Suitable Biomass for a **Sustainable Sugarcane** Industry

UPDATE 4:
Sweet Sorghum as a Supplementary Crop

This project is supported by CRC for Developing Northern Australia, Far Northern Milling Pty Ltd, Sugar Research Australia , and The University of Queensland

Introduction

A challenge facing all sugarcane mills that wish to diversify their income streams is to use the processing capacity for most of the year. The crushing season in Australia generally lasts about 22 weeks. For the Far Northern Milling Company, the problem is exaggerated by the fact that there is already a shortfall in available biomass during the short crushing season.

The length of the cropping cycle is largely determined by the physiology of the sugarcane crop and the weather conditions. Sugar milling is usually confined to the period from June to December to take advantage of the higher sugar content of cane and to avoid the wet season which extends from late December through to March.

Ideally, the cane harvesting and crushing season must be completed by mid-to-late November. This is to allow sufficient time for ratoon crops to establish before the start of the rainy season sometime in January or February. Extending the crushing season into mid- or even late-December creates a problem as it reduces the growing season length of the ratoon crop cycle [15].

Harvesting under-aged or over-aged cane leads to losses in cane yield, sugar recovery, poor juice quality, and other milling problems due to extraneous matter.

For year-round operation, and to address the current shortfall in total biomass availability two options should be considered. Firstly, if sucrose is no longer the main emphasis then alterations to the sugarcane cropping cycle can be considered [20, 21]. This approach led to a farming system aimed at maximum biomass mass production i.e., “Energycanes”. Secondly, other feedstocks, besides sugarcane, as supplemental feedstock can be considered.

In a pioneering study, it was shown that both energy cane and sweet sorghum, which have harvest times different from sugarcane, were similar in gross structure and chemical composition and could be handled by a traditional sugarcane harvest and processing system [13]. Utilization of energycane and sweet sorghum outside the sugarcane season in Louisiana has the possibility to increase ethanol production as well as expand the feedstock supply. However, this study also shows that many challenges remain for the successful incorporation of new crops into the existing sugarcane

In this update

- Growth of sweet sorghum
- Flowering
- Pest and diseases issues
- Biomass composition

In the picture above sweet sorghum (Megasweet) 45 days after planting in Tablelands.

“Energycane and sweet sorghum has different harvest times than conventual sugarcane but can be processed for using the same equipment currently used in Sugarcane Mills”.

infrastructure and the possibility of partitioning feedstocks for both fuel and sugar during normal sugarcane processing [2].

In this update, the challenges in growing sweet sorghum in tropical conditions in Australia are highlighted. In addition, the chemical composition of the sweet sorghum under tropical conditions is presented.

Growth

A total of four different sorghum trials were conducted with 18 different sorghum genotypes at Singh Farming Pty Ltd ATF Singh Farming Business enterprise Trust in the Atherton Tablelands.

Seeds were treated with CONCEP II at a dose of 36g 20kg^{-1}) 24h before planting. Beds with a 1.8m spacing were formed with a bed former and Yaramila Complex fertilizer was broadcast to the top of beds at 665 kg ha^{-1}. This provided 80kg nitrogen, 33kg phosphate, 100kg magnesium, 100kg potassium, and 53kg sulphate per hectare.

In the first genotype screening trial two rows, and in all subsequent fully replicated trials, three rows were planted per bed at 40cm spacing. The planting depth was 30 – 35mm, and the seeding rate was 4 – 5 kg ha^{-1}.

Germination is rapid and the crop is well established in less than 25 days (Fig.1&2). Sorghum growth is characterised by a relative short lag phase between planting and exponential growth (Fig.3). At the completion of the vegetative growth phase approximately 70% of the total biomass is represented by the stems and 30% by the leaves.

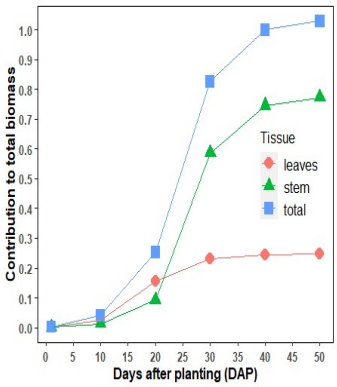


Figure 3: Pattern of biomass accumulation in sorghum. Data is expressed as relative to the final total biomass.

There are major differences in the yield between the sorghum genotypes (Table 1). Three of these sorghums (SE45, SE19 and Megasweet) from this trial plus two others (Dynasweet and SK106) were evaluated further in fully replicated trials.

Megasweet and SK106 were the highest yielding varieties and had the highest stalk populations (Fig.4).

Sweet sorghum has a 3-month crop cycle and can be cultivated twice per year. Research in Brazil and the USA showed that sweet sorghum can be grown and harvested before and after sugarcane season so as to extend the period of operation of a distillery [3, 6, 11].

However, it is also evident that planting in autumn results in much lower yields than a summer planting (Table 1 and Fig.4). Previous work has also shown that the time of planting has a profound effect on the juice quality and levels of soluble sucrose [6]

Figure 1: Sorghum seedlings 25 days after planting (DAP).

Figure 2: Sorghum (Megasweet) plants 45 days after planting.

Table 1: Yield of 14 sorghum genotypes in the Tablelands. Plants were harvested 65 days after planting. Planting was done in early December.

Genotype	Fresh Biomass Yld (tph)	Plant Population	Flowered
SE35	82.3	135844	N
SE23	78.4	172197	N
SE81	77.5	166457	Y
SE86	70.8	170284	Y
SE1	67	151151	N
SE42	67	130104	Flagging
SE2	64.1	151151	Y
SE78	61.2	156891	Y
SE45	58.4	107145	Y
SE5	57.4	130104	N
SE20	49.7	126278	N
SE19	47.8	89925	Y
SE106	47.8	132018	Y
MegaSweet	46.9	116711	Y

* Flagging= flag leaf visible start of transition to flowering

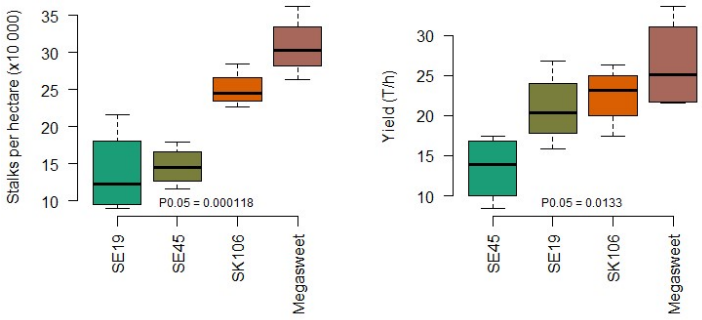


Figure 4: Stalk population (A) and yield (B) of four sweet sorghum genotypes in the Tablelands. Plants were planted in April and harvested 58 days after planting. All the varieties flowered more than 90% at this stage.

Flowering

Floral initiation marks the end of vegetative growth of sorghum. The transition of the vegetative apex into a reproductive apex is primarily controlled by genetics, temperature, and photoperiod. The grand period of growth in sorghum follows the formation of a floral bud and consists largely of cell enlargement.

All the sorghum varieties used in this project flowered. All 15 genotypes in the unreplicated small plot screening trial planted on 02/10/2021 flowered at 62 DAP. Genotypes SE1, SE5, SE20, SE23 & SE35 are slower to reach flowering than the other varieties (Table 1).

"Planting in spring and early summer results in much higher yields than a late summer/autumn planting"

Figure 4: Sorghum (SK106) in the background 135 days after planting in a trial at Toowoomba. Note the absence of flowering in a crop that is more than 3.2m tall.

"Early flowering is useful for grain production but a negative factor for vegetative growth and stem biomass production"

"The lack of long days and warm temperatures in the tropical production conditions in the Tablelands and Mossman are serious impediments for successful biomass production of sweet sorghum"

For successful integration of sorghum into a sugarcane-based production system genotypes will have to be found that have a much longer vegetative growth period. In sorghum delayed flowering increases the size of stems and the potential for sucrose accumulation [7, 9, 10]. Delayed flowering and long duration of vegetative growth is a key trait associated with high biomass yield and nitrogen use efficiency.

In our search for such a variety a new hybrid (SK106) that is ultra-late flowering, mid-range sugar and high biomass was identified (Fig 4)[1]. However, in fully replicated trials including SE19, SE45, Megasweet and SK106 all the genotypes were either flowering or flagging was already initiated 59 DAP.

What controls flowering

Sorghum is a typical short-day plant (SDP), and the variation in its response to photoperiod (day length) and temperature determines in which areas it can be successfully grown. As with many other physiological processes the transition to flowering is at least partially dependent on the accumulation of heat units above a base temperature around 9.5°C [17].

The genetic control of flowering has been studied extensively [5] and flowering under different conditions accurately modelled [17]. More than 40 QTL for flowering have been identified for sorghum [8]. The two main environmental factors controlling flowering are photoperiod and temperature.

Photoperiod sensitivity, and hence flowering time in sorghum is controlled through the maturity alleles Ma_1 through Ma_6 [5]. Ma_1 encodes *PRR37* an inhibitor of flowering in long days [12]. Ma_6 encodes the floral inhibitor *SbGhd7.* Expression of *SbGhd7* is controlled by the circadian clock and light signalling. Genotypes that are dominant for both Ma_1 and Ma_6 will flower very late under long day conditions. It is likely that a genotype such as SK106 would fall into this category.

Seasonal variation in photoperiod decreases with latitude and hence variation in daylength is small in the tropics compared to temperate regions. The small variation in daylength and warm temperature in the tropics are optimal for development from sowing to flowering. Under these circumstances most genotypes will flower and mature too early in the tropics to accumulate sufficient biomass.

Pest and disease challenges

In all the trials armyworm was a significant problem (Fig. 5) and needed to be controlled with a spray of Althachlor (active ingredient Chlorantraniliprole) at a rate of 150g ha^{-1}.

It is known that Pokkah boeng disease(PBD) caused by *Fusarium subglutinans* affects sugarcane and sorghum [4]. In fact, the first report on the occurrence of PBD in sorghum dates back to 1941 [22]. In the first season of this project and in all the initial screening trails PBD was not observed.

Figure 5: Damage caused by fall army worm (*Spodoptera frugiperda)* to the spindle leaves

Figure 6: Pokkah boeng disease (PBD) in the sorghum trials in the Atherton Tablelands.

[1] Ivan Calvert, Australian Research Operations Manager, GenTech Seeds Pty Ltd. Ivan.Calvert@GenTechSeeds.com

However, in the fully replicated trials in the second half of 2021PDB became a major issue. The varieties that suffered less severe symptoms are recovering but varieties that suffered top rot are severely compromised and forced the abandoning of trials (Fig.6).

Characteristic symptoms of the disease in sorghum include appearance of deformed or discoloured leaves near the top of the plant. Sometimes, the leaves become wrinkled, twisted, and do not unfold properly giving a ladder-like appearance. Other symptoms of the disease commonly noticed are wrinkling of leaf-bases and appearance of small, transverse cuts in the leaf margin, stem bending, and twisting of nodes and internodes. In extreme cases, infection may move from leaf and sheath into stem causing top rot [4 and references therein].

The presence of pokkah boeng in the sorghum trials was confirmed by Robert Magarey[2]. There were also numerous reports in 2021 of PBD in sugarcane on the wet tropical coast.

Fall armyworm damage is minor compared to damage caused by Pokkah boeng.

Biomass composition

Plant samples were collected in-field and transported to the laboratory where the stalks were separated into leaves and stem. The samples were dried for 96h at 70°C and then grinded to a particle size of less than 500µm.

Methods

Extraction

To prevent interference with the acid hydrolysis all extractable components were removed from the biomass before lignocellulosic analysis [14, 18]. A Dionex Accelerated Solvent Extractor (ASE) 200 was employed for extractives removal using water and/or 95% ethanol as solvents and a pressure of 1500 PSI, a temperature of 100°C for 5 min and a static cycle time of 7 min. Three static cycles were used for each sample and the total flush volume was 150%. After extraction the remaining solid was air dry for 2 days. Then, the moisture content of the sample was determined. Extractives were determined as the loss in dry matter associated with the extraction. As well as water and ethanol extractions, a "full" extraction was undertaken which involved a water extraction (3 static cycles) followed by an ethanol extraction (3 static cycles). For water extractions the weight of the liquid extract collected was recorded and a subsample was taken for soluble sugars analysis using ion chromatography.

Hydrolysis

A procedure similar to the Uppsala Method [1] was used for the acid hydrolysis of the fully extracted sample. This involved hydrolysis with 72% H_2SO_4 (3 mL) at 30° C, for 1 h with constant stirring. After dilution of the acid to 4% by addition of water the tubes were sealed and autoclaved at 121°C for 60 min. Standard sugar solutions were processed

"Care were taken to ensure that all soluble components are removed from the biomass to ensure proper acid hydrolysis and estimation of Klason lignin, acid soluble lignin, and structural carbohydrates"

[2] Dr Robert Magarey, Sugar Research Australia, Tully (r.magarey@sugarresearch.com.au)

at the same time to determine losses and to allow correction. After cooling to room temperature, the hydrolysates were filtered (using vacuum suction), through filter crucibles of known weight, and the resulting filtrate was stored. Residual solids were washed from the tube using deionised water until all the residue resided on the filter crucible. This was then dried overnight at 105° C and weighed to determine the Acid Insoluble Residue (AIR) content. The filter crucible was then ashed to determine the acid-insoluble ash (AIA) content. The Klason lignin content was determined as AIR minus AIA.

Acid soluble lignin (ASL)

The hydrolysate was placed in a 1 cm path-length (3 mL volume) quartz cuvette and diluted with water until the UV-absorbance was within a linear region. The spectrum of the sample was collected in transmission mode using a HP Agilent 8452A diode array spectrophotometer. The absorbance at 205 nm was used to determine the ASL content using an absorptivity constant of 110 [19].

Chromatography conditions

The hydrolysates were diluted and known concentration of the internal standard melibiose added. Analysis was done on a DIONEX ICS-3000 ion chromatography system comprising: an electrochemical detector (using Pulsed Amperometric Detection, PAD), a gradient pump, a temperature controlled column and detector enclosure, and an AS-AP autosampler [16].

For the analysis of the sugars in the water extract, the same conditions were used except the column temperature was reduced to 17°C to resolve sucrose and fructose.

Main components of biomass

For the purpose of this document the biomass composition of sorghum leaves and stems were pooled (Fig 7). The data shows that the leaves and stems have a similar composition at the higher level where approximately 30% of the total dry mass is water soluble (Fig 7A).

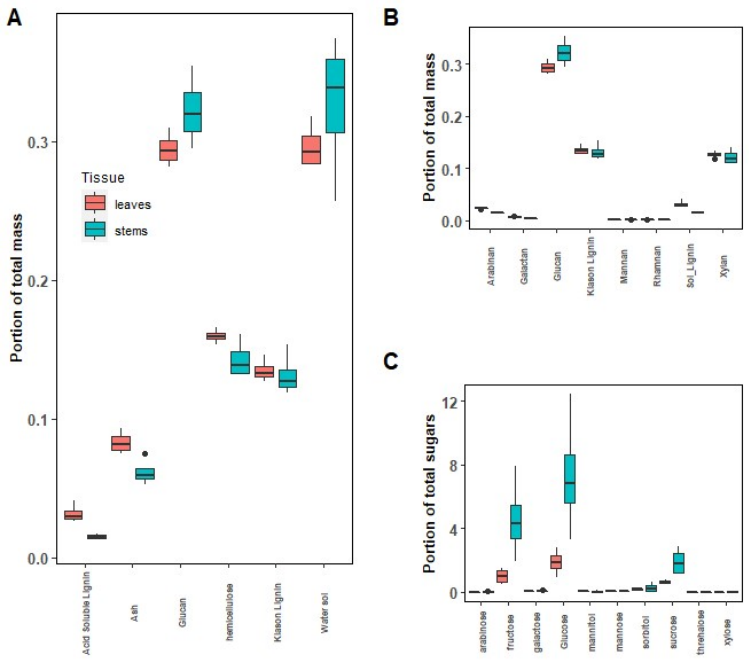
The large variation in cell wall composition and soluble sugar concentrations within the same tissue type reflects genotype differences and this aspect will be further analysed in subsequent work.

The cell wall is primarily made up of cellulose (glucan) and equal proportions of lignin and hemicellulose with no significant differences between the leaves and stems (Fig 7B). The glucan levels vary between 28-35%, lignin between 12-16% and hemicellulose 13-18%. The most abundant pentose sugar is Xylose (Fig 7B).

The main differences between the leaves and stems are in the water soluble sugars (Fig 7C). Total water-soluble sugars in the leaves vary between 2-5% and in the stems between 8-22%.

In the stems glucose, fructose and sucrose are more abundant than the other sugars. It is also obvious that glucose and fructose levels are significantly higher than that of sucrose in both stems and leaves. In the stems glucose and fructose represent between 60 and 90% of the total water-soluble sugar.

"The very high reding sugar content in sweet sorghum juice will make it unsuitable for sucrose recovery but would provide an excellent fermentation substrate"



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References

1. Theander, O, P Åman, E Westerlund, R Andersson, and D Pettersson, ***Total dietary fiber determined as neutral sugar residues, uronic acid residues, and klason lignin (the uppsala method): Collaborative study.*** Journal of AOAC International, 2020. **78**(4): 1030-1044.
2. Patil, V, HI Mcdermott, T Mcallister, M Cummins, JC Silva, E Mollison, R Meikle, J Morris, PE Hedley, R Waugh, C Dockter, M Hansson, and SM Mckim, ***Apetala2 control of barley internode elongation.*** Development, 2019. **146**(11): dev170373.
3. Mathur, S, AV Umakanth, VA Tonapi, R Sharma, and MK Sharma, ***Sweet sorghum as biofuel feedstock: Recent advances and available resources.*** Biotechnology for Biofuels, 2017. **10**(1): 146.
4. Das, IK, S Rakshit, and JV Patil, ***Assessment of artificial inoculation methods for development of sorghum pokkah boeng caused by fusarium subglutinans.*** Crop Protection, 2015. **77**: 94-101.
5. Murphy, RL, DT Morishige, JA Brady, WL Rooney, S Yang, PE Klein, and JE Mullet, ***Ghd7 (ma6) represses sorghum flowering in long days: Ghd7 alleles enhance biomass accumulation and grain production.*** The plant genome, 2014. **7**(2): 1-10.
6. Rao, PS, CG Kumar, and BVS Reddy, ***Sweet sorghum: From theory to practice,*** in *Characterization of improved sweet sorghum cultivars*, PS Rao and CG Kumar, Editors. 2013, Springer India: India. 1-15.
7. Olson, SN, K Ritter, J Medley, T Wilson, WL Rooney, and JE Mullet, ***Energy sorghum hybrids: Functional dynamics of high nitrogen use efficiency.*** Biomass and Bioenergy, 2013. **56**(0): 307-316.
8. Mace, ES, CH Hunt, and DR Jordan, ***Supermodels: Sorghum and maize provide mutual insight into the genetics of flowering time.*** Theoretical and Applied Genetics, 2013. **126**: 1377-1395.
9. Burks, P, TJ Felderhof, HP Viator, and WL Rooney, ***The influence of hybrid maturity and planting date on sweet sorghum productivity during a harvest season. Agron. J. 105:263–267.*** Agronomy Journal, 2013. **105**: 263-267.
10. Olson, SN, K Ritter, WL Rooney, A Kemanian, BA Mccarl, Y Zhang, S Hall, D Packer, and J Mullet, ***High biomass yield energy sorghum:***

Developing a genetic model for c4 grass bioenergy crops. Biofuels Bioprod Biorefin, 2012. **6**: 640-655.
11. Kim, M, K-J Han, Y Jeong, and DF Day, ***Utilization of whole sweet sorghum containing juice, leaves, and bagasse for bio-ethanol production.*** Food Science and Biotechnology, 2012. **21**(4): 1075-1080.
12. Murphy, RL, RR Klein, DT Morishige, JA Brady, WL Rooney, FR Miller, DV Dugas, PE Klein, and JE Mullet, ***Coincident light and clock regulation of pseudoresponse regulator protein 37 (prr37) controls photoperiodic lowering in sorghum.*** Proc. Natl. Acad. Sci. USA 2011. **108**: 16,469-416,474.
13. Kim, M and DF Day, ***Composition of sugar cane, energy cane, and sweet sorghum suitable for ethanol production at louisiana sugar mills.*** Journal of Industrial Microbiology & Biotechnology, 2011. **38**.
14. Villaverde, JJ, RMA Domingues, CSR Freire, AJD Silvestre, CP Neto, P Ligero, and A Vega, ***Miscanthus giganteus extractives: A source of valuable phenolic compounds and sterols.*** Journal of Agricultural and Food Chemistry, 2009. **57**: 3626-3631.
15. Di Bella, LP, S J.K., AW Wood, AR Royle, and GP Holzberger, ***What impact does time of harvest have on sugarcane crops in the herbert river district?*** Proceedings Australian Society of Sugar Cane Technologists, 2008. **30**: 337-348.
16. Anon, *: Analysis of carbohydrates by high performance anion exchange chromatography with pulsed amperometric detection (hpaepad).* Dionex, Editor. 2000, Dionex Corporation: , Sunnyvale, CA.
17. Craufurd, PQ, V Mahalakshmi, GL Hammer, FR Bidinger, SZ Mukuru, J Chantereau, PA Omanga, A Qi, EH Roberts, RH Ellis, and RJ Summerfield, ***Adaptation of sorghum : Characterisation of genotypic flowering responses to temperature and photoperiod.*** Theoretical and Applied Genetics, 1999. **99**(5): 900-911.
18. Thammasouk, K, D Tandjo, and MH Penner, ***Influence of extractives on the analysis of herbaceous biomass. .*** Journal of Agricultural and Food Chemistry, 1997. **45**: 437-443.
19. Anon, *Tappi standard um 250. Acid-soluble lignin in wood and pulp.* 1991, TAPPI J: Atlanta, GA
20. Alexander, AG, ***Sugarcane as a source of biomass*****,** in *Fao expert consultation on sugarcane as feed*, R Sansoucy, G Aarts, and TR Preston, Editors. 1988, FAO: Roma.
21. Alexander, AG, ***The energy cane alternative***. Sugar sciences. 1985, New York: Elsevier.
22. Ramakrishnan, TS, ***Top rot (twisted top or pakkah bong) of sugarcane, sorghum and cumbu.*** Current Science India, 1941. **10**: 406-408.

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