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Full Length Research Paper

Variability in sucrose content at grand growth phase in tissues of *Saccharum officinarum* × *Saccharum spontaneum* inter-specific hybrid progeny

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The variability for sucrose content in existing commercial sugarcane germplasm is not large and hence crosses were made between two *Saccharum officinarum* clones, 'Gungera' (cross I) and 'Keong' (cross II) with high sucrose content and a clone of *Saccharum spontaneum* 'SES 603' with low sucrose content. A high sucrose commercial var. CoS 8436 was also crossed with a low sucrose var. Co 1148 (cross III). Among the three crosses, only cross I germinated to give healthy twenty nine inter-specific hybrids. Inter-specific hybrids selected from cross I were graded into very high, high, low, and very low sucrose classes on the basis of morphological characters. Also anthrone method of sucrose estimation from internode, Brix value and quality analysis were done on whole sugarcane stem at different growth stages. Rate of photosynthesis, transpiration and stomatal conductance were analyzed using infrared gas analyzer and was found to be high for high sucrose hybrids. Rate of assimilate translocation of sucrose was also estimated with radio-labelled ^{14}C and was found to be highest in the midribs. On this basis, molecular markers for high and low sucrose content ISH-1, ISH-5, ISH-17 and ISH-23 were identified as very high and ISH-10, ISH-11, ISH-12 and ISH-25 as very low sucrose hybrids.

Key words: Inter-specific hybrids, sucrose content, sugarcane, TVD leaf.

INTRODUCTION

All the present day commercial sugarcane cultivars possess parental genes from a few inter-specific hybrids developed at Coimbatore during the early 20th century (Selvi et al., 2005). These cultivars, thus, have a narrow genetic base. Genus *Saccharum* consists of three cultivated species, that is, *Saccharum officinarum*, *Saccharum barberi* and *Saccharum sinensis*, and two wild species of *Saccharum robustum* and *Saccharum spontaneum*. Sugarcane varieties presently under cultivation have been derived through artificial crosses between two or more of these *Saccharum* species as the source of genetic materials. The maturation of sugarcane is characterized by the accumulation of sucrose in developing internodes (Glasziou and Gayler, 1972). It is evident that a cycle of sucrose synthesis and degradation exists in all of the internodes (Whittaker and Botha, 1997). Furthermore, carbon assimilation rate during maturation is influenced by factors like light intensity, plant age, and soil and leaf water contents (Grantz, 1989). Extensive work has been carried out in improving the sucrose accumulation in sugarcane at the CCS HAU Regional Research Station, Karnal. It was observed that sucrose accumula-tion was recorded on the basis of Brix and sucrose content (Dendsay et al., 1992) in sugarcane var. CoJ 64 was three fold more in lower internodes and highest during the peak growth period as compared to sugarcane var. Co1148 made them suitable for selection as parents for crossing so as to obtain hybrids of varying sucrose content. Sehtiya et al. (1991) showed that different zones of internodes vary in the sucrose content and sucrose accumulation was slightly higher in the central core than in the peripheral regions. Parenchyma cells were five to six times larger in the central core than in the periphery of the stalk. Attan et al. (2003) observed that *in vitro*

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sucrose uptake from sucrose containing medium by internodal slices of high sugar content var. CoJ 64 was higher than that of low sugar content var. Co 1148, irrespective of the maturity status of the internodes. It was concluded that the high sucrose clones store higher quantities of sucrose in their apparent free space (AFS) during their maturation phase.

Vinayak, et al. (2010) identified molecular markers for high and low sucrose genotypes and for this there was need to create germplasm with range of sucrose content from very low to very high so that inter-specific hybrids selected on the basis of their sucrose content by anthrone method, Brix, quality analysis and morphological findings can be compared to the inter-specific hybrids selected by high and low identifying sucrose markers. Therefore, high sucrose clones of *S. officinarum* were crossed with a low sucrose clone of *S. spontaneum*, to create variabilities. For early selection in breeding work, molecular markers have been employed to determine high sucrose types in the hybrids formed from a cross between high sucrose and low sucrose *Saccharum* genotypes. To study the contribution of *Sus2* to sugar accumulation process, PCR markers have been developed for different *Sus2* alleles (Lingle and Dyer, 2004). Map study of sugarcane shows *Shrunken1* (*Sh1*) gene, a probe from maize (*Zea mays*) is linked to the Brix character in sugarcane (Ming et al., 2001). *Sus2* gene from sugarcane is identical to *Shrunken1* gene from maize. However, northern analysis of diverse *Saccharum* genotypes shows that the *Sus2* gene is differentially expressed among the genotypes (Lingle et al., 2001), probably because of variable indels in promoter region of the gene. Clearly, genetic modification for sucrose accumulation requires a complete knowledge of rate limiting steps that are involved in sucrose synthesis, transport and accumulation. Some advancement towards elucidating of these processes have been made in the past few years and yet clear identification of genes in rate limiting steps is far from complete. The identification of DNA or protein markers related to high sucrose could, therefore, have significant consequences for genetic manipulation. The progeny provides materials with a wide range of sucrose contents. This paper includes observations on morphological characters, photosynthetic rates and assimilates translocation for these hybrid progeny and the parents.

MATERIALS AND METHODS

Crossing experiments were carried out at the Sugarcane Breeding Institute, Coimbatore, where parental clones were maintained. Sugarcane germplasm was planted in the fields on 15th March 2005. Plant materials included in this work were raised at CCS Haryana Agricultural University, Regional Research Station, Karnal. The following two inter-specifics (Cross I and II) and one inter-varietal cross (Cross III) were attempted:

Cross I : *S. officinarum* 'Gungera' × *S. spontaneum* 'SES 603'. Cross II: *S. officinarum* 'Keong' x *S. spontaneum* 'SES 603'. Cross III: Sugarcane var. CoS 8436 x Sugarcane var. Co1148.

Seeds packed in polythene laminated aluminum foil were transported from Coimbatore to Karnal.

The seeds were very susceptible to high temperature. Its viability was checked in the laboratory by germination tests in two ways. In test I, seeds were surface sterilized with 0.1% sodium hypochlorite and placed on absorbent cotton wool in petridish, moistened and covered. In test II, a mixture of sand and farm yard manure was sterilized in autoclave bags at 15 psi pressure and 121°C temperature. Sterilized mixture was spread in steel trays; moistened seeds were embedded into the soil and covered with polythene sheets. Samples from both tests were placed in culture room at 25 ± 2°C. Light intensity of 100 µE m^{-2} sec^{-1} was provided using fluorescent tubes and bulbs over a light and dark period of 16 and 8 h, respectively, and observations for germination of seeds were made after 10 days. Seeds from cross II showed no germination and hence were discarded. One hundred seeds from cross I and cross III were planted in the screen house in sterilized potting mixture containing soil, sand and FYM (1:1:1). After one month, the young seedlings were transferred to root trainers and then transferred to the field.

Height of field grown plants was recorded at three stages during grand growth on July 17 (stage 1), August 2 (stage 2) and August 21 (stage 3). Cane girth was recorded at stage 3 with vernier calipers and the number of tillers counted on stage 2. Mother tillers of fifteen randomly chosen plants were used for recording height and girth of genotypes. Photosynthetic rate, stomatal transpiration and stomatal conductance was measured using IRGA (Bioscientific Ltd., England) taking standard leaf of surface area 11.4 cm^2 (length 5.7 cm and breadth 2.0 cm). From the differences in gas concentration and air flow rate, the photosynthetic and transpiration rates were calculated every 20 s.

Rate of ^{14}C fixation into the leaves and its translocation from leaves to internodes in *S. spontaneum* 'SES 603', *S. officinarum* 'Gungera' and their progenies ISH-1, ISH-5, ISH-11, ISH-12, ISH-23 and ISH-25 was measured at about 30 cm middle part after removing 25 cm long tip part of TVD leaf which was inserted in the assimilation chamber, containing a few drops of water to maintain humidity. A disposable vial attached to assimilation chamber was added 1.0 ml of $NaH^{14}CO_3$ (specific gravity 51.9 mCi /mM) and 4.0 ml of 0.1 M phosphate buffer (pH 7.5) with the help of 5 ml syringe. The $^{14}CO_2$ (400 µCi) was released by injecting 2 ml of 1 N HCl with 5 ml syringe through the rubber cork of high tensile strength fitted on upper surface of chamber just above the attached vial. The test leaf in each experiment was allowed to assimilate $^{14}CO_2$ in sunlight for 2 h. Translocation rate was determined as radioactive counts after 24 h translocation period in six different samples of plant, namely leaf lamina, midrib, TVD internode, TVD+1 internode, TVD+ 3 internode and TVD+5 internode.

The sample intermodal tissue was boiled in 80% ethanol at 80 ± 2°C and decanted after cooling. The process was repeated thrice. The prepared ethanol soluble extract was then transferred to 20 ml scintillation vials and dried at 50 ± 2°C. 10 ml of Brays scintillation fluid (8 g PPO and 0.5 g POPOP in 1 litre of toluene) was added to the dried vials. The radioactive counts in the sample were determined by using the Liquid Scintillation Analyser (Model No. A 21000 TR, Packard Instrument Company, Neriden, U.S.A.).

Sucrose was estimated from the 6th internode at stage 3 by anthrone method of Van (1968) with O.D measured at 620 nm and sucrose content calculated as mg sucrose g^{-1} tissue. The values were confirmed by measuring Brix with refractometer at great grand growth phase on September 15 (stage 4) and by polarimetry at maturation phase on November 14 (stage 5) from the whole cane juice of a sugarcane stem.

Table 1. Plant height, Average number of tillers and cane girth in sugarcane clones *S. officinarum* "Gungera", *S. spontaneum* "SES 603" and their twenty-nine interspecific hybrids. Observations of the number of tillers and cane girth were recorded on June 15 and plant height on August 21. Values in the table are mean ± S.E.

Genotype	Average number of tillers	Cane girth (cm)	Plant height (cm)
'SES 603'	13.67 ± 1.19	0.62 ± 0.04	149.30 ±15.37
'Gungera'	2.00 ± 0.47	2.61 ± 0.12	108.30 ±10.90
ISH-1	4.67 ± 1.19	1.72 ± 0.07	199.40 ±13.58
ISH-2	8.67 ± 1.91	1.63 ± 0.08	132.10 ±10.48
ISH-3	4.67 ± 0.98	1.62 ± 0.06	139.60 ± 7.93
ISH-4	4.33 ± 0.54	1.82 ± 0.09	155.50 ±13.60
ISH-5	6.33 ± 1.09	1.71 ± 0.04	138.70 ± 5.78
ISH-6	5.00 ± 0.47	0.40 ± 1.19	133.40 ±13.48
ISH-7	9.67 ± 2.13	1.58 ± 0.03	145.80 ±14.24
ISH-8	2.67 ± 0.72	1.28 ± 0.02	134.70 ±11.62
ISH-9	4.67 ± 0.72	1.82 ± 0.06	154.00 ± 7.31
ISH-10	10.33 ±0.27	2.15 ± 0.04	146.40 ±10.96
ISH-11	3.33 ± 0.27	1.27 ± 0.04	126.00 ± 8.82
ISH-12	4.67 ± 0.27	1.52 ± 0.06	150.00 ±11.99
ISH-13	5.00 ± 0.47	1.55 ± 0.05	199.30 ± 5.70
ISH-14	6.00 ± 2.16	1.61 ± 0.05	144.30 ±12.43
ISH-15	5.33 ± 0.54	1.73 ± 0.06	160.50 ± 7.58
ISH-16	6.33 ± 0.98	1.62 ± 0.06	133.20 ±11.27
ISH-17	3.00 ± 0.94	1.50 ± 0.06	103.10 ±15.69
ISH-18	9.33 ± 2.37	1.56 ± 0.07	129.20 ±11.82
ISH-19	3.00 ± 0.82	2.12 ± 0.14	151.30 ± 9.67
ISH-20	0.67 ± 0.27	1.40 ± 0.00	-
ISH-21	5.33 ± 0.27	1.65 ± 0.05	144.40 ± 6.86
ISH-22	4.33 ± 0.72	1.52 ± 0.04	155.50 ± 9.75
ISH-23	3.67 ± 0.72	1.68 ± 0.05	143.90 ± 9.92
ISH-24	5.00 ± 0.82	1.61 ± 0.06	142.70 ±16.54
ISH-25	4.00 ± 1.25	1.53 ± 0.07	158.50 ± 9.27
ISH-26	6.33 ± 0.98	1.47 ± 0.08	175.50 ±20.04
ISH-27	5.33 ± 0.98	1.61 ± 0.06	168.40 ±14.31
ISH-28	2.33 ± 0.54	1.35 ± 0.03	134.90 ± 9.14
ISH-29	4.33 ± 0.54	1.93 ± 0.07	136.10 ± 6.38

RESULTS

Sets of *S. spontaneum* 'SES 603', *S. officinarum* 'Gungera' and *S. officinarum* 'Keong' showed 88.88, 80.95 and 72.22% germination, respectively, in the screen house and 83.33, 82.35 and 61.53% germination, respectively, in the field. Seed germination rates were zero in Cross II, while it was 50 and 60% in Cross I and Cross III, respectively, in the net house and 58 and 56.66% germination in the field (data not shown), indicating no differences in the germination rates between the two crosses. The plantlets of Cross I were named as interspecific hybrids (ISH) and those from Cross III were named as commercial hybrids (CH). A total of twenty-nine ISH and thirty-four CH survived in the field. However, CH showed relatively poor and stunted growth, only twenty-nine ISH from cross I were selected for further work.

Of the two parental species, *S. spontaneum* 'SES 603' showed much higher tillering (13.67) compared to *S. officinarum* 'Gungera' (2.00) (Table 1). Among the inter-specific hybrids, profuse tillering (average number of tillers) was observed in ISH-2 (8.67), ISH-7 (9.67), ISH-10 (10.33) and ISH-18 (9.33), while very poor tillering was seen in ISH-8 (2.67), ISH-11(3.33), ISH-17 (3.00), ISH-19 (3.0), ISH-20 (0.67) and ISH-28 (2.33). Cane girth of second internode from bottom was only 0.62 cm in *S. spontaneum* clone 'SES 603' compared to 2.61 cm in *S. officinarum* clone 'Gungera' (Table 1). Even though none of the progeny showed cane girth

Table 2. Photosynthetic rate (µ mole m–2s–1), stomatal transpiration (mole m–2s–1) and stomatal conductance (mole m–2s–1) in sugarcane clones *S. spontaneum* 'SES 603', *S. officinarum* 'Gungera' and their interspecific hybrids observed using infra red gas analyzer. Values in the table are mean ± S.E.

Genotype	Photosynthetic Rate (µ mole m–2s–1)	Stomatal transpiration (mole m–2s–1)	Stomatal conductance (mole m–2s–1)
'SES 603'	67.78 ± 4.67	11.68 ± 2.08	0.94 ± 0.18
'Gungera'	22.62 ± 0.71	3.84 ± 0.28	0.35 ± 0.06
ISH-1	22.20 ± 1.31	5.58 ± 0.31	0.23 ± 0.03
ISH-2	21.63 ± 1.72	5.75 ± 0.35	0.23 ± 0.03
ISH-4	25.66 ± 2.03	6.76 ± 0.40	0.36 ± 0.07
ISH-5	22.03 ± 3.22	6.17 ± 0.52	0.26 ± 0.06
ISH-6	25.78 ± 1.55	7.99 ± 0.39	0.23 ± 0.03
ISH-7	29.88 ± 1.16	9.79 ± 0.40	0.24 ± 0.02
ISH-8	29.16 ± 0.30	10.16 ± 0.80	0.23 ± 0.04
ISH-9	24.55 ± 1.03	6.22 ± 0.25	0.26 ± 0.03
ISH-10	23.56 ± 2.90	8.57 ± 0.73	0.18 ± 0.04
ISH-11	22.69 ± 2.51	7.88 ±0.70	0.18 ± 0.03
ISH-12	22.45 ± 0.85	6.32 ± 0.30	0.26 ± 0.04
ISH-13	23.90 ± 0.92	6.84 ±0.22	0.20 ± 0.01
ISH-14	25.28 ± 0.67	6.56 ± 0.18	0.28 ± 0.03
ISH-15	25.78 ± 1.51	6.71 ± 0.19	0.28 ± 0.02
ISH-16	20.78 ± 2.06	7.30 ± 0.39	0.14 ± 0.02
ISH-17	25.22 ± 0.67	6.69 ± 0.18	0.25 ± 0.02
ISH-19	23.43 ± 1.36	6.01 ± 0.14	0.28 ± 0.02
ISH-20	21.69 ± 1.47	6.07 ± 0.27	0.22 ± 0.03
ISH-21	33.26 ± 3.75	9.81 ± 0.88	0.30 ± 0.05
ISH-22	28.83 ± 3.52	9.09 ± 0.78	0.25 ± 0.04
ISH-23	22.68 ± 3.02	7.24 ± 0.83	0.21 ± 0.05
ISH-24	24.84 ± 1.32	6.94 ± 0.16	0.22 ± 0.03
ISH-25	21.81 ± 2.14	6.54 ± 0.45	0.18 ± 0.03
ISH-26	25.56 ± 1.50	7.56 ± 0.32	0.21 ±0.02
ISH-27	24.02 ± 2.35	8.09 ± 0.48	0.18 ± 0.02
ISH-28	22.16 ± 0.73	7.49 ± 0.23	0.21 ± 0.01

comparable to Gungera, hybrids ISH-10, ISH-19 and ISH-29 showed cane girth of more than 1.90 cm, while ISH-1, ISH-4, ISH-5, ISH-9 and ISH-15 showed cane girth between 1.70 to 1.90 cm morphologically indicating high sucrose hybrids like clone 'Gungera' as cane girth is directly proportional to sucrose accumulation. The remaining progeny had girth shorter than 1.70 cm morphologically falling into low sucrose hybrids like clones 'SES 603'. Clone *S. spontaneum* 'SES 603' attained a cane height of 149.30 cm at stage 3, whereas clone *S. officinarum* 'Gungera' attained cane height of 108.3 cm at this stage. Tall hybrids as indicated by cane height at this stage include ISH-1 (199.40 cm); ISH-4 (155.50 cm); ISH-9 (154.00 cm); ISH-12 (150.00 cm); ISH-13 (199.30 cm); ISH-15 (160.50 cm); ISH-19 (151.30 cm); ISH-22 (155.50 cm); ISH-25 (158.50 cm); ISH-26 (175.50 cm) and ISH-27 (168.40 cm). The remaining hybrids showed cane height between 103 and 148 cm.

S. spontaneum 'SES 603' showed highest photosynthetic rate (µmole $m^{-2}s^{-1}$) of 67.78 which is three times greater than *S. officinarum* 'Gungera' which showed low photosynthetic rate of 22.62 (Table 2). The inter-specific hybrids; ISH-4, ISH-6, ISH-7, ISH-8, ISH-14, ISH-15, ISH-17, ISH-21, ISH-22 and ISH-26 showed photosynthetic rate between 25.22 to 33.26 µmole $m^{-2}s^{-1}$ while the rest of the hybrids had less than that. The stomatal transpiration rate was also higher for *S. spontaneum* 'SES 603' when compared to *S. officinarum* 'Gungera'. The inter-specific hybrids

Table 3. Percent of 14C counts in different plant parts in clones *S. spontaneum* 'SES 603', *S. officinarum* 'Gungera' and their six interspecific hybrids (ISH-1, ISH-5, ISH-11, ISH-12, ISH-23 and ISH-25). Leaves were allowed to photosynthesize in the assimilation chamber in radiolabelled $^{14}CO_2$ air for 2 h in the morning. Samples were collected after 24 h of translocation period. Percent 14C counts in lamina, midrib and internodes were calculated.

S/no	Percent	SES 603	Gungera	ISH-1	ISH-5	ISH-11	ISH-12	ISH-23	ISH-25
		Percent ^{14}C counts							
1.	Leaf lamina	46.02	31.33	34.39	43.60	27.25	45.15	31.30	38.68
2.	Midrib	36.16	50.20	63.43	47.63	62.19	44.12	55.31	58.99
3.	TVD internode	3.23	5.84	0.70	2.67	2.37	2.44	1.37	0.50
4.	TVD+1 internode	7.93	8.76	1.00	3.13	6.27	6.23	10.5	1.04
5.	TVD+3 internode	2.45	3.10	0.30	2.55	0.72	1.13	0.77	0.44
6.	TVD+5 internode	4.18	0.66	0.15	0.38	1.16	0.89	0.57	0.32

showing stomatal transpiration rate above 7.0 mole $m^{-2}s^{-1}$ were ISH-6, ISH-7, ISH-8,ISH-10,ISH-11,ISH-16, ISH-21, ISH-22, ISH-23, ISH-26, ISH-27 and ISH-28. The stomatal conductance rate (mole $m^{-2}s^{-1}$) was again greater for clone 'SES 603' (0.94) when compared to 'Gungera' (0.35). Hybrids showing stomatal conduc-tance between 0.25 and 0.36 mole $m^{-2}s^{-1}$ were ISH-4, ISH-5, ISH-9, ISH-12 ISH-14, ISH-15, ISH-17, ISH-19, ISH-21 and ISH-22 and the remaining ISH lines showed stomatal conductance below them.

Rate of translocation of sucrose using radiolabelled ^{14}C showed that radioactive counts were found to be at the highest in lamina and midrib (Table 3). *S. spontaneum* 'SES 603' showed comparatively high total incorporation of ^{14}C compared to high sucrose parent, *S. officinarum* 'Gungera'. The percent incorporation of the midrib was greater in clone 'Gungera' than clone 'SES 603'. Thus, the percentage of radioactivity in midrib of clone 'SES 603' was 36.16% which is far lesser than that in clone 'Gungera' (50.2%). From Table 3, it is also clear that the percentage of photosynthates entering translocation stream in clone 'SES 603' and clone 'Gungera' was 53.97 and 68.6%, respectively. The pattern of translocation of ^{14}C was same in all the genotypes studied, that is, ISH-1, ISH-6, ISH-11, ISH-12, ISH-23 and ISH-25.

They was a very high increase in midrib and a decrease towards lower internodes, that is, from TVD internode to TVD+1, TVD+3 and TVD+5.

Sucrose content estimated by Van Handel method at stage 3 in 6th internode from bottom for all the sugarcane genotypes is shown in Table 4. *S. spontaneum* 'SES 603' and *S. officinarum* 'Gungera' had 45.5 mg sucrose g tissue^{-1} and 56.1 mg sucrose g tissue^{-1}, respectively. The ISH hybrids having sucrose content above 70 mg g tissue^{-1} were graded into very high sucrose types, viz., ISH-1, ISH-5, ISH-9, ISH-13, ISH-17 and ISH-23. High sucrose types having sucrose content between 50 to 70 mg g tissue^{-1} include hybrids like ISH-3, ISH-4, ISH-6, ISH-26 and ISH-28. On the other hand, ISH hybrids having sucrose content between 30 to 50 mg g tissue^{-1} were graded into low sucrose types, they were hybrids like ISH-8, ISH-14, ISH-16, ISH-18, ISH-20, ISH-21 and ISH-22, whereas ISH hybrids which showed very low sucrose content less than 30 mg g tissue^{-1} were ISH-2, ISH-7, ISH-10, ISH-11, ISH-12, ISH-15, ISH-19, ISH-24, ISH-25, ISH-27 and ISH-29. Brix values in juice of sugarcane were estimated at alternate internodes at stage 4 (Table 5). Also quality analysis of sugarcane clones and its hybrids was done at stage 5 which showed variable values of Brix, Sucrose percentage and Commercial Cane Sugar (CCS) (Table 6). Thus, the two clones of 'SES 603' and 'Gungera' had large difference in their growth and morphological characters. Indeed, this was the basis of selecting these two as parents in the present study. The present studies showed that the two parents and twenty nine inter-specific hybrids obtained satisfy the initial goal proposed and present a complete range of variation for tillering, cane girth and other characters.

DISCUSSION

This study attempts to develop materials in specifically designed crosses to obtain a complete range of genotypes. Vinayak, et al. (2010) identified four high sucrose PCR markers: AI +AIR (Acid invertases); MSSCIRI + MSSCIRIR; A +AR; and B + BR and one low sucrose PCR marker SMC226CG + SMC226CGR which graded ISH-1, ISH-5, ISH-17 and ISH-23 into high sucrose inter-specific hybrids and ISH-10, ISH-11, ISH-12 and ISH-25 into low sucrose inter-specific hybrids with variable sucrose content for the study of sucrose accumulation process.

Seeds from cross II showed no germination and hence could not be continued. Cross I and cross III showed good germination, but plants from cross III showed poor and stunted growth in the field and hence progeny of only cross I formed experimental material for further work. Sugarcane has sexually developed true

Table 4. Sucrose content (mg sucrose g tissue^{-1}) in internode number 6 from bottom in sugarcane clones *S. officinarum* "Gungera", *S. spontaneum* "SES 603"and their twenty-nine inter-specific hybrids. Values in the table are mean ± S.E. Observations were recorded on September 15 at stage 3.

Genotypes	Sucrose (mg sucrose g tissue^{-1})
'SES 603'	45.5 ± 6.79
'Gungera'	56.1 ± 0.55
ISH-1	78.9 ± 2.16
ISH-2	28.6 ± 0.60
ISH-3	57.7 ± 0.55
ISH-4	53.9 ± 0.32
ISH-5	91.8 ± 2.56
ISH-6	58.1 ± 2.27
ISH-7	18.2 ± 0.99
ISH-8	44.3 ± 1.15
ISH-9	80.6 ± 1.13
ISH-10	26.8 ± 0.57
ISH-11	4.4 ± 1.60
ISH-12	12.9 ± 0.76
ISH-13	78.1 ± 2.71
ISH-14	45.0 ± 1.82
ISH-15	26.2 ± 0.37
ISH-16	49.3 ± 0.64
ISH-17	74.4 ± 3.23
ISH-18	45.5 ± 3.25
ISH-19	15.3 ± 2.85
ISH-20	37.6 ± 2.22
ISH-21	44.8 ± 0.94
ISH-22	46.3 ± 2.25
ISH-23	71.4 ± 1.72
ISH-24	16.8 ± 1.93
ISH-25	17.9 ± 0.78
ISH-26	55.4 ± 0.54
ISH-27	4.7 ± 1.81
ISH-28	68.3 ± 2.64
ISH-29	17.2 ± 1.00

seed like any other grain crop and follows Mendel's law of inheritance in crossing. *S. spontaneum*, a wild type cane, has a very poor sink and extremely low sucrose accumulation capacity; whereas the cultivated form of sugarcane, viz. *S. officinarum,* has efficient sucrose accumulating sinks. The cross between clones of these two divergent species, *S. officinarum* 'Gungera' with *S. spontaneum* 'SES 603', in the present work resulted in progeny that provided ideal study material with a wide range of sucrose content.

'SES 603', clone from wild *S. spontaneum*, had profuse tillering, greater plant height and hence thin canes. 'Gungera', on the other hand, a clone of noble species of *S. officinarum,* had lesser tillering, less plant height and thicker canes. The progeny of the two selected as ISH-1 to ISH-29 hybrids had variable cane girth, number of tillers and height. MacColl (1976) has described the relationship of growth patterns with sucrose content and had shown that tillering at times when early formed tillers are elongated, reduces the sugar content.

Thus, since determination of sucrose accumulation capacity of parents and hybrids used in this study was very critical to interpretation of results, different methods (Brix, Anthrone reagent, Polarimetry) were employed several times to arrive at a conclusion. Even though there were occasional variations in results, an overall conclusion of the repeated measurement of Brix, sucrose and juice quality assigned parent clone *S. officinarum* 'Gungera' and four hybrids, viz., ISH-1, ISH-5,

Table 5. Brix values in juice in sugarcane clones *S. officinarum* "Gungera" and *S.spontaneum* "SES603' and their twenty-nine interspecific hybrids. Internodes were numbered from 1 to 9 with 1 being the uppermost newly formed (≥ 1 cm long) internode. Observations were recorded on September 15. Values in the table are mean ± S.E.

Genotype	Internodes number				
	1	3	5	7	9
'SES 603'	6.9 ± 0.42	9.0 ± 0.05	10.9 ± 0.05	10.8 ± 0.16	12.3 ± 0.19
'Gungera'	6.2 ± 0.30	6.0 ± 0.00	8.9 ± 0.05	11.0 ± 0.47	13.0 ± 0.09
ISH-1	7.1 ± 0.19	6.2 ± 0.76	10.2 ± 0.14	15.2 ± 0.56	18.2 ± 0.21
ISH-2	6.2 ± 0.30	5.0 ± 0.09	9.2 ± 0.24	12.8 ± 0.43	15.2 ± 0.09
ISH-3	5.2 ± 0.11	8.2 ± 0.68	11.6 ± 0.51	16.8 ± 0.46	18.6 ± 0.80
ISH-4	6.8 ± 0.33	7.6 ± 0.18	11.7 ± 0.64	13.2 ± 0.09	12.3 ± 0.27
ISH-5	4.8 ± 0.46	6.5 ± 0.09	13.5 ± 0.43	15.6 ± 0.35	16.4 ± 0.05
ISH-6	9.2 ± 0.97	8.4 ± 0.19	13.4 ± 0.35	10.2 ± 0.11	13.1 ± 0.44
ISH-7	7.0 ± 0.05	6.9 ± 0.33	10.1 ± 0.05	12.0 ± 0.37	–
ISH-8	5.9 ± 0.05	4.4 ± 0.23	6.4 ± 0.24	9.9 ± 0.39	11.2 ± 0.14
ISH-9	6.3 ± 0.12	6.8 ± 0.48	10.3 ± 0.12	12.3 ± 0.14	14.2 ± 0.09
ISH-10	5.1 ± 0.07	8.9 ± 0.78	9.1 ± 0.04	11.1 ± 0.08	–
ISH-11	5.0 ± 0.47	5.4 ± 0.24	9.8 ± 0.15	–	–
ISH-12	5.0 ± 0.02	5.7 ± 0.15	8.3 ± 0.07	10.5 ± 0.17	–
ISH-13	5.1 ± 0.07	7.7 ± 0.46	13.5 ± 0.23	16.0 ± 0.26	14.0 ± 0.05
ISH-14	7.0 ± 0.94	5.7 ± 0.21	7.3 ± 0.54	10.4 ± 0.24	11.2 ± 0.09
ISH-15	4.6 ± 0.27	5.0 ± 0.42	8.3 ± 0.10	12.8 ± 0.16	–
ISH-16	5.6 ± 0.24	5.0 ± 0.09	7.1 ± 0.10	10.5 ± 0.23	12.5 ± 0.30
ISH-17	6.7 ± 0.54	8.1 ± 0.11	11.6 ± 0.24	14.2 ± 0.33	12.3 ± 0.72
ISH-18	7.6 ± 0.54	8.0 ± 1.24	11.4 ± 0.23	14.2 ± 0.33	12.3 ± 0.72
ISH-19	6.1 ± 0.10	4.1 ± 0.13	6.8 ± 0.24	12.0 ± 0.47	14.8 ± 0.33
ISH-20	4.8 ± 0.49	6.8 ± 0.05	11.3 ± 0.12	15.4 ± 0.28	15.3 ± 0.14
ISH-21	4.2 ± 0.30	5.9 ± 0.42	8.5 ± 0.23	12.4 ± 0.23	14.4 ± 0.19
ISH-22	8.0 ± 0.79	4.0 ± 0.05	8.6 ± 0.98	12.1 ± 0.46	13.2 ± 0.09
ISH-23	9.8 ± 0.16	9.5 ± 0.48	13.3 ± 0.27	16.4 ± 0.23	17.0 ± 0.00
ISH-24	5.0 ± 0.47	6.0 ± 0.47	7.9 ± 0.05	1.6 ± 0.24	10.5 ± 1.03
ISH-25	4.3 ± 0.27	5.0 ± 0.00	6.9 ± 0.16	7.0 ± 0.05	8.0 ± 0.00
ISH-26	5.8 ± 0.46	5.0 ± 0.48	7.6 ± 0.24	12.5 ± 0.40	14.4 ± 0.19
ISH-27	7.1 ± 0.10	7.3 ± 0.54	8.3 ± 0.27	11.0 ± 0.47	–
ISH-28	5.1 ±0.37	5.0 ±0.14	8.6 ±0.47	13.2 ±0.21	16.8 ±6.13
ISH-29	5.3 ±6.72	4.2 ±0.30	5.2 ±0.16	9.0 ±0.25	12.5 ±0.23

ISH-17, ISH-23 as high sucrose types and parent clone *S. spontaneum* 'SES 603' and four hybrids ISH-10, ISH-11, ISH-12 and ISH-25 as low sucrose types for experimental work.

From the radioactive counts and the percentage radioactivity determined in six different sample parts of each selected variety, it appears that the photosynthates entered and accumulated in the midrib and were then translocated to the other regions; primarily to lower internodes. The TVD internode above the TVD leaf showed lesser radioactive counts and percentage radioactivity, since the translocation of photosynthetic radiolabelled assimilates is mainly downwards. TVD + 1 internode had higher radioactivity than TVD internode. The lower internodes like TVD+3 and TVD+5 have very low percentage of radioactivity and radioactive counts since the TVD leaf may accumulate its photosynthates only to nearby upper internodes. Therefore, percentage of radioactivity estimated in TVD+5 internodes is less than 1%. Interestingly, the wild type parent 'SES 603' has higher number of total radioactive counts incorporated (47578) compared to the high sucrose parent 'Gungera' (14099). This is perhaps because in clone 'SES 603' the leaves are very narrow and the total surface area of leaf is very small, so the amount of radiolabelled sucrose accumulated per cell in the leaves is more than the amount of radiolabelled sucrose accumulated in 'Gungera'. Thus, for sucrose accumulation, the capacity of the sink to accumulate seems more important than

Table 6. Quality analysis showing Brix, sucrose percent and CCS percent in sugarcane clones *S. officinarum* "Gungera", *S. spontaneum* "SES 603" and their twenty-nine inter-specific hybrids. Observations were recorded on November 14.

Genotypes	Brix	Suc %	CCS %
'SES 603'	12.32	8.94	3.79
'Gungera'	13.02	9.40	3.98
ISH-1	15.24	9.83	3.83
ISH-2	15.34	8.84	3.12
ISH-3	14.64	8.88	3.29
ISH-4	15.34	9.33	3.46
ISH-5	14.64	8.38	2.94
ISH-6	15.14	8.35	2.82
ISH-7	16.14	10.27	3.96
ISH-8	15.94	9.83	3.69
ISH-9	15.34	9.83	3.81
ISH-10	14.64	8.35	2.92
ISH-11	14.44	8.38	2.98
ISH-12	12.24	6.46	2.07
ISH-13	17.44	10.23	3.67
ISH-14	16.24	9.79	3.61
ISH-15	16.74	10.27	3.84
ISH-16	13.44	7.42	2.51
ISH-17	15.94	10.32	4.04
ISH-18	14.24	8.38	3.02
ISH-19	14.64	8.88	3.29
ISH-20	–	–	–
ISH-21	12.44	6.46	2.03
ISH-22	14.24	7.89	2.68
ISH-23	14.34	8.38	3.00
ISH-24	13.74	8.91	3.49
ISH-25	13.94	8.41	3.10
ISH-26	13.84	7.42	2.43
ISH-27	15.24	9.33	3.48
ISH-28	13.34	7.42	2.53
ISH-29	12.24	6.46	2.07

the rate of photosynthesis. Thus, the two clones 'SES 603' and 'Gungera' had large difference in their growth and morphological characters. Indeed, this was the basis of selecting these two as parents in the present study. Twenty-nine inter-specific hybrids obtained from crossing of the two parents showed a complete range of variation for tillering, cane girth and other characters. Thus, the two parents and twenty-nine ISH hybrids provided the complete variation in sucrose content and selection of high and low sucrose inter-specific hybrids.

REFERENCES

Attan PS, Dendsay JPS, Dhawan AK (2003). *In vitro* sucrose uptake by internodal slices of sugarcane genotypes varying in maturity and sucrose accumulation potential. Proc. S.T.A.I. 65: 97-109.

Dendsay JPS, Dhawan AK, Sehtiya HL (1992). Biochemical approaches for predicting high sucrose content in sugarcane. Coop. Sugar, 23: 597-604.

Glasziou KT, Gayler KR (1972). Storage of sugars in stalks of sugarcane. Bot. Rev. 38: 471-490.

Grantz DA (1989). Effect of cool temperature on photosynthesis and stomatal conductance in field grown sugarcane in Hawaii. Field Crops Res. 22: 143-155.

Lingle SE, Dyer JM (2001). Cloning and expression of sucrose synthase-1 cDNA from sugarcane. J. Plant Physiol. 158: 129-131.

Lingle SE, Dyer JM (2004). Polymorphism in the promoter region of the sucrose synthase-2 gene of Saccharum genotypes. J. Am. Soc. Sugarcane Technol. 24: 241-249.

MacColl D (1976). Growth and sugar accumulation of sugarcane. I. Percentage of sugar in relation to pattern of growth. Exp. Agric. 12: 369-377.

Ming R, Liu SC, Moore PH, Irvine JW, Peterson AH (2001). QTL Ming, R., Liu, S.C., Moore, P.H., Irvine, J.W. and Peterson, A.H. (2001). QTL analysis in a complex autopolyploid : genetic control of sugar content in sugarcane. Genome Res. 11: 2075-2084.

Sehtiya HL, Dendsay JPS, Dhawan AK (1991). Internodal invertases and stalk maturity in sugarcane. J. Agric. Sci. Cambridge 116: 239-243.

Selvi A, Nair NV, Noyer JL, Singh NK, Balasundaram N, Bansal KC, Koundal KR, Mohapatra T (2005). Genomic constitution and Genetic Relationship among the tropical and subtropical Indian Sugarcane Cultivars revealed by AFLP. Crop Sci. 45: 1750-1758

sucrose Saccharum genotypes. Int. J. Physiol. Mol. Biol. Plants Springer 2010; 16(1): 107-111.

Van HE (1968). Direct microdetermination of sucrose. Anal. Biochem., 22: 280-283.

Vandana V, Dhawan AK (2010). PCR Primers for identification of high

Whittaker A, Botha FC (1997). Carbon partitioning during sucrose accumulation in sugarcane internodal tissue. Plant Physiol. 115: 1651-1659.