17	125

SES147B	1222.60	112.96	57
SES189	3485.82	162.48	163
SES205A	3210.77	186.58	150
SES231	4171.09	59.46	195
SES234A	4003.07	163.71	187
SES234B	4757.40	285.61	222
SH249	5667.48	72.86	264
SPONT#17	3433.36	115.86	160
SPONT#24	1988.89	505.11	93
SPONT#37	4077.61	209.91	190
TAINAN	3800.54	10.65	177
US56-13-7	4921.89	177.13	230
US56-15-8	2928.04	685.42	137

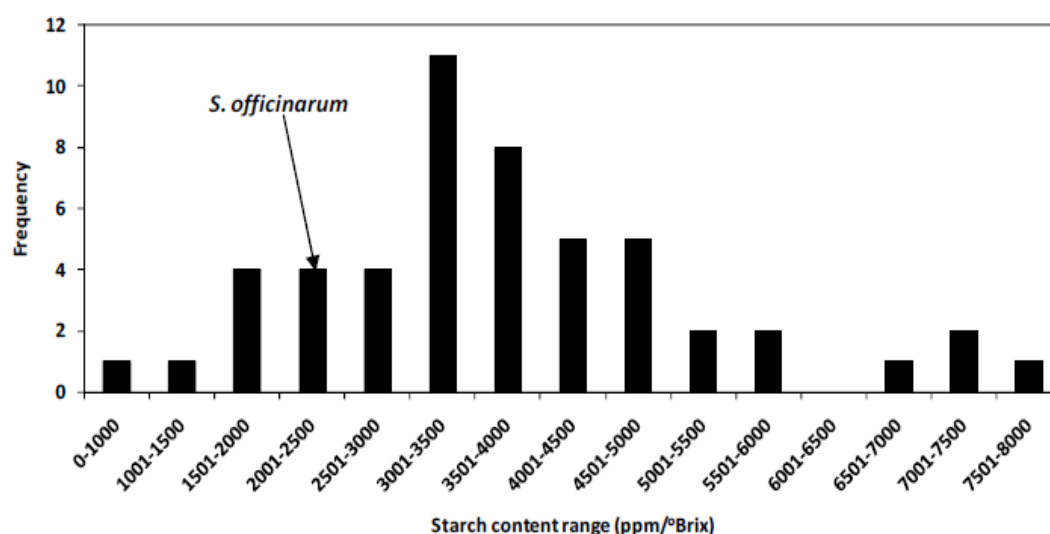


Figure 1. Frequency distribution of starch content among the *S. spontaneum* clones sampled from the wild species collection growing in cans at the USDA, ARS Sugarcane Research Station at Houma (Zhou et al., 2008)

3.2 *Saccharum* species at USDA, ARS Sugarcane Research Station at Houma

Samples of juice were collected from *S. barberi*, *S. bengalense*, *S. officinarum*, *S. robustum*, *S. sinense*, *S. spontaneum*, *Erianthus*, and *Miscanthus* species. There were highly significant ($P < 0.0001$) differences for starch content among the species. The cultivated species (*S. officinarum*, *S. robustum*) produced significantly ($P < 0.05$) less starch than their wild relatives (Table 4). Among the entries that had at least five clones, *S. officinarum* produced the lowest starch content followed by *S. robustum*, *S. barberi* and *S. sinense*, whereas *S. spontaneum* had the highest starch content. The clones from within the wild species generally produced more starch content than the cultivated species (Table 5). There were highly significant differences ($P < 0.0001$) among clones within each species grouping. The *S. spontaneum* and *S. sinense* species showed the greatest within species variability for starch content (highest standard deviations) (Tables 4 and 5). The cultivated species produced lower genetic variability for starch content, probably because of selection for high sucrose (Zhou et al., 2008).

Table 4. The mean, standard deviation (Std Dev) and mean % of *S. officinarum* for starch content (ppm/°Brix) of the *Saccharum* and related species sampled at USDA, ARS Sugarcane Research Station at Houma (Zhou et al., 2007)

Species	Number of clones	Mean	Std Dev	% of <i>S. officinarum</i>
<i>S. barberi</i>	13	1912.96	243.37	131
<i>S. bengalense</i>	1	2580.65	53.45	176
<i>Erianthus</i>	1	2453.53	12.00	168
<i>Miscanthus</i>	1	1536.53	332.46	105
<i>S. officinarum</i>	9	1463.62	270.01	100
<i>S. robustum</i>	11	1747.58	423.42	119
<i>S. sinense</i>	8	1929.42	843.02	132
<i>S. spontaneum</i>	5	2348.57	846.01	160

Table 5. The mean, standard deviation (Std Dev) and mean % of *S. officinarum* for starch content (ppm/°Brix) among clones from *Saccharum* and related species

Clone	Species	Mean	Std Dev	% of <i>S. officinarum</i>
CHIN	<i>Barberi</i>	1760.54	60.94	120
DHAULA	<i>Barberi</i>	1926.81	27.72	132
GANAPATHY	<i>Barberi</i>	1758.25	63.16	120
HATUNI	<i>Barberi</i>	1984.76	31.30	136
KALARI	<i>Barberi</i>	1571.67	42.61	107
KETARI	<i>Barberi</i>	1604.87	21.55	110
KHAGZI	<i>Barberi</i>	2156.72	57.10	147
MATNA-SHAHJ	<i>Barberi</i>	2143.81	26.83	146
NARGORI	<i>Barberi</i>	1606.33	43.74	110
NEWRA	<i>Barberi</i>	2387.70	24.52	163
PANURA	<i>Barberi</i>	1883.32	50.19	129
RENA	<i>Barberi</i>	2077.51	55.77	142
TERERU	<i>Barberi</i>	2006.18	49.43	137
IMP9751	<i>Bengalense</i>	2580.65	53.45	176
NG77-214	<i>Erianthus</i>	2453.53	12.00	168
MISCANTHUS-JV1	<i>Miscanthus</i>	1536.53	332.46	105
BADILLA	<i>Officinarum</i>	1292.19	13.61	88
FIJI1	<i>Officinarum</i>	1550.54	46.57	106
FIJI147	<i>Officinarum</i>	1296.49	24.59	89
GREEN-GERMAN	<i>Officinarum</i>	1828.41	28.85	125
IN84-68A	<i>Officinarum</i>	1340.77	8.58	92
LA-PURPLE	<i>Officinarum</i>	1999.33	70.22	137
LA-STRIFE	<i>Officinarum</i>	1175.68	42.10	80
MENTOR-4745	<i>Officinarum</i>	1424.22	28.02	97
OI-DEONG	<i>Officinarum</i>	1264.88	21.02	86
CHINA	<i>Robustum</i>	1673.86	18.23	114
CHUKCHE	<i>Robustum</i>	1726.62	25.08	118

IMP72-232	<i>Robustum</i>	1852.21	17.46	127
IS76-184	<i>Robustum</i>	2331.06	53.19	159
MERTHI-ZELL	<i>Robustum</i>	1601.16	29.35	109
MOLOKIA	<i>Robustum</i>	1303.08	2.88	89
NG77-147	<i>Robustum</i>	2695.67	128.71	184
NG77-159	<i>Robustum</i>	1737.24	28.87	119
TUKUYU#1	<i>Robustum</i>	1390.14	9.72	95
UBA-DEL-NATAL	<i>Robustum</i>	1704.65	27.97	116
UBA-INDIA	<i>Robustum</i>	1207.71	28.30	83
IMP3057	<i>Sinense</i>	3959.17	509.54	271
KATHA	<i>Sinense</i>	1597.41	43.93	109
MCIKUM	<i>Sinense</i>	1573.70	20.60	108
MERTHI	<i>Sinense</i>	1156.36	87.04	79
RHEA-SPORT	<i>Sinense</i>	2173.15	81.47	148
RUCKRI	<i>Sinense</i>	1722.82	39.71	118
TEKCHAOKINAWA	<i>Sinense</i>	1547.88	49.81	106
UBA-NAQUIN	<i>Sinense</i>	1704.87	118.55	116
MPTH97-260	<i>Spontaneum</i>	2356.64	179.71	161
MPTH98-326	<i>Spontaneum</i>	2615.01	149.60	179
MPTH99-476	<i>Spontaneum</i>	3681.71	70.16	252
NG57-54	<i>Spontaneum</i>	1804.49	9.02	123
SES-288	<i>Spontaneum</i>	1285.00	192.62	88

3.3 Replicated Germplasm at Houma

Sixty-three clones, made up of six *Saccharum* hybrids, four *S. officinarum*, three *S. robustum* and 50 *S. spontaneum* were grown in replicated pots at the USDA, ARS Sugarcane Research Station at Houma. There were highly significant ($P < 0.0001$) differences among the hybrids and species. The hybrids produced 6% less starch content than *S. officinarum* while *S. robustum* and *S. spontaneum* clones produced more than double the starch content produced by *S. officinarum* (Table 6). The *S. robustum* and *S. spontaneum* produced greater within species variability as evidenced by their high standard deviations. Frequency distribution of starch content among the *S. spontaneum* clones produced a non-discrete distribution (Figure 2), similar to that of Figure 1. All the *S. spontaneum* clones produced more starch content than the *S. officinarum* clones (Table 6). The *S. officinarum* clones produced the least variability for starch content (Table 6).

Table 6. The mean, standard deviation (Std Dev) and mean % of *S. officinarum* for starch content (ppm/^oBrix) for *Saccharum* hybrids, *S. officinarum*, *S. robustum* and *S. spontaneum* clones sampled from a replicated experiment growing in pots at USDA, ARS Sugarcane Research Station at Houma, Louisiana

Species	Number of clones	Mean	Std Dev	% of <i>S. officinarum</i>
<i>Saccharum</i> hybrid	6	788.97	225.48	94
<i>S. officinarum</i>	4	841.30	125.13	100
<i>S. robustum</i>	3	2064.08	582.72	245
<i>S. spontaneum</i>	50	2231.44	998.65	265

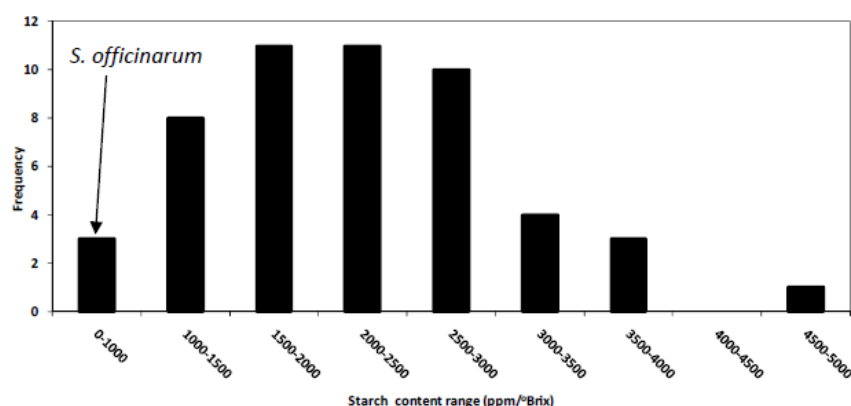


Figure 2. Frequency distribution for starch content among *S. spontaneum* clones sampled from a replicated experiment growing in pots at the USDA, ARS Sugarcane Research Station at Houma

3.4 Canal Point Nursery, Florida

At the Canal Point nursery, juice samples were collected from seven *S. barberi*, 14 *Saccharum* hybrids, 36 *S. sinense*, four *S. spontaneum* and one *S. officinarum* which was used as a control. There were highly significant ($P < 0.0001$) differences among the species. The wild species germplasm (*S. barberi*, *S. sinense*, *S. spontaneum*) produced significantly ($P < 0.001$) and at least three times more starch content than *S. officinarum* (Table 7). The *Saccharum* hybrids produced two and half times more starch content than *S. officinarum*. Some of the hybrids (L01-290, L04-417, TCP89-3505) produced more than five times the amount of starch content produced by *S. officinarum* (Table 8). Other hybrids (Q158, Q160, CP02-2365) produced more than double the amount of starch content produced by *S. officinarum*.

Thirty-six *S. sinense* clones were sampled from the Canal point nursery. While the majority of the clones produced more starch content than *S. officinarum* and the hybrids (Tables 7 and 8, Figure 3), the *S. sinense* produced less starch content than *S. spontaneum*. The distribution of the *S. sinense* clones (Figure 4) showed that the majority of the clones produced lower starch content than *S. spontaneum*. Thirty-four out of 36 clones produced less starch content than *S. spontaneum*. The clones within the *S. sinense* population also produced a narrow variability for starch content.

Table 7. The mean, standard deviation (Std Dev) and mean % of *S. officinarum* for starch content (ppm/°Brix) for *Saccharum* hybrids, *S. barberi*, *S. officinarum*, *S. robustum* and *S. spontaneum* clones sampled at Canal Point nursery, Florida

Species	Number of clones	Mean	Std Dev	% of <i>S. officinarum</i>
<i>S. barberi</i>	7	476.55	289.51	381
<i>Saccharum</i> hybrids	14	319.86	244.46	256
<i>S. officinarum</i>	1	124.97	9.28	100
<i>S. sinense</i>	36	380.19	406.67	304
<i>S. spontaneum</i>	4	737.01	340.19	590

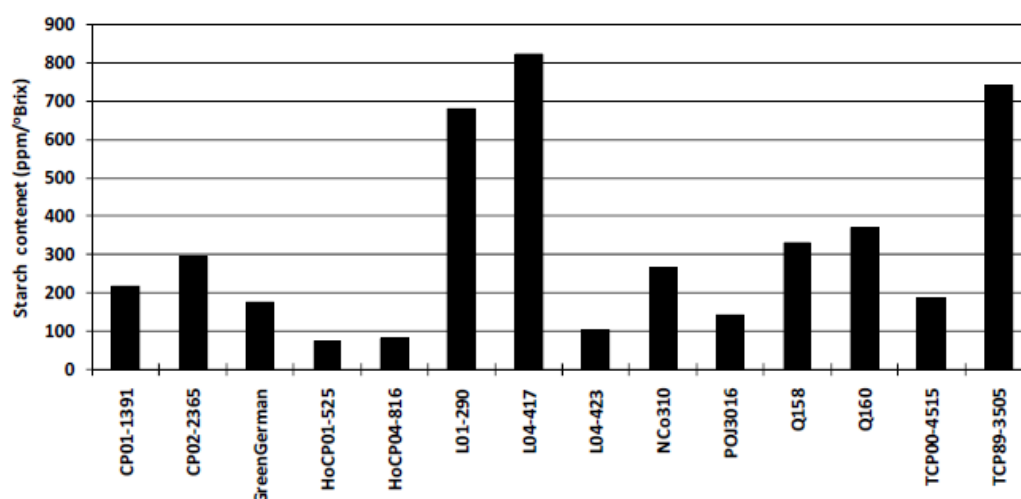


Figure 3. The mean starch content for *Saccharum* hybrids sampled from the Canal Point nursery, Florida

Table 8. The mean, standard deviation and mean % of *S. officinarum* for starch content (ppm/°Brix) among the *Saccharum* species sampled at Canal Point nursery, Florida

Variety	Species	Mean	Std Dev	% of <i>S. officinarum</i>
Grandsheni	<i>Barberi</i>	318.98	35.19	255
Hatooni	<i>Barberi</i>	450.90	39.51	361
Kalari	<i>Barberi</i>	229.44	10.40	184
Kinar	<i>Barberi</i>	1010.98	11.69	809
Lalri	<i>Barberi</i>	731.88	16.41	586
Mol6427	<i>Barberi</i>	449.60	108.89	360
Rhea	<i>Barberi</i>	144.08	6.75	115
CP01-1391	Hybrid	216.69	18.98	173
CP02-2365	Hybrid	294.38	61.67	236
GreenGerman	Hybrid	174.44	7.73	140
HoCP01-525	Hybrid	74.19	13.32	59
HoCP04-816	Hybrid	81.27	14.06	65
L01-290	Hybrid	678.49	60.26	543
L04-417	Hybrid	823.84	18.63	659
L04-423	Hybrid	104.47	21.03	84
NCo310	Hybrid	265.90	10.59	213
POJ3016	Hybrid	142.44	11.07	114
Q158	Hybrid	329.62	3.03	264
Q160	Hybrid	368.29	0.80	295
TCP00-4515	Hybrid	184.19	10.47	147
TCP89-3505	Hybrid	739.83	15.77	592
LAPurple	<i>Officinarum</i>	124.97	9.28	100
Agaul	<i>Sinense</i>	252.85	7.51	202
Archi	<i>Sinense</i>	265.32	4.62	212

Berlin	<i>Sinense</i>	369.64	28.73	296
China	<i>Sinense</i>	329.15	32.44	263
Chukche	<i>Sinense</i>	158.68	14.38	127
Chynia	<i>Sinense</i>	1199.84	20.57	960
DesiPaunda	<i>Sinense</i>	2380.33	8.14	1905
Guilin-1	<i>Sinense</i>	163.10	6.53	131
Japonesa	<i>Sinense</i>	529.52	478.91	424
Kacai	<i>Sinense</i>	228.71	20.54	183
Kavengire	<i>Sinense</i>	689.75	129.60	552
Kerah	<i>Sinense</i>	274.27	3.79	219
Ketari-II	<i>Sinense</i>	394.72	15.00	316
Khakai	<i>Sinense</i>	228.85	8.93	183
Louje	<i>Sinense</i>	597.58	9.49	478
Lucane	<i>Sinense</i>	651.22	48.61	521
Maneria	<i>Sinense</i>	222.73	7.89	178
Mankia	<i>Sinense</i>	244.63	5.76	196
Mcilkrum	<i>Sinense</i>	128.93	6.65	103
Merthi	<i>Sinense</i>	187.90	15.73	150
Merthi-Zel	<i>Sinense</i>	196.62	3.97	157
Mialan	<i>Sinense</i>	246.91	5.40	198
Nepal3	<i>Sinense</i>	174.19	19.56	139
Oshima	<i>Sinense</i>	147.43	6.97	118
Pansahi204	<i>Sinense</i>	177.64	9.50	142
Sinense	<i>Sinense</i>	222.49	7.90	178
TanzhouBamboo	<i>Sinense</i>	313.52	0.87	251
Tekcha	<i>Sinense</i>	107.89	6.24	86
TekchaChiisland	<i>Sinense</i>	247.00	16.03	198
TekchaOkinawa	<i>Sinense</i>	424.42	44.44	340
Tekchungts	<i>Sinense</i>	380.95	21.39	305
Tukuyudist#1	<i>Sinense</i>	201.32	6.11	161
UbaDelNatal	<i>Sinense</i>	133.85	8.66	107
UbaIndia	<i>Sinense</i>	251.11	5.06	201
UbaNaquin	<i>Sinense</i>	250.58	11.72	201
Zwinga	<i>Sinense</i>	254.17	10.63	203
MPTH97-3	<i>Spontaneum</i>	916.13	6.40	733
MPTH98-326	<i>Spontaneum</i>	287.73	12.23	230
MPTH98-388	<i>Spontaneum</i>	595.36	16.68	476
US02-1339	<i>Spontaneum</i>	1148.81	25.77	919

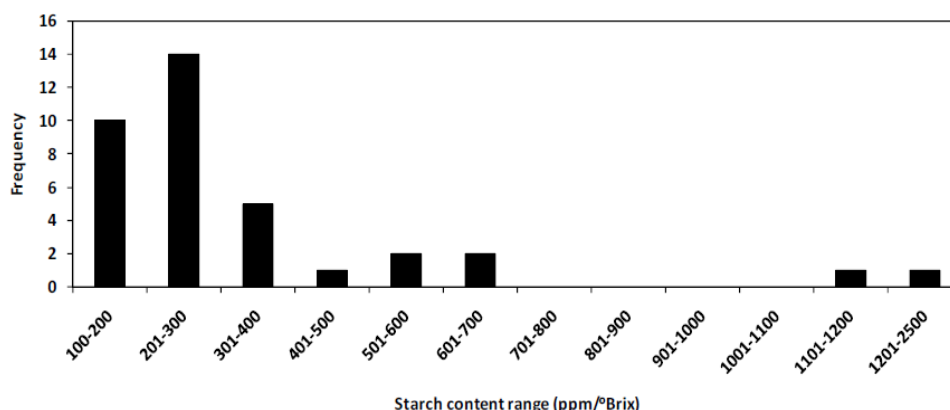


Figure 4. Frequency distribution of starch content among *S. sinense* clones sampled from the wild *Saccharum* species collection growing at Canal Point nursery, Florida

3.5 Spontaneum Clones in Canal Point Cans, Florida

Twenty *S. spontaneum* clones growing in cans were sampled at Canal Point, Florida. The frequency graph showed a non-discrete distribution for starch (Figure 5). A few clones produced extremely high starch values (Table 9, Figure 5).

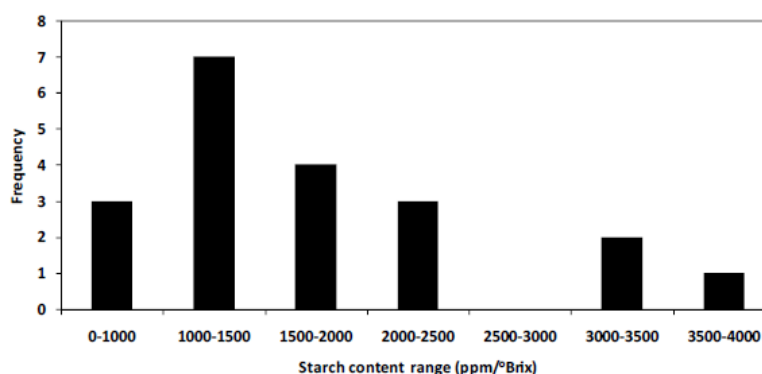


Figure 5. The distribution of starch content among 20 *S. spontaneum* clones growing in cans at Canal Point, Florida

Table 9. The mean and standard deviation (Std Dev) for starch content (ppm/°Brix) of 20 *S. spontaneum* clones sampled at Canal Point, Florida

Variety	Mean	Std Dev
Holes	1867.92	89.70
IN84-88	1160.87	74.18
IN84-91	1199.94	213.84
IND81-146	1035.39	142.41
IND82-257	1430.31	684.15
IND82-311	3900.68	3051.52
Okinawa1	2054.60	555.49
S.spont	1029.41	153.01
S.spont#10	922.15	111.15

S.spont#28	671.18	567.48
S.spontIran	2564.50	472.33
S66-121	879.70	170.20
SES11	1329.26	234.31
SES208	2146.15	983.93
SES234	1128.62	302.83
SES297A	3229.32	2133.51
SES92	3283.76	1838.38
SLC92-51	1632.92	715.59
Taiwan2n=96	1805.14	192.40
US84-1058	1924.79	178.99

3.6 Miami Collection

Juice was extracted from Four *S. barberi*, one *S. edule*, 15 *S. officinarum*, nine *S. robustum* and six *S. sinense* clones which were located at the wild species collection at Miami, Florida. There were highly significant ($P < 0.0001$) differences among species and clones within species. The *S. robustum* clones produced more starch content than *S. officinarum* clones (Table 10). Among the *S. officinarum* clones, Barbados white spot and IJ76-521 clones produced much higher starch content than expected (Figure 6, Table 11).

Table 10. The mean, standard deviation (Std Dev) and mean % of *S. officinarum* for starch content (ppm/°Brix) of *Saccharum barberi*, *S. edule*, *S. officinarum*, *S. robustum* and *S. sinense* clones sampled at Miami, Florida

Species	Number of clones	Mean	Std Dev	% of <i>S. officinarum</i>
<i>S. barberi</i>	4	571.59	300.84	96
<i>S. edule</i>	1	1019.26	27.70	172
<i>S. officinarum</i>	15	593.45	275.32	100
<i>S. robustum</i>	9	661.43	323.41	111
<i>S. sinense</i>	6	619.68	219.45	104

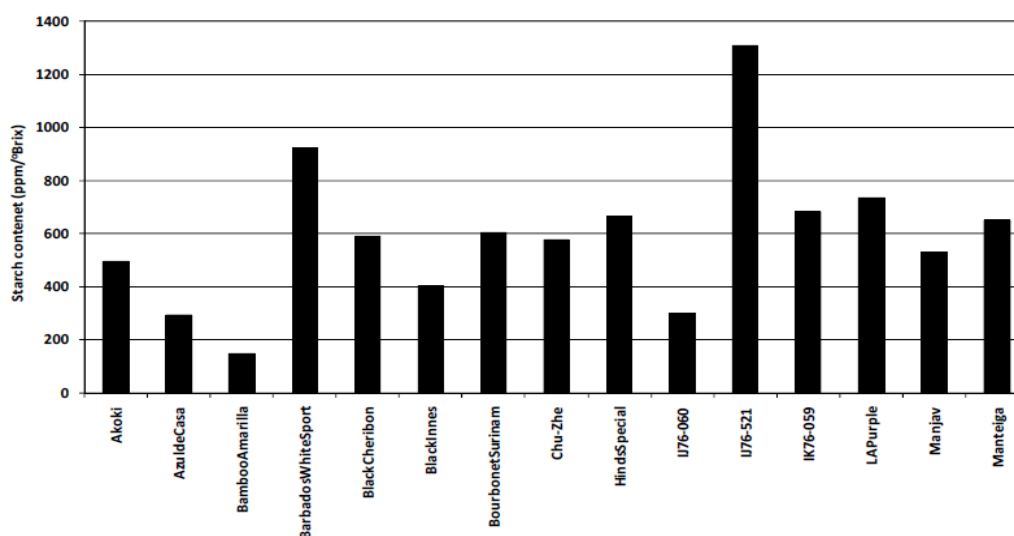


Figure 6. The mean starch content for *Saccharum officinarum* clones hybrids sampled from the collection at Miami, Florida

Table 11. The mean, standard deviation (Std Dev) and mean % *S. officinarum* for starch content (ppm/°Brix) among the clones derived from *Saccharum barberi*, *S. edule*, *S. officinarum*, *S. robustum* and *S. sinense* species sampled at Canal Point nursery, Florida

Clones	Species	Mean	Std Dev	% of <i>S. officinarum</i>
Baroukha	Barberi	1066.59	66.06	180
Kinar	Barberi	368.75	1.88	62
ManeiraCoimbatore	Barberi	429.34	8.71	72
Tereru	Barberi	421.67	5.23	71
NG77-079	Edule	1019.26	27.70	172
Akoki	Officinarum	495.07	5.35	83
AzuldeCasa	Officinarum	293.99	4.63	50
BambooAmarilla	Officinarum	146.90	16.56	25
BarbadosWhiteSport	Officinarum	921.55	35.25	155
BlackCheribon	Officinarum	588.78	3.34	99
BlackInnes	Officinarum	402.07	14.01	68
BourbonetSurinam	Officinarum	604.31	75.44	102
Chu-Zhe	Officinarum	577.63	14.08	97
HindsSpecial	Officinarum	666.23	6.56	112
IJ76-060	Officinarum	301.08	28.14	51
IJ76-521	Officinarum	1308.53	24.40	220
IK76-059	Officinarum	682.00	33.76	115
LAPurple	Officinarum	734.80	17.28	124
Manjav	Officinarum	529.08	8.80	89
Manteiga	Officinarum	649.69	189.73	109
IJ76-424	Robustum	387.15	4.79	65
IJ76-496	Robustum	774.25	23.25	130
IJ76-547	Robustum	410.85	3.18	69
M3035/66	Robustum	763.06	16.57	129
Mol6077	Robustum	603.36	15.28	102
NG57-024	Robustum	509.31	22.21	86
NG57-054	Robustum	895.28	13.47	151
NG77-055	Robustum	247.69	27.98	42
NG77-084	Robustum	1361.95	29.83	229
DesiPaunda	Sinense	528.26	14.50	89
TeckhaOkinawa	Sinense	802.85	21.84	135
Tukuyudist#1	Sinense	432.12	3.07	73
UbaDelNatal	Sinense	998.68	29.55	168
UbaIndia	Sinense	404.07	4.72	68
Yuegsen	Sinense	552.08	9.48	93

4. Discussions

This study provided the first comprehensive evaluation of starch content in a wide sugarcane germplasm collection. The results obtained in this study are expected to provide a resource to guide the selection of parental

material for introgression programs from the wild germplasm collection. The results also provide a guide as to the variability in starch content among the *Saccharum* species germplasm collection. This study could also be used by other sugar industries to evaluate their collections in their environments. Additionally, sugarcane breeding programs that have similar genotypes in their germplasm collection could use the results to select the clones suitable for use in introgression programs. This would be possible because starch content was shown to be stable across environments with very little influence from genotype by environment interaction (Zhou et al., 2008).

There were non-discrete frequency distributions of starch content among the species. The non-discrete distribution implies continuous variation for starch among the wild germplasm clones particularly for *S. spontaneum*. The implication of the non-discrete distribution patterns showed that there is potential for identifying *S. spontaneum* clones with low starch content and such clones would be desirable for germplasm introgression and enhancement programs. Clones with low starch, when used as parents in breeding programme are expected to require fewer backcross cycles with recurrent parent before they are acceptable for use in commercial breeding programs. Based on the frequency distributions, the clones could be classified into low, medium and high starch content.

This study also showed that low starch was important for successful sucrose production. The cultivated species, *S. officinarum* and *S. robustum* produced significantly less starch content than the wild relatives. Studies by Zhou et al. (2007, 2008) also showed a negative association between sucrose content and starch content in a breeding population. In this study, *S. officinarum* produced the lowest starch followed by *S. robustum*, *S. barberi* and *S. sinense* whereas *S. spontaneum* consistently produced the highest starch content. Our studies are in agreement with the results from a previous study by Dutt and Narasimhan (1951). The study of Dutt and Narasimhan (1951) tested 215 wild germplasm clones and cultivars and found that *S. officinarum* and *S. robustum* produced traces of starch whereas *S. spontaneum*, *S. barberi* and *S. sinense* accumulated much higher levels of starch.

There were highly significant differences for starch content among species and among clones within species. Selection for low starch can therefore be done at the species level as well as within the species. The data showed large variability for starch content within *S. spontaneum* species. *S. spontaneum* has been and will be used continuously for germplasm enhancement because it possesses desirable traits for hardiness, ratooning ability and high biomass production and also exhibits extensive genetic diversity because it is found in almost all continents. Studies by Alwala et al. (2006) and Suman et al. (2010) demonstrated the existence of large genetic diversity among *S. spontaneum* clones. The disadvantage of *S. spontaneum* is in its low sucrose content and high starch content. However, the large variability for starch content among clones within *S. spontaneum* provides an opportunity for selecting those clones that produce low starch content for use in germplasm introgression programs.

It is important to evaluate germplasm, but it is much more important to provide strategies for utilization of the germplasm. It gets difficult for plant breeders to select and add more traits in their breeding population as it complicates the selection procedure. Adding starch content as one of the selection criteria would not be a feasible option for plant breeders. We suggest that utilizing the knowledge of starch content in germplasm can be done at the crossing level. If parents with low starch content are used, the progeny produced by the introgressions are expected to produce low starch content. This study showed that there was a wide range of variation in starch content. By using low starch content parents, fewer backcrossing cycles would be required to get progeny with the desired levels of sucrose content. Conversely, if it becomes necessary to use a parent known to produce high starch content (because it possesses other desirable traits), then the breeders would be able to plan ahead the possible high number of backcrossing cycles that would be required to lower starch content in the resulting progeny populations. Previous studies by Zhou et al. (2007, 2008) showed that starch decreased from F₁ to BC, indicating backcrossing to a low starch recurrent parent would reduce the starch content.

The clones can be classified into low, medium and high starch content. Previous studies by Zhou et al. (2008) showed that the starch trait was stable across environments. These studies by Zhou et al. (2008) showed that the starch content for low starch clones was particularly more stable than that of high starch content clones. Therefore, once the parents have been characterized for starch content, the information can be used across breeding programs in different locations. The studies by Zhou et al. (2008) also showed high broad sense heritability for starch content, indicating that the genotypes within the species can be selected to identify parents that produce low starch. Furthermore, introgression programs could also be used to develop parents with low starch content, in addition to other important traits, through selection. Such developed parental genotypes would be expected to be stable for starch content across environments and would reduce the required backcrossing cycles in commercial breeding programs.

In our study, we also compared *Saccharum* species hybrids. This study included the cultivar NCo310 that is known to possess high starch content (Agarwal et al., 1998). The cultivar NCo310 provided difficulties to sugar processing, particularly in South Africa, a subtropical environment. In this study, cultivars L01-290, L04-417 and TCP89-3505 were found to produce significantly higher levels of starch content than NCo310. These cultivars that produce high starch content levels could be managed in sugar cane production systems. By harvesting the cultivars towards the end of the harvesting season when their starch content has significantly decreased as suggested by Zhou et al. (2008), the impact of starch at the mill could be reduced. But late harvesting could have adverse effect on the varieties, if pest and disease pressure are taken into account. It would be beneficial for any breeding programme to incorporate low starch content in varieties via introgression breeding rather than tweaking the management practices.

5. Conclusions

The non-discrete frequency distributions of starch content among the species indicated that the clones could be classified for starch into low, medium and high starch content. Introgression with low starch content clones would require fewer backcrosses to the recurrent parent to eliminate the excess starch and raise the sucrose content of the progenies. Starch content was important for successful sucrose production as evidenced by the cultivated species, *S. officinarum* and *S. robustum*, that produced significantly less starch content than the wild relatives. The significant differences among species and among clones within species showed that selection for low starch content can be done at the species level as well as clones within the species. The large variability for starch content among clones within species particularly *S. spontaneum* together with the high broad sense heritability indicated that low starch content clones for use as parents can be identified. The high broad sense heritability also meant low starch content parents can be developed during introgression programs. The best strategy for utilizing the germplasm characterized for starch content is at the parent selection stage and at crossing rather than selecting for starch content as an extra trait in breeding populations. During introgression programs, starch content can be evaluated before the developed parents are recommended for the commercial breeding programs. Because starch content has been found to be stable across environments, the germplasm characterization for starch content can be applied across breeding programs. Cultivars known to produce high starch content such as NCo310, L01-290, L04-417 and TCP89-3505 can be managed by planting and harvesting in late season when starch levels are low.

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