NORTHERN HEALTH SERVICE DELIVERY

TRADITIONAL OWNER-LED DEVELOPMENT

AGRICULTURE & FOOD

Suitable Biomass for a Sustainable Sugarcane Industry

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CRCNA
DEVELOPING NORTHERN AUSTRALIA

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Acronyms

AGR	Absolute growth rate (g time^{-1})
AIR	Acid insoluble residue
ASE	Accelerated Solvent Extractor
ASL	Acid soluble lignin
BMP	Biomethane potential
Brix%	Amount of dissolved solids in solution
CCS	Commercial Cane Sugar (calculated using Brix%, Pol% and Fibre)
CDD	Culm growth day degrees
CONCEP II	Formulation that ontains 700g/kg OXABETRINIL to protect against herbicide damage
DAP	Days after planting
DD	Day degrees
DW	Dry weight
Fibre	Residue after removal of all the water soluble components from sugarcane biomass
FNM	Far North Milling company
FW	Fresh weight
GC-MS	Gas chromatography followed by mass spectrum analysis
GL	Green leaf
GSD	Ground sampling distance
HMDB	Human Metabolome Database (https://hmdb.ca/about)
HSD	Tukey HSD pot hoc tests to compare group means
LC	Cabbage or cane tops
LSD	Least significant difference
MS	Millable stalk
NDVI	Index calculated from the red and near-infrared radiation reflectance
NIR	Near-infrared radiation with a wavelength spanning 780 to 2500 nanometers
PBD	Pokkah boeng disease caused by the fungus *Fusarium subglutinans*
PDD	Phyllochron production day degrees
Phyllochron	The intervening period between the sequential emergence of leaves on the main stem of a plant, also rendered as leaf appearance
PI	Phyllochron interval
Pol%	For solutions containing pure sucrose in water, pol % juice is the same as sucrose % juice
RGB	Red, green and blue radiation
RGR	Relative growth rate (g g^{-1} time^{-1})
RPA	Remote pilotted aircraft
SD	Standard deviation
SE	Standard error
SRA	Sugar Research Australia
SVM	Support vector machine
TCH	Tonne cane per hectare
TH	Tonne per hectare
UAV	Unmanned aerial vehicle, commonly know as a drone
UV	Ultraviolet radiation
VIP	variable importance in projection

Project Participants

Executive Summary

The project aimed to achieve three main objectives: first, to explore the feasibility of enhancing total biomass production in the FNM production area using sugarcane genotypes that aren't solely chosen for high sucrose yield; second, to assess the potential of supplementing the sugarcane biomass delivery to the mill by planting sweet sorghum; and third, to investigate the possibility of extracting other high-value products from any of the biomass components of sugarcane or sweet sorghum.

In the Mossman and Tablelands production areas, we tested seventeen different sugarcane genotypes, including commercial standard varieties and type I energy canes. Our findings showed that increasing biomass yield by up to 25% is possible, but this would result in a significant decrease in sucrose concentration in the biomass. As a result, cane quality will be lower and sucrose recovery during processing would decrease. Despite this, total sucrose and sugar yields per hectare would remain high, and the juice could serve as an excellent fermentation substrate for producing biofuels or other chemicals.

The accumulation of sucrose is negatively correlated with the growth rate of sugarcane. It has been observed that environments with small oscillations between day and night temperatures have poorer production of recoverable sucrose. This means that cane quality, in terms of recoverable sucrose, is better in the Tablelands compared to Mossman, and would deteriorate further in more tropical production conditions.

The sugarcane crop experiences its highest growth rate around six months after planting and earlier in a ratoon crop. Although biomass gain during the last three months of the cycle is low, it plays a crucial role in the maturation and accumulation of sucrose. Nonetheless, the findings indicate that the sugarcane cropping cycle could be notably shortened if sucrose is no longer the primary focus.

The data shows a significant difference in the lignocellulosic fraction between the sugarcane genotypes in terms of the total portion of biomass, the ratio between cellulose and hemicellulose, lignin, acetylation levels, and uronic acid content. This suggests that the process of extracting cell wall sugars will vary greatly between the different genotypes. As a result, when developing and selecting energy canes, it is essential to consider cell wall properties rather than simply looking at the total fibre content.

Incorporating sweet sorghum as a supplementary crop in tropical conditions poses a significant challenge due to the lack of variation in day length, resulting in early flowering and reduced vegetative biomass yield. Studies conducted in Tablelands and Toowoomba in Queensland showed a production loss of over 50% in tropical growth conditions. Although the lignocellulosic fraction of sweet sorghum is similar to sugarcane and can be used in bagasse applications, its juice contains lower sucrose levels, making it unsuitable for sucrose crystallisation. However, the juice can be an ideal fermentation substrate, similar to the juice from type I energy canes.

Sugarcane and sweet sorghum leaves contain over 200 stable metabolites, while the stalks contain around 100. The secondary metabolites found in leaf extracts are essential for plant health and have various uses in food processing and pharmaceuticals. However, probably only 19 of these metabolites can be extracted from the juice in sufficient amounts. Some of these metabolites have a high market value, but their world market is relatively small. Fermentation processing can produce all of these metabolites, making it a more cost-effective alternative to extracting them from sugarcane juice. The best probable use of sugarcane juice would be through concentration to maximise nutritional and pharmacological uses.

Project Motivation

Far Northern Milling Pty Ltd (FNM) in Mossman and the wider Australian sugar industry face three major obstacles in achieving the abovementioned goals. Firstly, there is inadequate knowledge about the best tropical varieties. The current variety profile in Mossman is optimised for sucrose-only production, neglecting the full potential of the feedstock. Secondly, there is no information available about biomass composition apart from sucrose and fibre. Lastly, there needs to be more diversification and value addition regarding bagasse and molasses.

Sugarcane is a significant component of the tropical and subtropical Australian economy, but low international market prices for sucrose necessitate alternative and additional revenue streams from sugarcane biomass. The project aims to identify genotypes that can increase total biomass production per unit of land area. High biomass genotypes that can retain the current sucrose production while increasing the total bagasse and molasses will be given preference. A comprehensive chemical analysis will be conducted to identify potential ways to add value to the fibre, molasses, and cane tops.

During the project, commercial and near-commercial varieties, energy canes, and a few sweet sorghum genotypes will be evaluated for their potential to ensure a year-long feedstock supply to the Mossman mill. These trials will cover the production conditions of Mossman and Tableland.

The Far Northern Milling Company faces a challenge in using the processing capacity for most of the year to diversify its income streams. The crushing season in Australia generally lasts about 22 weeks, and there is already a shortfall in available biomass during that time. Three options should be considered to address the current shortfall and enable year-round operation. Firstly, alterations to the sugarcane cropping cycle can be considered if sucrose is no longer the primary emphasis (Alexander, 1988, 1985). Secondly, energy canes, Type I or II, can be used to increase biomass yield (de Souza Barbosa et al., 2020; Tew and Cobill, 2008). Thirdly, a supplementary crop such as sweet sorghum can be considered to add to total biomass production, especially at the front and backend of the sugarcane cropping cycle (Kim and Day, 2011).

Towards a biomass economy

The world is currently facing two significant issues: changes in global climate and the depletion of fossil fuel reserves. The primary cause of these problems is the high consumption of fossil fuels, which comprise approximately 80% of global energy usage (Seh et al., 2017; Alalwan et al., 2019). Given the growing world population and worsening climate change conditions, developing, and implementing sustainable methods for producing biomass has become increasingly crucial.

There are several ways to create clean energy, including wind, solar, and hydropower. However, biomass is significant because it provides liquid fuels for transportation. Biofuels are classified as first, second, third, or fourth generation, depending on the feedstocks and production processes used (Saladini et al., 2016; Alalwan et al., 2019).

Biomass utilisation as a renewable carbon source involves more than just biomass conversion to biofuels. The concept of biorefineries was introduced to make the most of biomass and reduce waste and emissions (Cherubini, 2010). Biorefineries are used to efficiently produce valuable items from various sources, such as lignocellulosic biomass, algal biomass, microbial-treated wastes, and manures (Ubando et al., 2020). Enzymatic techniques have also been integrated with biorefineries to create advanced biofuels (Singh et al., 2019). The circular bioeconomy, which involves using biomaterials in technical and production cycles beyond the biological cycle, offers opportunities for reuse, recycling, and remanufacturing (Benetto et al., 2018).

As the world faces mounting pressure to reduce global warming and environmental pollution by moving away from fossil fuels, new opportunities are emerging to develop a sustainable biomass-based economy. This has enormous potential to create jobs, but it will require entrepreneurship and innovation to unlock those opportunities. Specifically, we need to utilise hemicellulose and lignin fully, convert sugar into different high-value products, improve low-cost biomass pre-treatment technologies, use more efficient fermentation technologies, and gain a better understanding of crop chemical composition.

Plants that can produce a lot of biomass have the potential to help with global issues like energy security and climate change. Often, when crops are harvested, only part of the plant is used for food, feed, or fibre, while

the rest (like wheat straw, corn stover, or sugarcane bagasse) is burned for power or turned into biofuel through processes like pyrolysis or fermentation (Botha and Moore, 2013 and references therein)

One of the main obstacles to creating advanced biomass technologies is obtaining affordable feedstock. The cost of acquiring feedstock makes up 80 to 90% of the total fuel price for many processes, making it essential for the long-term viability of biomass-dependent technologies.

Sugarcane as a biomass crop

This extraordinarily high yield and well-established farming and processing technologies make sugarcane a leading candidate for bioenergy production and suitable feedstock for bio-refineries.

Sugarcane is a highly advantageous biomass crop due to its extensive history of industry research and development and the existing infrastructure currently used for traditional sugar production. The agronomic infrastructure comprises large-scale systems for planting, cultivation, irrigation, fertilisation, weed control, pest, and disease control, and harvesting. The processing infrastructure includes sugar mills, which are sometimes connected to downstream manufacturing or refining facilities. Along with growing and processing, extensive breeding research and development programs are conducted to produce new sugarcane varieties that are improved for yield and can overcome problems associated with existing varieties.

Sugarcane, like most crops, is a seasonal bioenergy crop. This is a drawback for bioenergy refineries that need to operate year-round. In sugar production, sugarcane is typically harvested when the sucrose concentration of the juice is at its maximum or when the cane is ripe. In subtropical regions, the season for maximum sucrose yield is limited to 3-4 months of late autumn and early winter. However, the harvest season can last as long as 12 months in tropical regions. If sugarcane is the only biomass feedstock for a biorefinery, adjustments must be made to the sugarcane cropping system to allow year-round harvesting and processing.

The aboveground (aerial) sugarcane biomass is partitioned between green leaves, trash (dead leaves), and the stalk (Inman-Bamber, 2013). In sugarcane, it is customary to refer to the living aboveground biomass as the cabbage and millable stalk. The cabbage is generally considered to be the green leaves and young internodes above the ‘natural breaking’ point. In very young cane, the cabbage is the total aerial biomass, but in fully mature cane, it comprises approximately 10% of the biomass. The immature internodes comprise a small portion (<10%) of the cabbage biomass. The total number of green leaves per stalk depends on the genotype, growth conditions, water availability, and row spacing (Inman-Bamber, 2013). The number of green leaves increases to a maximum of around 15 and then decreases to around 8-10. Water stress (Inman-Bamber, 2013) and other physiological stresses (Botha, 2019; Botha et al., 2023; Inman-Bamber, 2013; Lingle, 1999; Singels and Inman-Bamber, 2011) can significantly reduce the number of green leaves per stalk. Some dead leaves, also known as trash, will stay on the stalk, while others will fall off. The amount of trash that sticks to the stalk differs depending on the genotype, with an average of around 15% of the above-ground biomass being trash. During harvesting, about 70-80% of the trash is left in the field, while the remaining amount is taken to the mill as extraneous matter.

The crop age and development stage are key drivers of profitable sugarcane production if the emphasis is on maximising sucrose yield. Sucrose accumulation in sugarcane only occurs after growth and cellular elongation have slowed (Botha, 2019; Botha et al., 2023; Inman-Bamber, 2013; Lingle, 1999; Singels and Inman-Bamber, 2011).

The production of biomass in sugarcane is closely tied to how the plant's carbohydrates are distributed throughout the entire plant. The factors determining how much the plant requires, or "sink strength," have been a topic of discussion for a long time. Nevertheless, it is commonly agreed upon that a plant organ's ability to import photoassimilates is dependent on its competitive capacity, which is influenced by both the size and activity of the sink (Bihmidine et al., 2013; Herbers and Sonnewald, 1998; Ho, 1988; Slewinski, 2012). Sink strength refers to the rate at which a specific organ or tissue increases in dry weight. The dry weight accumulation is determined by the amount of nutrients imported minus the amount exported and used during respiration (Doehlert, 1993).

The volume of the stalk primarily determines the amount of biomass accumulated in sugarcane stalks. A larger volume requires more osmolytes to maintain the necessary water potential gradients, and the

expanding cells need to synthesise more cell walls, cell membranes, proteins, and other precursors to sustain cellular functions. This increased biosynthesis demand is reflected in demand functions associated with cell wall biosynthesis, secondary metabolism, and respiration. (Botha et al., 2023). The hexose and hexose-phosphate pools derived from the imported sucrose are the source to meet these metabolic demands.

Sucrose accumulation in the sugarcane stalk can be classified into two stages (Inman-Bamber et al., 2002; Robertson et al., 1996; van Dillewijn, 1952). The top 8 to 10 internodes have a sharp gradient in sucrose

content. In the rest of the stalk, sucrose content has reached a maximum and remains constant (mature culm). Maximum sucrose levels in internodes are reached after 800-1000 degree days (Lingle, 1999).

It should be evident from the diagram (Figure 1) that the faster the growth and/or the higher the maintenance respiration component, the less sucrose will accumulate. In addition, because growth and maintenance respiration require sucrose to be broken down into glucose and fructose, the purity of the juice will be poor.

Hence, high biomass sugarcane (energy canes) will have high structural carbon content, lower sucrose and higher reducing sugar concentrations than conventional sugarcane.

Two options should be considered for year-round operation and to address the current shortfall in total biomass availability. Firstly, if sucrose is no longer the main emphasis, alterations to the sugarcane cropping cycle can be considered (Alexander, 1988, 1985). This approach led to a farming system aimed at maximum biomass mass production, i.e., “Energy canes”. Alternative sugarcane varieties can be developed that have superior growth rates and biomass yield (Carvalho-Netto et al., 2014; de Souza Barbosa et al., 2020; Tew and Cobill, 2008). Energy canes might also be the ideal feedstock for a farming system aimed at biomass yield rather than sucrose yield. Secondly, other feedstocks, besides sugarcane, as supplemental feedstock can be considered.

Figure 1: Sucrose links photosynthesis, growth and cellular maintenance.

Worldwide, there is an interest in further developing the energy cane concept. Broadly, sugarcane can be classified into three groups (Tew and Cobill, 2008)

- Traditional sugarcane varieties contain about 75% water, 12% fibre, and 13% sugar. This raw material provides juice for sugar and ethanol production, and fibre for electricity production,
- Type I energy can varieties bred to maximise sugar and fibre yield. This type of cane, conceptualised by (Alexander, 1985), has lower water content (65%), fibre ranging from 13% to 17% and a small reduction of the sugar content. This raw material, in addition to providing juice for sugar and ethanol production, contributes with more fibre for the greater production of electricity, lignocellulosic ethanol, as well as other derivatives of economic value for the sugarcane industry,
- Type II energy cane variety selected to maximise fibre yield (fibre> 30%), with insignificant sugar content and lower water content (60%). This raw material is not of interest to the current sugarcane industry, being required by other Agro-industry sectors that need biomass for energy generation.

Within the Type II energy canes, it is possible to differentiate two types (Santchurn et al., 2014). Those clones where the compromise between high fibre and sucrose content is moderate and those where the compromise is severe.

Sorghum as a complementary crop

Sorghum is a versatile crop that can supplement the biomass supply at the Mossman mill. Originating from Africa, sorghum has many uses, including food from its grains, feed from its leaves, fibre from its bagasse, and fuel from its sugary juice. It can be grown worldwide in tropical, semi-tropical, and semiarid regions due to its high photosynthesis rate, water resistance, and nutrient efficiency (Mathur et al., 2017).

Like sugarcane, sweet sorghum produces a high sugar concentration in its culm/stalk. The juice contains a mixture of sugars, including sucrose, glucose, and fructose, which can be directly fermented into a first-generation biofuel. The bagasse can be used as fodder, as heat generation through burning, or as a raw material for second-generation biofuels after pretreatment (Bihmidine et al., 2015; Mathur et al., 2017)).

A study showed that sweet sorghum and energy cane, which have different harvest times from sugarcane, have similar chemical compositions and structures and can be processed by traditional sugarcane harvest and processing systems (Kim and Day, 2011). This presents an opportunity to increase ethanol production and expand the feedstock supply outside the sugarcane season in Louisiana. However, incorporating new crops into the existing sugarcane infrastructure and partitioning feedstocks for both fuel and sugar during normal sugarcane processing remain challenging (Mathur et al., 2017).

Opportunities and challenges

For sugarcane mills to maintain success, they must address two significant factors: feedstock availability and market volatility. Securing a consistent and high-quality supply of sugarcane feedstock is a constant challenge due to climate changes, seasonal fluctuations, and competition for land use. The global sugar and ethanol markets are also prone to price fluctuations, which can impact the financial sustainability of sugarcane mills and their ability to manage costs and revenues.

Feedstock supply can only be addressed by producing more biomass from sugarcane or through integration with rotational crops or intercropping systems. Energy canes, which are bred for enhanced biomass production, have higher cellulose content in their stalks, making them suitable for advanced biofuel production like cellulosic ethanol. However, this comes with a significant penalty in sucrose yield.

There are many opportunities for sugarcane diversification, including biofuels, bioplastics, energy generation, and platform chemicals to generate value-added products. The two main components of sugarcane processing, namely bagasse and juice, can be better exploited to derive more economic value than with the current focus at Australian sugarcane mills.

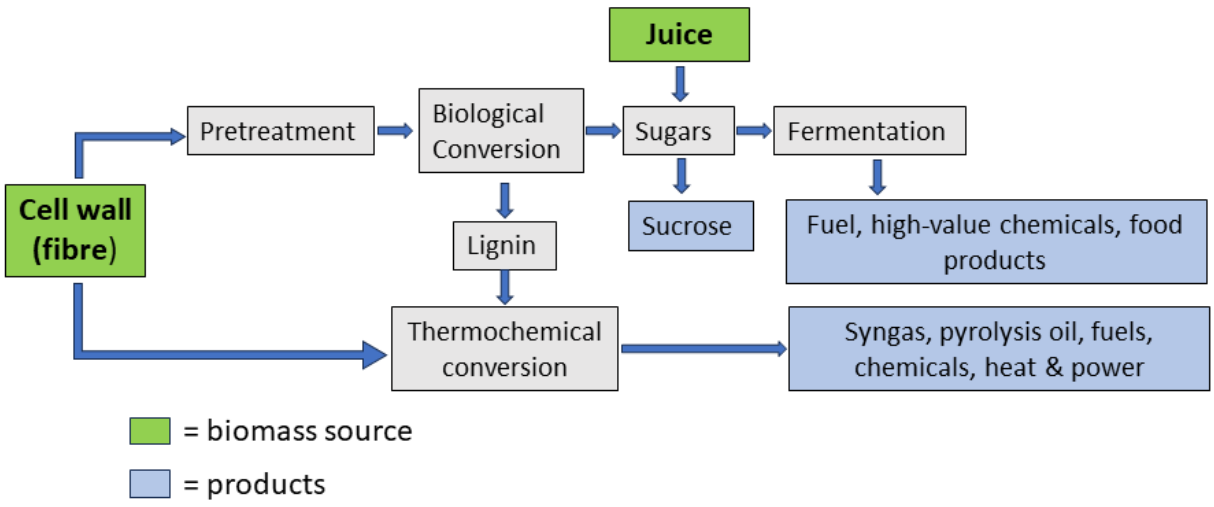

Figure 2: Processing opportunities of the soluble (juice) and insoluble (bagasse) fractions of sugarcane

Value from juice

Sugarcane juice is a nutritious and tasty drink with vitamins, carbohydrates, and amino acids. It has phytochemicals useful for pharmacological research and has been used in Ayurvedic medicine. Sugarcane juice is promising for future studies due to its potent biological activities ((for review Arif et al., 2019).

Sugarcane juice offers a variety of applications that extend beyond a simple beverage.

- It can be infused with fruit extracts, herbs, or spices to create a unique flavour profile or blended with healthy smoothie ingredients.
- It can be concentrated as a sweetener or flavouring agent in food and beverages and transformed into juice-based jellies jams or syrup for pancakes, waffles, and desserts.
- High-value products can be directly extracted from the juice.
- It can be fermented to produce alcoholic beverages or ethanol as a fuel.
- It can be fermented and distilled into high-value alternative products.

The composition and concentration of the juice may vary during the growth cycle of sugarcane. For all the abovementioned applications, knowledge regarding the initial composition of the juice is important.

Utilising the lignocellulosic fraction

There are four primary methods to utilise water-insoluble biomass fibre as an energy source (Carvalho-Netto et al., 2014):

- Directly burn it to create thermal energy and electricity via cogeneration.
- Employ chemical or enzymatic hydrolysis to extract fermentable sugars from the cellulose, hemicellulose, and lignin and generate liquid fuels.
- Gasification to produce synthesis gas, comprising carbon monoxide and hydrogen, or biogas.
- Pyrolyze it to produce bio-oil or coal/coke.

Sugarcane juice has long been used to produce ethanol, but utilising the lignocellulosic fraction for ethanol, biodiesel, and aviation fuels is still in the development phase. This is due to challenges in technology and economics, as well as differences in the composition of the lignocellulose biomass[1]. Nevertheless, many of these technologies for utilising lignocellulose are ready for upscaling (Jatoi et al., 2023).

There are several methods for producing aviation fuel from sugarcane bagasse. One such approach is the alcohol-to-jet (ATJ) method. This involves fermenting the sugars found in biomass into ethanol or other alcohols and then dehydrating and oligomerizing them to create jet fuel (Yao et al., 2017). Another method is called catalytic pyrolysis. It involves heating the biomass without oxygen and using a catalyst to transform the resulting vapors into jet fuel through the alkylation of aromatics (Zhang et al., 2023). A third method involves using furfural as an intermediate product. Furfural is created by dehydrating the pentosans in the bagasse and then reacting it with acetone to produce jet fuel precursors. These precursors are then further processed into long-chain alkanes through dehydration and hydrogenation (Tian and Lee, 2017).

The fist pilot biorefinery in Queensland has been built by Mercurius Australia and aims at the production of biodiesel and aviation fuel.

The biofuels market size is expected to grow from 1.80 million barrels of oil equivalent per day in 2023 to 2.32 million Barrels of oil equivalent per day by 2028, at a compound annual growth rate of 5.20% during the period 2023-2028.

Material and methods

Material

Sugarcane

The growth of the 17 sugarcane genotypes (Appendix 1) was monitored over a plant and two ratoon crops. Fifteen sugarcane genotypes were planted at each of the two trial sites at Mossman and Atherton Tablelands in Northern Queensland. These included six current commercial varieties and nine non-commercial genotypes.

The Mossman trial was established at the Mango Park Cane Farm Company, Farm number: 5185 (16°28'38.16"S 145°20'59.16"E). The clones were planted on 2 September 2020. The Tablelands trial was established at the Salvetti Farming Company, Farm number: 6207 (17°6'8"S 145°20'28"E).

The trials were planted in a completely randomised design, including three replicate plots per treatment. Each replicate consists of 4 x 10 meters of cane. Billets obtained from disease-free stalks were used as planting material.

Sorghum

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 fertiliser 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 and growth

To gather data on germination and crop establishment, both on-ground measurements and aerial photography using drones were utilised. In each plot, ten primary shoots for sugarcane and six primary shoots for sweet sorghum were tagged for easy identification. Non-destructive measurements of stalk elongation and phyllochron development were taken in the field to ensure minimal disruption to canopy development. Monthly measurements were taken for sugarcane throughout the first six months of crop development, while sweet sorghum measurements were taken every two weeks following the first visible unfolded leaf. The canopy height for sugarcane was measured from the base of the stalk to the first visible dewlap, while the culm height for sweet sorghum was measured from ground level to the tip of the spindle leaf.

UAV analysis

A customised quadcopter (DJI Matrice 100) with a flight time of 15 min and a payload capacity of approx. 1.2 kg was used in the analysis (Natarajan et al., 2019). Six flights (two UAV flights per growth cycle (plant, first ratoon and second ratoon crops) were conducted at the Mossman and Tablelands sites. Briefly, the analyses comprised of the following.

Multispectral images were captured using a five narrow-band camera (MicaSense, Inc., USA); the bands centred at 475 nm, 560 nm, 668 nm, 717 nm, and 840 nm corresponding to the blue, green, red, red edge, and near-infrared (NIR) regions, respectively. Images of a calibration panel captured before and after each flight along with a sun irradiance sensor mounted on the UAV were used for calibrating multispectral images.

The UAV was programmed to fly autonomously along a pre-defined single grid path controlled by a ground control station. The UAV mission plan was optimised by varying flight height and speed in order to capture the experimental area within the 15 min flight time, with a GSD less than 5 cm, and a minimum overlap of 80% in both X and Y directions. The multispectral and visual cameras captured images at one-second intervals.

The images were triangulated and mosaicked using a photogrammetric software (Pix4dMapper, Switzerland) based on the structure from motion algorithm to generate an orthomosaic and a 3D reconstruction of the experimental field.

Canopy cover was estimated from the visual orthomosaic by classifying the orthomosaic to vegetation, soil, and other background using the support vector machine (SVM) algorithm. Canopy height was determined as the difference in elevation of the canopy surface (digital surface model) and elevation of bare earth (digital elevation model). Spectral index NDVI was calculated from the 5 band multispectral images using the NIR and red band reflectance by using

$$NDVI = \frac{NIR - Red}{NIR + Red} \quad (1)$$

Individual plot boundaries were identified on the orthomosaic by segmenting the experimental field into regularly shaped plot polygons along with a buffer around the plots to minimise neighbouring plot edge effects based on a supplied field experimental design (Natarajan et al., 2019).

Environmental conditions

Daily maximum and minimum air temperatures, rainfall, daily global incoming radiation for each of the trial sites were extracted from (Jeffrey et al., 2001).https://www.longpaddock.qld.gov.au/silo/point-data/ -responseTab2 . Photosynthetic active radiation was calculated (Meek et al., 1984). Thermal time (°C d) defined as the cumulative heat units above the base temperature of a growth process, was calculated for phyllochron production and culm growth, using a base temperature as specified in the text.

In sugarcane, the base temperature for germination is around 12ºC, leaf appearance is 9ºC and internode elongation (growth) is 18ºC. The thermal time for leaf appearance and internode elongation was calculated from

$$\left(\frac{Max\ temp + Min\ temp}{2}\right) - t_{base}\ ) \quad (2)$$

There is a notable contrast between the two locations in terms of their daily minimum temperature (Appendix 2). The tablelands site has a lower temperature compared to the Mossman site, which consequently leads to a faster accumulation of heat units at the latter (Figure A2. 1). However, the accumulation of photosynthetic active radiation remains comparable between the two research sites, as shown in (Figure A2. 2A&B).

The time and temperature conditions occurring between the appearance of two successive leaves is termed the phyllochron and is measured in heat units or thermal time (◦Cd). In this study a base temperature of 9ºC was used for phyllochron formation. The Phyllochrons do not appear at a fixed thermal time but instead can be best described by biphasic model (Inman-Bamber, 1994), or a power function (Bonett, 1998). We used the biphasic model approach in this study.

Culm growth rate (biomass accumulation)

Culm growth, and biomass accumulation was modelled by applying the following equation.

$$\int (growth) = \frac{asymptote}{1 + exp\ ((t_{mid} - t) * scale))} \quad (3)$$

where (growth) is the change in the phenotype parameter (height or biomass) and (t) represents time. The parameters to be fitted were maximum height or biomass (asymptote), and t_{mid} the time where half of the maximum was reached. The steepness of the growth curve is represented by the scale parameter.

The slope, or rate of growth, at any time point, can be calculated from

$$\frac{d}{dt}f(t) = asymptote * \frac{scale * \exp\left((t_{mid} - t) * scale\right)}{(1 + \exp\big((t_{mid} - t) * scale\big))^2} \quad (4)$$

A the midpoint (4 reduces to

$$slope\ at\ midpoint = aymptote * \frac{scale}{4} \quad (5)$$

Biomass composition
Samples from 6 randomly selected stalks were divided into millable stalk (MS), green leaf (GL) and cabbage (LC). Samples were disintegrated using a garden mulcher or Dedini laboratory disintegrator at room temperature. The mulched material was weighed to determine the fresh weight (FW) and then transferred to a paper bag and dried at 60ºC until a constant dry weight (DW) was attained (usually 6 to 7 days). The dried material was then processed with a grinder fitted with a 0.5mm sieve and stored in zip lock bags for compositional analysis.

Millroom analysis for primary quality components
In a breeding program focused on the development of type I sugarcane, Brix, Pol, purity and fibre content are routine measured as the primary quality components of sugarcane (Berding and Marston, 2010) . Six culm samples were taken from the field plots at two time points during the season approximately six and twelve months from planting or ratooning.

Samples were analysed with a modified method (Berding and Marston, 2010). Culm samples were disintegrated using a Dedini laboratory disintegrator and then processed using the SpectraCane™ automated NIR-based system [@berding2010]. At the end of each harvesting season, SpectraCane™ is re-calibrated against the conventional laboratory data. In addition, every tenth sample through SpectraCane™ is automatically saved and processed through the conventional laboratory where juice is squeezed from the shredded cane using a hydraulic press. The remaining fibre is then dried and weighed to calculate the fibre content.

Wet chemistry
All the analyses were conducted at Celignis Analytical using the analytical packages P19 (Deluxe lignocellulose: Sugars, Lignin, Extractives, and Ash, protein-corrected lignin, water-soluble sugars, uronic acids, acetyl content and starch) and P81(Biomethane potential: Biomethane Potential (BMP), Total Biogas Volume, Total Solids, Volatile Solids, pH, Biogas Methane Content, Biogas Carbon Dioxide Content, Biogas Oxygen Content, Biogas Hydrogen Sulphide Content, Biogas Ammonia Content).

Briefly, the analyses involved the following: Before analysing the lignocellulosic components, all extractable parts were removed from the biomass to avoid interference with acid hydrolysis. The Dionex Accelerated Solvent Extractor (ASE) 200 was used to remove extractives using water and/or 95% ethanol as solvents under a pressure of 1500 PSI and a temperature of 100°C for 5 minutes with a static cycle time of 7 minutes. Each sample underwent three static cycles, and the total flush volume was 150%. The remaining solid was air-dried for 2 days, and the sample's moisture content was determined. The loss in dry matter associated with the extraction was considered as the extractives. Apart from water and ethanol extractions, a "full" extraction was performed, which involved a water extraction (3 static cycles) followed by an ethanol extraction (3 static cycles). The weight of the liquid extract collected during water extractions was recorded, and a subsample was analysed for soluble sugars using ion chromatography.

In order to analyse a sample, we utilized a method similar to the Uppsala Method. The sample was treated with 3 mL of 72% H_2SO_4 at 30ºC and stirred continuously for an hour to break it down. Once the acid was diluted to 4% with water, the tubes were sealed and autoclaved at 121ºC for an hour. To ensure accurate measurements, we also processed standard sugar solutions. The hydrolysates were filtered and stored at room temperature using known-weight filter crucibles and vacuum suction. We then used deionized water to remove any remaining solids until only residue was left on the filter crucible. The filter crucible was left to dry overnight at 105ºC and weighed to determine the Acid Insoluble Residue (AIR) content. We also ashed the

filter crucible to determine the acid-insoluble ash (AIA) content. Finally, we calculated the Klason lignin content by subtracting the AIA from the AIR.

Acid soluble lignin (ASL)

After preparing the hydrolysate, water was added to dilute it until the UV absorbance was within a linear range. The UV-Visible (190-520nm) transmission spectrum of the sample is then obtained using the HP 8452A diode-array spectrophotometer. The ASL content was determined by measuring the absorbance at 205 nm, using an absorbtivity constant of 110 M^{-1} cm^{-1}.

Chromatography conditions

To analyse the hydrolysates, they were diluted, and a known concentration of the internal standard melibiose was added. The analysis was conducted using a DIONEX ICS-3000 ion chromatography system that included an electrochemical detector utilising Pulsed Amperometric Detection (PAD), a gradient pump, a temperature-controlled column and detector enclosure, and an AS-AP autosampler. For the examination of the sugars in the water extract, the same conditions were used, except the column temperature was reduced to 17^{o}C to separate sucrose and fructose.

Metabolome analysis

Sample preparation

Dried plant material was extracted in 100% methanol (containing ^{13}C,^{15}N-valine and ^{13}C-sorbitol) in a chilled cryo-mill (Precellys/Cryolys – Bertin Technologies) at 6800 rpm agitation. The sample was then centrifuged and the supernatant transfer to a clean vessel. The pellet was extracted twice with Milli-Q water and the supernatants combined with the original methanol supernatant. A 30ul aliquot was then dried under vacuum.

Derivatisation and GC-MS

Dried samples for targeted analysis were derivatised online using the Shimadzu AOC6000 autosampler robot. Derivatisation was achieved by the addition of 25 µL methoxyamine hydrochloride (30 mg/mL in pyridine, Merck) followed by shaking at 37°C for 2h. Samples were then derivatised with 25 µL of *N*,*O*-*bis* trimethylsilyl)trifluoroacetamide with trimethylchlorosilane (BSTFA with 1% TMCS, Thermo Scientific) for 1h at 37°C. The sample was allowed to equilibrate at room temperature for 1 h before 1 µL was injected onto the GC column using a hot needle technique. Split (1:10) injections were performed for each sample.

The GC-MS system used comprised of an AOC6000 autosampler, a 2030 Shimadzu gas chromatograph and a TQ8040 triple quadrupole mass spectrometer (Shimadzu, Japan). The mass spectrometer was tuned according to the manufacturer's recommendations using tris-(perfluorobutyl)-amine (CF43). GC-MS was performed on a 30m Agilent DB-5 column with 0.25mm internal diameter column and 1µm film thickness. The injection temperature (inlet) was set at 280°C, the MS transfer line at 280°C and the ion source adjusted to 200°C. Helium was used as the carrier gas at a flow rate of 1 mL/min and argon gas was used in the collision cell to generate the MRM product ion. The analysis of TMS samples was performed under the following oven temperature program; 100°C start temperature, hold for 4 minutes, followed by a 10°C min^{-1} oven temperature ramp to 320°C with a following final hold for 11 minutes. Approximately 520 targets were collected using the Shimadzu Smart Metabolite Database, where each target comprised a quantifier MRM along with a qualifier MRM, which covers approximately 350 endogenous metabolites and multiple stable isotopically labelled internal standards. Resultant data was processed using Shimadzu LabSolutions Insight software, where peak integrations were visually validated and manually corrected where required.

Two approaches were followed for metabolome analysis. Firstly a untargeted metabolomics detection was used to identify more than 200 metabolites in the sugarcane and sorghum samples. Univariate and multivariate statistical analysis were performed on metabolomic profiles to screen for "important metabolites", which were determined by variable importance in projection (VIP) scores and P value. Data was log-transformed and median- normalised prior to statistical analysis using the software program Metaboanalyst .

One limitation of untargeted metabolite profiling is that it only reveals the relative abundance of a metabolite. This means that a single metabolite can be compared across samples to draw conclusions about changes in its abundance. However, it is not possible to compare metabolites to each other or make conclusions about their concentration.

To overcome this limitation, targeted analyses were carried out. The first round of analyses indicated that around 20 metabolites were present at levels that could be sufficient for commercial purposes. Three-point

calibration curve were plotted with low (0.062 mM), medium (0.25 mM) and high (1 mM) concentrations to obtain the concentration of the endogenous metabolites. Concentrations were normalised to the internal standard and weight of the material and reported as moles g^{-1}. Using the metabolites' molecular weight, the concentration in tonnes ha^{-1} were calculated.

Biomethane potential (BMP)

This biomethane potential (BMP) test was performed using active inoculum from an anaerobic digester. The inoculum has been tested for its quality and degassed to avoid interference of organic matter present in the inoculum with the test sample. The volume to headspace ratio of the digester is 7:3 and the inoculum to substrate ratio is 4:1. German standard methods (VDI 4630) were used in determining the BMP of the sample.

The average moisture of the samples was 5% with total solids of 95% and the percentage of ash in the samples a DW basis was 1.1%. The average volatile solids (VS) percentage was 98% on a DW basis.

Data analyses

Statistical analyses were performed in R (version 3.61) using the package Agricolae (De Mendiburu and Reinhard, 2015). One-way ANOVA tests were used to make multiple comparisons followed by a Least Significant Difference Test (LSD) (Steel et al., 1997). The TukeyHSD post hoc tests were used to compare the group means shown in the graphs with different letters and corresponding colours. All graphs in the boxplot format were prepared in R using the package MultiCompview, in which the default is to present the upper and lower ides of the box as the first and third quartile.

Sugarcane biomass production

Germination

The germination of sugarcane clones showed significant variability at both locations (Table 1) . The data indicated that five genotypes exhibited better germination rates and had a larger stalk population compared to KQ228. At the Tablelands site, QN13-609 and QN12-512, and at Mossman QN12-520 and QN-512 demonstrated poor germination.

Table 1: Shoot emergence after planting of 15 sugarcane genotypes in the Tablelands

Clone	Shoot emergence								
	28 DAP[1]			42 DAP[1]			56 DAP[1]		
	Shoots[2]	sd	TUKEY[3]	Shoots[2]	sd	TUKEY[3]	Shoots[2]	sd	TUKEY[3]
QS10-7123	33.3	14.6	b	76.0	14.8	abc	172.7	37.6	a
QS08-8662	34.0	6.4	b	93.0	1.6	a	147.0	6.5	ab
QS10-8770	36.3	1.2	b	80.3	7.3	ab	144.0	12.8	ab
SRA3	52.3	3.8	a	77.7	1.7	ab	135.3	31.5	abc
QN13-173	26.7	7.4	bcd	54.0	8.6	cd	117.7	31.4	bcd
KQ228	33.7	8.3	b	73.3	15.4	abcd	113.3	15.2	bcd
WSRA24	34.3	3.3	b	68.0	7.3	bcd	100.7	9.3	cd
QS09-8348	28.3	10.0	bc	69.3	16.0	bcd	98.0	18.1	cd
Q240	14.3	4.5	cde	52.3	12.3	d	94.0	18.4	d
Q208	12.7	9.4	de	56.7	19.2	cd	92.7	24.1	d
QS09-8404	26.7	1.9	bcd	59.0	4.3	bcd	92.7	4.6	d
QS08-7370	12.7	4.2	de	61.7	13.7	bcd	91.0	17.1	de
QS07-9185	24.0	8.5	bcd	53.3	8.2	d	87.3	13.6	def
QN13-609	3.3	2.1	e	25.7	8.4	e	50.3	13.7	ef
QN12-512	8.7	5.4	e	28.7	7.8	e	47.0	15.9	f

[1] Days after planting

[2] Shoots per 20 meters

[3] TUKEY HSD ($P_{0.05}$)

Canopy development

Stalk elongation and leaf development were measured throughout the first seven months of crop development.

To estimate the canopy cover, we classified the RGB orthomosaic into three categories: vegetation, soil, and other background pixels. The proportion of vegetation pixels within a plot was then used as the measure for canopy cover. It was observed that the canopy growth in Tablelands is more consistent and faster compared to Mossman.

Based on the ANOVA analysis, it was evident that there are notable distinctions in canopy growth ($P<0.001$) among various genotypes (Figure 3). This variation was further supported by the canopy coverage observed three months after planting (Table 1). However, it should be noted that the canopy development was comparatively more consistent at the Tablelands site.

The phytomer serves as the basic unit for a crop canopy(McMaster 2005, and references therein). In the case of sugarcane, a phytomer is comprised of a leaf, an attached axillary bud, a node, and an internode (Evans, 1940). As the shoot apex grows, phytomer units are added sequentially to build the culm. The time between the appearance of a leaf and its internode is known as the phyllochron interval (PI).

Table 2: Phyllochron thermal time (°Cd) for 17 sugarcane genotypes. Letters correspond to significant differences among groups after the TukeyHSD post hoc test. Means with the same letter are not significantly different.

Genotype	Phyllochron	SD[1]	TUKEY[2]
	°C d^{-1}		
Q200	117.5	9.3	a
QS08-8662	101.1	17.5	b
Q240	95.3	18.1	bc
QS09-8404	94.3	15.1	bcd
QS10-7123	94.2	13.0	bcd
QS09-8348	93.1	15.5	bcd
QS10-8770	92.8	14.6	bcde
QN13-173	92.7	15.7	bcde
QN12-512	91.8	22.6	bcde
WSRA24	91.6	12.4	bcde
QS08-7370	89.2	12.2	cde
QS07-9185	89.1	20.4	cde
QN13-609	88.3	16.5	cde
Q208	87.9	18.2	cde
SRA3	83.1	7.6	cde
QN12-520	80.2	7.9	de
KQ228	78.7	10.1	e

[1] = Standard deviation
2 = TUKEY HSD (P0.05)

The crop accumulated heat units at a faster rate under the coastal conditions in Mossman (see Appendix 2). The warmer night conditions in Mossman have led to a faster accumulation of phyllochron degree days (PDD). These accumulated degree days were used to calculate the PI for all genotypes.

Phyllochrons differ significantly between the genotypes (Table 2). The two commercial standards Q200 and KQ228 represented the extremes in PI values. It's fascinating that modelling analysis suggests that sucrose accumulation is influenced by sugarcane phenology. To enhance sucrose and biomass production, an alternative approach could be to breed genotypes with appropriate phenological and structural partitioning traits, such as the rate of phytomer development (Singels and Inman-Bamber, 2011).

Crop height

In all the grasses, such as sugarcane, there is a strong correlation between stalk length, internode length and aboveground biomass (Kebrom et al., 2017; Lingle and Thomson, 2012). Cooler temperatures and reduced moisture availability lead to shorter internodes (Bonnett et al., 2006) and reduced biomass yield.

Canopy height was measured from the base of the stalk to the first visible dewlap (van Dillewijn, 1952). In addition, canopy height was derived from the visual images after constructing a digital surface model (DSM) and a digital terrain model (DTM).

Both methodologies displayed a similar trend, but there is a significant correlation ($p<0.01$) between the RPA height measurement and the "on the ground" measurement of culm length, especially for crops that are younger than four months. This correlation is expected since the RPA analysis considers the total length of the plant (culm and tops), whereas the on-ground measurement only accounts for the stalk length and not the crop height. During the early stages of crop growth, the leaves constitute a more significant portion of the stool. As the crop grows, the stalk becomes a more substantial part of the total height.

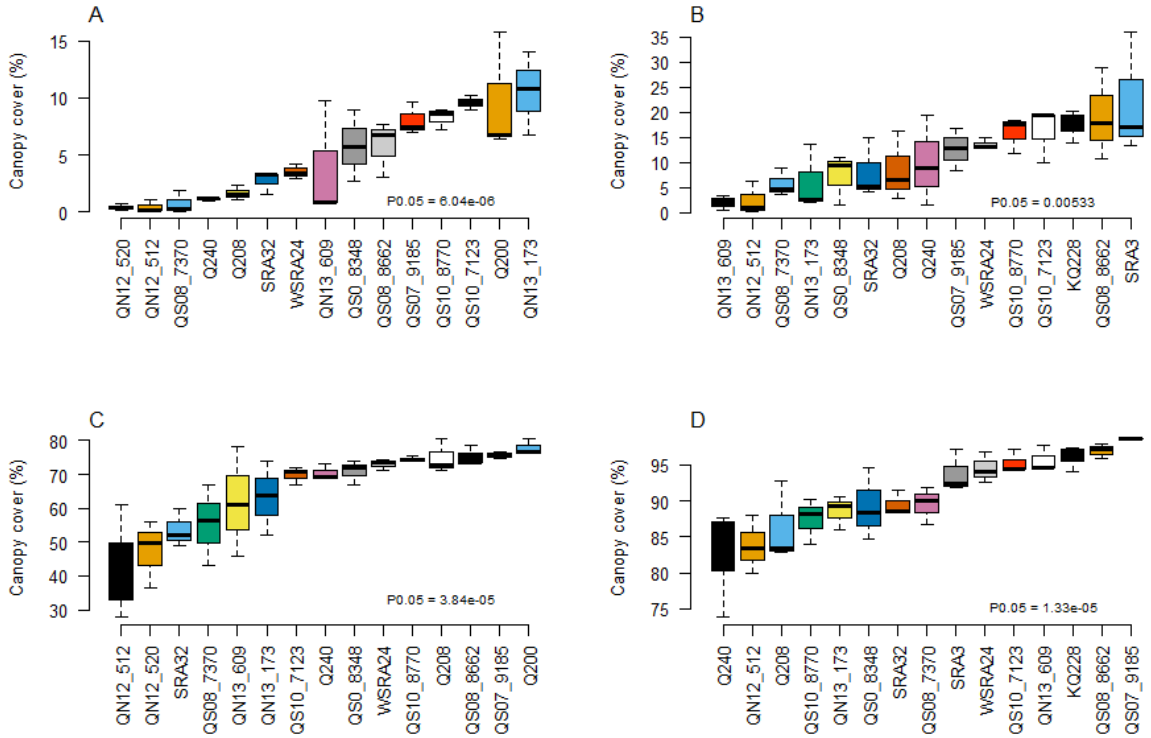

Figure 3: Canopy cover of the 15 test genotypes at Mossman (A, C) and Tablelands (B, C) 3 months ((A, B) and 7 months (C, D) after planting. Canopy cover (%) was determined by classifying the images into vegetation or soil and determining the percentage vegetation within each plot. Varieties were sorted with the highest at the right.

A one-way ANOVA revealed that there was a statistically significant difference in canopy height and genotype at both locations and in 3- and 7-month-old cane (**Figure 4**). According to a Tukey's HSD test for multiple comparisons, three genotype groups were significantly different (P=0.05).

Stalk biomass

The crop yield at the Mossman and Tableland trial sites are presented in (Appendix 3: Biomass production). A two-way ANOVA was performed to analyse the effect of genotype and geographic location on cane yield.

Simple main effects analysis showed that genotype did have a statistically significant effect on cane yield at Mossman ($P<0.001$) and the Tablelands ($P<0.042$). There was also a statistically significant effect of the stage in the crop cycle (plant or ratoon) and cane yield (Table 3).

In the Mossman trial, there was a significant difference of over 45 TCH between the various sugarcane genotypes (Table A3. 3Table A3. 3: Cane yield (TCH) at the Tablelands and Mossman trial sites.). However, this can be mostly attributed to the QN12-512 and QN12-520 varieties having poor establishment and slow growth. Among the other varieties, Q240, QS08-7370, and QN13-609 also performed worse than Q208 at Mossman. The top four clones for TCH were experimental clones QN12-512, QN13-609 and QS08-7370 also performed poorly at the Tablelands site. At this site three clones had yields significantly higher than Q208 namely, QS10-8770, WSRA24 and QS08-8662. Unfortunately, the yield of four clones were extremely variable and this confounded statistical analyses. analysis.

It should be noted that this variation was not observed at the Mossman site, indicating that it is likely due to site variability rather than differences in genotype.

There is a statistically significant variation ($P<0.05$) in the moisture content of the different genotypes at the time of harvest. As the purpose of this study is focussed on biomass production potential all data report in other sections of this report has been converted to dry weight and yield data is expressed as tonne dry weight of cane per hectare.

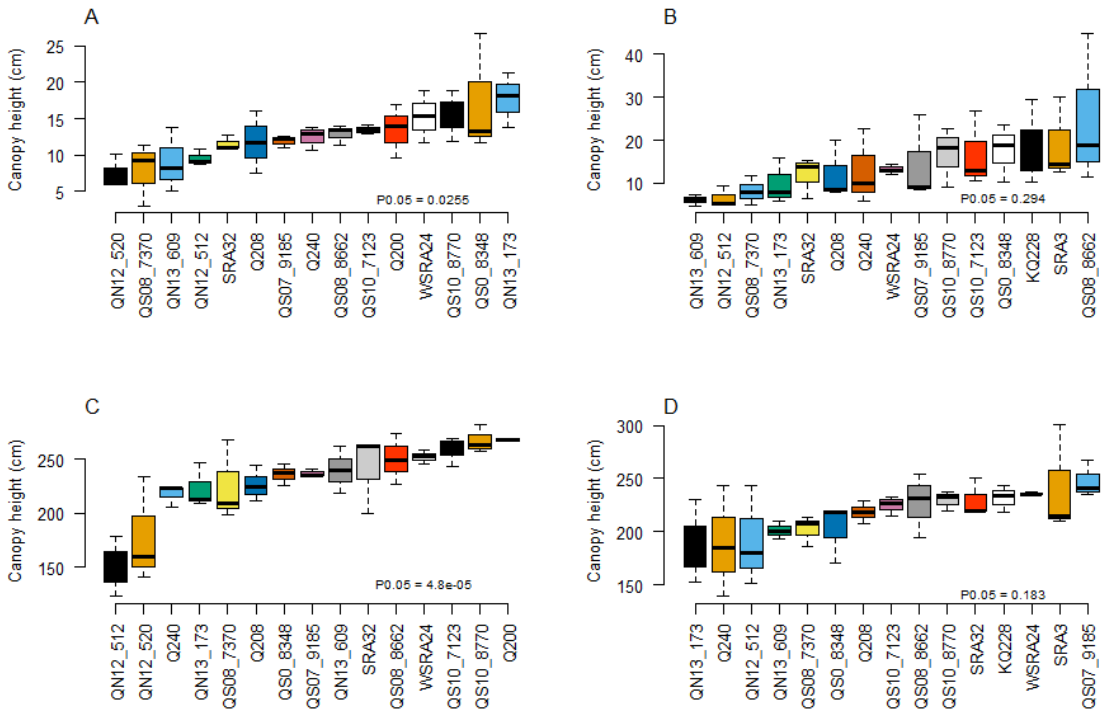

Figure 4: Canopy height of the 15 test genotypes at Mossman (A, C) and Tablelands (B, C) 3 months ((A, B) and 7 months (C, D) after planting.

Table 3: Two-way ANOVA results of crop yield (TCH) of sugarcane genotypes at two locations (Mossman and Tablelands) in the FNM production area.

Location	Main effects	Sum Sq	F value	P value
Tablelands		**Fresh weight**		
	Genotype	8158.829	2.472	0.00422
	Crop cycle	11435.744	24.254	1.50E-09
Mossman				
	Genotype	24256	9.426	1.14E-13
	Crop cycle	35209	95.775	2.00E-16
Tablelands		Dry weight		
	Genotype	782	1.796	0.0468
	Crop cycle	1128	18.139	1.25E-07
Mossman				
	Genotype	2355	8.057	8.11E-12
	Crop cycle	4561	109.244	2.00E-16

The tonne dry weight yield per hectare over the crop-ping cycle (plant plus ratoon) of Q208 was 29.02 ± 1.14 and 25.99 ± 5.9 for Mossman and the Tablelands respectively. In the data presented in Figure 6 the yield data has been normalised against the yield from Q208 in both pro-duction environments.

At the Mossman site only 4 genotypes (QS08-7370, SRA32, QS10-7123 and QS10-8770) had a higher average cane production than Q208 (Figure 6). Only the yield from QS10-8770 was significantly higher (9.2%) than that of Q208. None of the other commercial standards in the trial did better than Q208.

Several genotypes did better than Q208 at the Tablelands site (Figure 6.) Four genotypes (SRA3, QS10-7123, QS07-9185 and WSRA24) had a yield advantage of >20% over Q208. It is important to note that Q208 yield was lower at the Tablelands than Mossman trial sites.

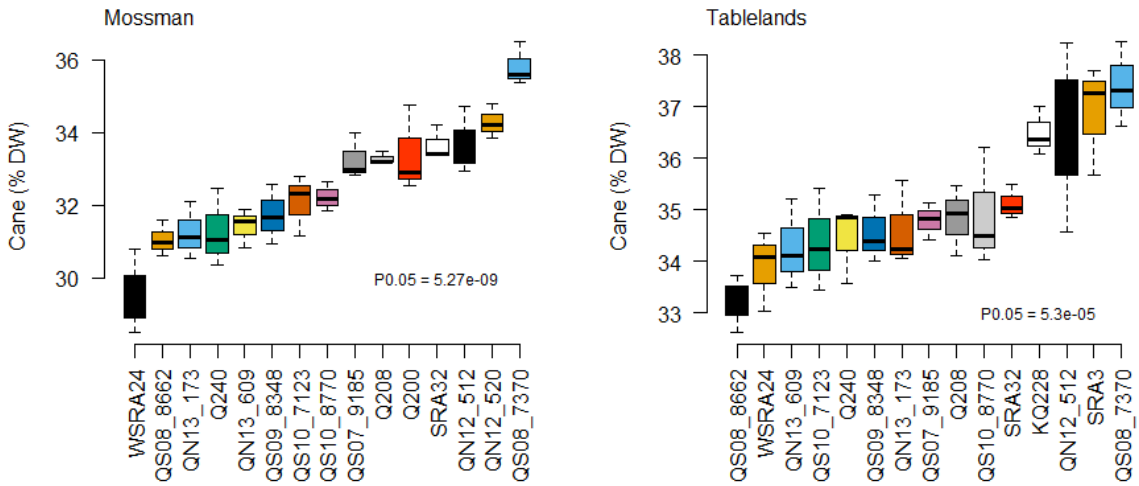

Figure 5: Dry weight percentage of the harvested culms from the varieties at the Mossman and Tableland trail sites.

Simulation of biomass accumulation

Sugarcane height and biomass do not increase in a linear fashion. Instead, growth can be best modelled by the application of a logistic function. Growth of the genotypes (expressed as increase in height) is presented in (Figure A3 1A). The genotypes differ significantly in the time point where the maximum growth rate is achieved (Figure A3 1B)

The experimental data from this and previous studies were used to simulate the increase in height (Table A3 1)and stalk biomass (Table A3. 2), of all the genotypes.

Both the maximum, and rate of growth differ statistically significant between the genotypes (Appendix 3: Biomass production). The maximum biomass accumulation rate is reached at 116 DAP (SRA3) and 163 DAP (QS07-9185).

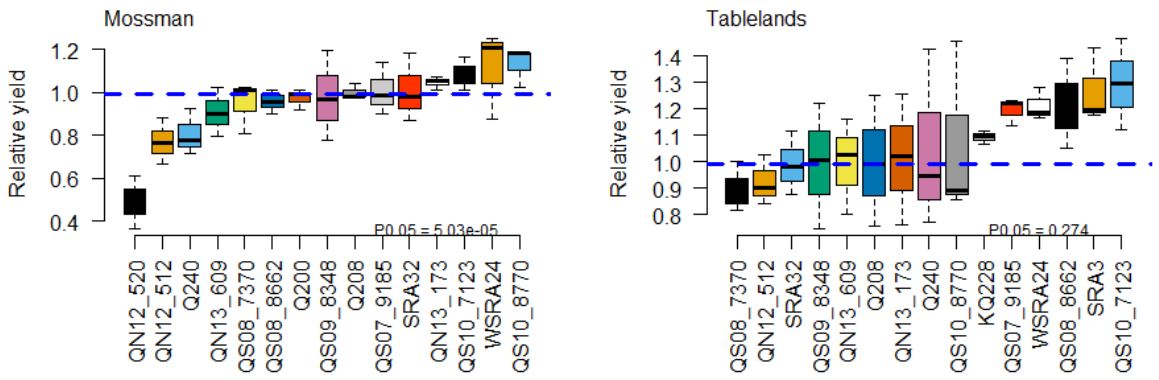

Figure 6: Relative crop yield (TCH dry weight) of the cane genotypes included in the field trials in Mossman and Tablelands. The data is the average yield of the plant and ratoon crop. Data is expressed relative to that of Q208 which is the dominant in variety in the FNM production area.

The rate at which the crop grow differs between a plant and ratoon crop (Inman-Bamber, 2014; Riajaya et al., 2022; Robertson et al., 1996; Thompson, 1988)

The aerial biomass is comprised of the foliage (cabbage), the culm and the dead leaves (trash) that clings to the culm. As the growth season progress a portion of the dead leaves is shed, a phenomenon often referred to as “self-trashing”. The contribution of these components to total biomass varies during the season (Figure A3 2).

To contribution of the different biomass components to the total biomass is demonstrated by the data in (Table 4). These calculations do not consider the dead leaves that are dropped as trash during the season. Important to note that the portion of clean stalk material that is harvested represents only 70% of the produced biomass. The data also highlight the little gain in biomass during the last portion of the growth cycle.

However, as will be pointed out later this slow growth at the end of the season significantly contribute to better cane quality and higher sucrose recovery.

Table 4: Biomass composition (TH) of QS-8770 and the commercial standard variety Q208.

DAP	Biomass Tonnes per hectare (TH)							
	Leaves		Culm		Trash		Total	
	QS10_8770	Q208	QS10_8770	Q208	QS10_8770	Q208	QS10_8770	Q208
100	16.1	19.5	13.8	16.7	0.0	0.0	29.9	36.3
180	18.5	16.4	41.0	36.5	9.1	8.1	68.6	61.0
240	16.6	11.7	55.3	39.1	11.0	7.8	82.9	58.6
300	15.3	10.5	61.0	42.0	11.5	7.9	87.8	60.4

Biomass composition

Sugarcane biomass offers opportunities for electricity, second-generation ethanol, chemicals, and bioproducts. However, as many sugarcane mills are already crushing below capacity, there is a growing interest in energy canes to provide additional biomass. Generally, high biomass is associated with high fibre and reduced sucrose levels.

However, there needs to be more information on the chemical composition of sugarcane leaves, stalks, and the total composition of energy canes. Such information is essential for total biomass utilisation and efficient biorefinery applications.

The insoluble component (bagasse)

Earlier in this report, we provided data that compared the amount of fibre produced by different genotypes at both locations. Regarding sugarcane, fibre typically refers to the leftover material (known as bagasse) that remains after the stalks are crushed to extract juice for sugar production. Bagasse is composed of cellulose, hemicellulose, lignin, and pectin.

Cell wall composition of Q208

In the cell wall of Q208, there are 13 primary components depicted in Figure 7. The major components found in bagasse are glucan (cellulose), xylan (hemicellulose), and lignin. The stalk has a greater concentration of these three components than the leaves.

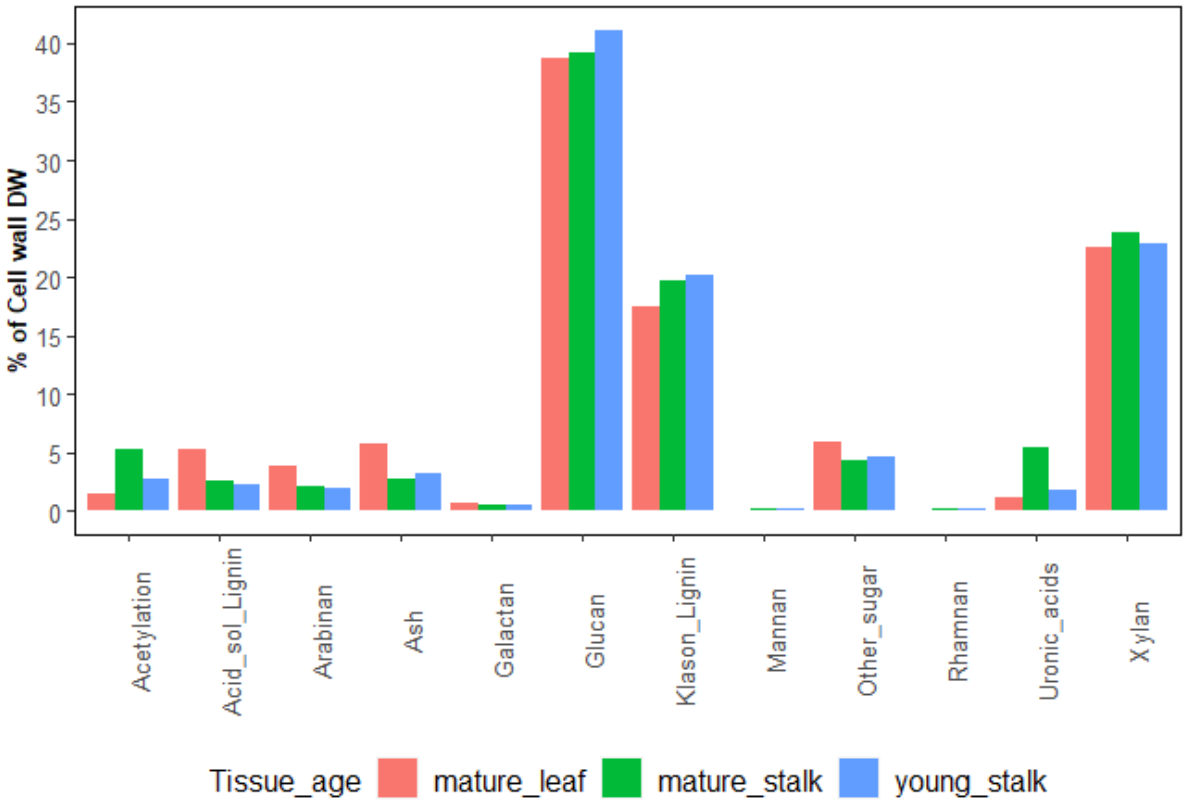

Figure 7: Composition of the insoluble fraction (bagasse) from leaves and the stalk of Q208 at different stages in the cropping cycle. Data is expressed as a percentage of the total cell wall dry mass.

Table 5: Anova of the cell wall components from Q208 green leaves and stalks at 6 and 11 months of growth.

Trait	Stalk[1]		Leaf[1]	
	p - value	Significance[2]	p - value	Significance[2]
Acetylation	1.92E-06	***	6.06E-01	ns
Acid soluble lignin	6.78E-01	ns	6.34E-01	ns
Arabinan	4.33E-05	***	5.87E-01	ns
Ash	3.15E-02	*	5.88E-01	ns
Galactan	3.91E-02	*	8.82E-01	ns
Glucan	6.65E-01	ns	7.95E-01	ns
Klason lignin	3.43E-01	ns	8.22E-01	ns
Mannan	1.10E-02	*	8.38E-01	ns
Other sugar	9.59E-02	ns	7.26E-01	ns
Rhamnan	1.82E-01	ns	5.78E-01	ns
Uronic Acids	2.48E-08	***	2.49E-01	ns
Xylan	2.62E-02	*	3.16E-01	ns

[1] = Comparison of material at 6 and 11 months of growth

ns = not significant

The cell wall composition in sugarcane leaves grown at Mossman and the Tablelands was similar. Furthermore, it was observed that the cell wall composition did not undergo any significant changes during the period spanning 6 to 11 months.

This finding is unsurprising given that 6-8 mature leaves are in the cane top, and only two are actively growing and expanding. Moreover, the green leaves of sugarcane and other grasses primarily serve as a source of tissue and do not store carbon in response to changes in environmental conditions.

However, a statistically significant difference was observed in the cell wall composition of immature (6 months) and mature (11 months) stalks (Table 5). The data suggest limited variability in the amount of hexose and pentoses produced from saccharification of the bagasse derived from the leaf, immature or mature sugarcane.

Efficient utilisation of bagasse for biorefinery applications is challenging due to the lignocellulose's recalcitrance to saccharification. Lignocellulosic feedstocks contain essential components such as uronic acids, lignin, and acetylation, significantly affecting the processing of these materials for biofuel production.

The positioning and quantity of acetyl groups in cell walls can have a notable impact on the arrangement and breakdown of the cell walls. Uronic acids can create ester bonds with lignin and acetyl groups, complicating the saccharification process. Tackling these bonds necessitates the use of particular enzymatic combinations.

In Q208, there was no significant difference in lignin content between the stalks and leaves at six and eleven months into the cropping cycle. However, there are significant differences in the acetylation and uronic acid levels in the material derived from different tissue types and stages of development. (Table 5).

The cooler temperatures at Tablelands compared to Mossman likely affect stalk growth. Temperature significantly impacts stalk growth, requiring a minimum temperature of 16-18^{o}C. During cool nights, there is less demand for cellular intermediates, resulting in less allocation of glucan to the cell wall and cellular respiration. The availability of acetyl-CoA directly controls the acetylation of cell wall polysaccharides. Due to the overall reduction in respiration during cooler periods, acetyl-CoA levels are low.

Cell wall differences between genotypes

All the cell wall constituents from all the genotypes and at different stages of development are presented in Appendix 4.

Most of this section presents the data of four genotypes: QS10-8770, QS10-7123, WSRA24, and Q208. These genotypes were selected due to their reputation for producing a significant amount of biomass [refer to section xxx]. Furthermore, Q208 is widely recognised as the industry standard for commercial production in both environments. The average leaf composition from both early and late-season collections has been consolidated for analysis and is presented in Appendix 4.

There is a statistically significant difference in the primary cell wall sugars (Figure 8). WSRA24 has a significantly lower glucan and higher xylan content than the other three genotypes. This results in a very low glucan-to-pentan ratio in this genotype. This characteristic was already evident in immature cane six months into the season (Table 7). Other genotypes that fall into this category are QS09-8348, Q240 and QN13-609.

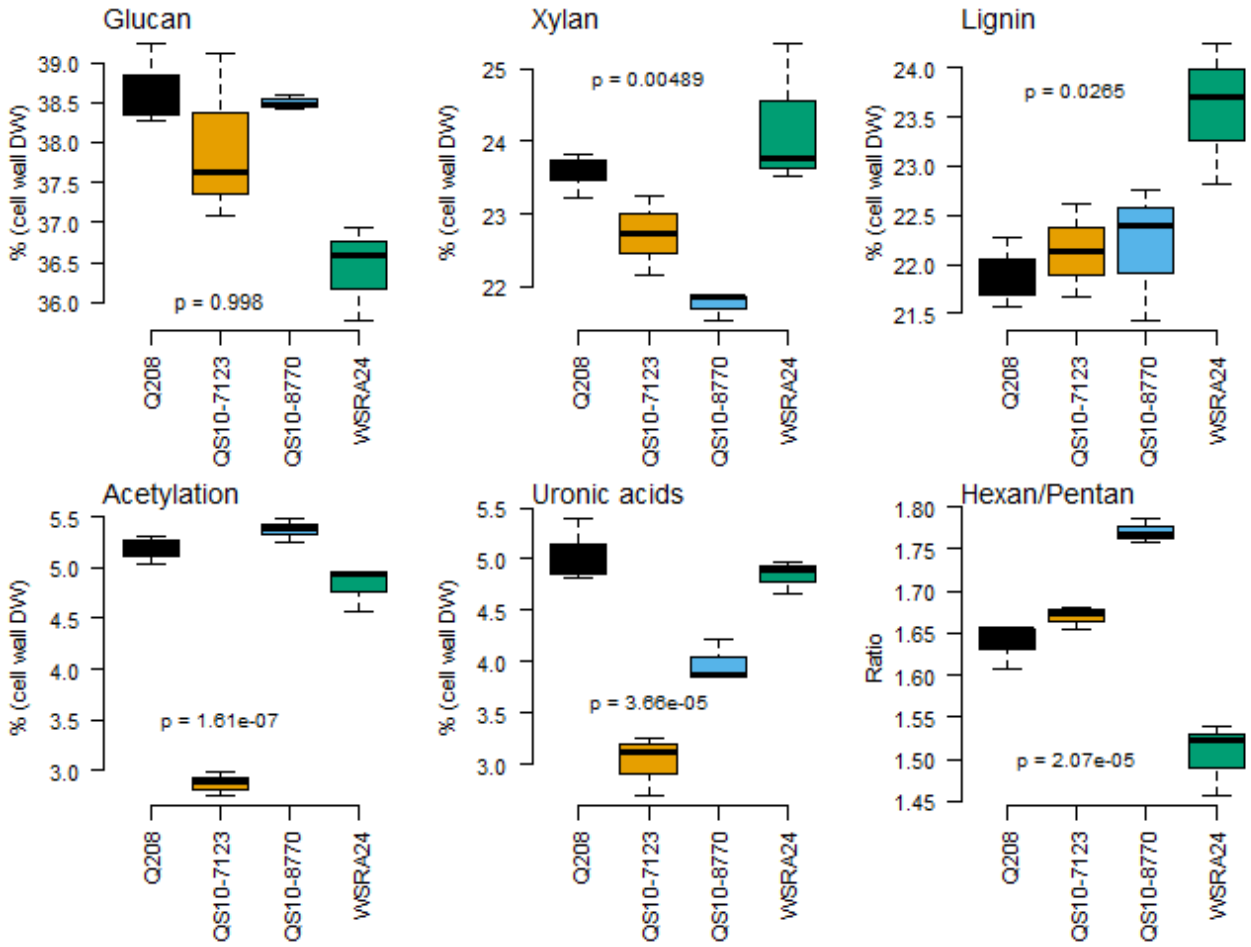

Figure 8: Differences in the cell wall components would be significant in saccharification and bioprocessing of four contrasting biomass sugarcane genotypes.

The ratio of glucan to pentose sugars in lignocellulosic biomass is a crucial factor in the production of lignocellulosic biofuels (Himmel et al. 2007; Burton and Fincher 2014; Alalwan et al. 2019). This ratio plays a significant role in both biochemical and thermochemical conversion methods. A higher concentration of glucan sugars is generally preferred for fermentation-based bioprocessing since many microorganisms can easily ferment glucose. Higher glucan content can increase biofuel production and more efficient fermentation processes.

WSRA24 has significantly higher total lignin content than the other genotypes. QS10-7123 has significantly lower acetylation, and QS10-7123 and QS10_8770 have lower uronic acid levels in the cell wall than the other genotypes (Figure 8).

The process of acetylating polysaccharides is essential for plant growth and development, but it can also make it difficult to break down plant material for biofuel production. This is because it reduces the efficiency of fermentation. As a result, scientists are working to decrease O-acetylation in wall polymers to improve biofuel production (Biely 2012; Gille and Pauly 2012; Zhang et al. 2017).

The data from this project emphasises the importance of detailed analyses of potential biomass feedstocks. It is not simply the amount of bagasse that will be important for bioprocessing, but more so the quality of the feedstock.

The hexoses and pentoses content obtained from bagasse varies significantly between genotypes. The efficiency of saccharification for bagasse will also vary greatly depending on the genotype and time of the season due to differences in lignin, acetylation, and uronic acid levels. The ratio between hexosans and pentosans in the cell wall is strongly influenced by genetics, as demonstrated by significant differences between genotypes during growth stages and in different geographic locations.

There was a significant difference in the lignocellulosic sugar yield that can be derived from the different genotypes and also between the time point in the cropping cycle (Table 6). There were also significant differences in the ratio between cellulose and hemicellulose in the cell wall between the genotypes (Table 7).

The cell wall contains two major polysaccharides, cellulose and hemicellulose, which can both be broken down into fermentable sugars for ethanol production. The ratio of cellulose to hemicellulose in lignocellulosic biomass plays a significant role in ethanol production through bioconversion processes. A higher ratio of cellulose to hemicellulose is advantageous for ethanol production as cellulose is more resistant to degradation but can be efficiently fermented into ethanol once broken down into glucose. On the other hand, a higher proportion of hemicellulose can still contribute to ethanol production potential as it is more easily hydrolysed and certain microorganisms are capable of fermenting these sugars into ethanol. Therefore, a higher hemicellulose content can diversify the sugar sources for ethanol production and potentially lead to higher ethanol yields.

The data in Table 6 suggest that the bagasse derived from young QSI0-7123, QN12-520, QS09-8348 and QN13-173 cane would be the best and that from KQ228, QN12-512 QN13-609, Q208, Q240 and SRA3 the worst for ethanol production. However, this theoretical calculation does not take into account the variation in cell wall hydrolysis and recalcitrance to breakdown.

An ideal scenario for ethanol production would involve a balanced ratio of cellulose and hemicellulose, along with a low lignin, uronic acid and acetylation content. Based on these criteria it would appear that a variety like WSRA24 might not be a good candidate for efficient ethanol production for the lignocellulosic fraction (Figure 8)

Biomethane potential

In all cases, anaerobic digestion resulted in very low concentrations of H_2S and there was no ammonia is noticed in the biogas. From the Buswell equation calculations, the stoichiometric methane potential of the feedstock was much higher than the actual BMP yield. The average biodegradability index of the feedstocks was 65.7%.

There were a significant difference in the biomethane potential between the bagasse derived from he different genotypes. The highest methane production potential was evident in QN13-609, QS10-7123, SRA3, QS08-8662 and WSRA24 (Table 8).

Digestibility

The average C/N ratio for the sugarcane leaf samples was 27:1 and for the stalk samples 70:1. A C/N ratio of 20-30 is considered most suitable for anaerobic digestion. The high C/N ratio indicates that moderate supplementation of nitrogen-rich feedstock is necessary for the leaf samples and significant supplementation for the stalk samples to achieve stable digestion in a continuous upscale AD process. The sulphur content in the sample indicated that the possibility of accumulation of hydrogen sulphide to toxic levels in the digester is very low.

Fertiliser suitability

Based on the analysis of the leaf and stalk samples, the average ratio of N, P, and K was found to be 11:1:12 and 13:1:4, respectively. This indicates that the material cannot be utilized as a sole fertilizer as it

does not meet the recommended N:P:K ratio of 3:1:1. Moreover, the high amount of biodegradable organics (> 0.25 l biogas / g volatile solids) present in the sample restricts its direct application to the soil.

Table 6: The cellulose and hemicellulose content, and theoretical ethanol production potential of the sugarcane genotypes.

Genotype	Cellulose		Hemicellulose		Ethanol	
	kg tonne^{-1}				litre tonne^{-1}	
	Mid-season cane					
QSl0-7123	424.54	a	256.98	a	234.15	a
Q200	420.49	a	253.04	a	223.34	abc
QN13-173	417.99	ab	248.13	ab	223.56	abc
QS10-8770	404.15	ab	244.91	abc	214.96	bcde
SRA32	403.23	abc	242.03	bcd	221.02	abc
QN12-512	403.05	abc	238.83	bcde	211.84	cde
QS09-8348	402.85	abc	238.31	bcde	223.90	abc
QN12-520	400.61	abc	236.29	cde	224.95	abc
QS08-7370	399.00	abc	236.04	cde	216.47	bcd
QS07-9185	393.87	abc	235.88	cde	214.49	cde
Q208	392.17	abc	235.44	cde	208.75	cde
QS08-8662	391.85	bc	234.16	cde	213.37	cde
WSRA24	391.13	bc	230.41	def	216.04	bcde
QN13-609	386.00	bc	227.68	ef	210.09	cde
KQ228	385.93	bc	221.36	f	212.98	cde
SRA3	380.50	bc	220.19	fg	199.68	e
Q240	366.76	bc	207.04	g	205.87	de
	Mature cane					
QS08-8662	391.88	a	243.63	a	211.03	a
QN13-173	389.47	a	242.10	a	209.88	a
QS08-7370	389.32	a	239.05	ab	211.08	a
Q208	386.44	ab	238.08	ab	212.20	a
QS10-8770	384.99	abc	235.74	abc	204.96	ab
KQ228	382.24	abc	230.18	abcd	203.50	ab
QS10-7123	379.36	abcd	227.81	abcd	206.75	ab
QS09-8348	376.17	abcd	227.21	abcd	211.78	a
WSRA24	364.32	bcde	226.82	abcd	207.37	ab
SRA32	363.80	bcde	225.10	abcd	200.93	abc
SRA3	361.50	cde	220.65	bcd	198.55	abcd
QN12-512	355.75	def	217.41	cde	188.64	cd
Q240	340.31	efg	215.85	de	198.47	abcd
QS07-9185	332.18	fg	213.77	de	186.51	d
QN13-609	330.75	g	198.85	e	195.01	bcd

Table 7: The ratio between glucan and xylan between the sugarcane genotypes at two season-time points. One-way ANOVA followed by HSD. Values followed by the same letters are not significantly different (p=0.05).

Genotype	Young cane		Genotype	Mature cane	
	Ratio			Ratio	
SRA3	1.84	a	QN12-512	1.79	a
QS10-8770	1.78	b	KQ228	1.77	ab
QN12-512	1.77	c	QS10-8770	1.77	ab
Q208	1.75	d	QS08-8662	1.72	abc
QN13-173	1.74	e	QN13-173	1.72	abc
QS08-7370	1.68	f	QS08-7370	1.69	bcd
QSl0-7123	1.65	g	QS10-7123	1.67	cd
SRA32	1.65	h	Q208	1.64	cd
QS07-9185	1.63	i	SRA3	1.64	cde
KQ228	1.62	j	SRA32	1.62	def
QS08-8662	1.61	k	QS07-9185	1.55	efg
WSRA24	1.60	l	QS09-8348	1.54	fg
QN13-609	1.57	m	WSRA24	1.51	gh
QS09-8348	1.55	m	Q240	1.42	hi
Q240	1.52	n	QN13-609	1.39	i

Utilising the lignocellulosic fraction

Ethanol production potential

For the determination of the ethanol production potential, the following two equations were used

$$EtoH_{hexosans} = \left(\frac{1110^{d}.0.5111^{b}}{0.789^{c}}\right).\left(\frac{H_{hc} - Cl_{con}}{100}\right).\left(\frac{H_{cl}}{100}\right).(\frac{F_{cl}}{100}) \quad (6)$$

$$EtoH_{pentosans} = (\frac{1136^{d}.0.5111^{b}}{0.789^{c}}).(\frac{H_{hc}}{100}).(\frac{F_{hc}}{100}) \quad (7)$$

The ethanol produced is expressed as litres per tonne of feedstock. Cl_{con} is the cellulase consumed by cellulases, H_{hc} is the hydrolysis efficiency of pentoses, H_{cl} is the hydrolysis efficiency of hexoses, F_{hc} and F_{cl} are the fermentation efficiency of pentoses and hexoses, respectively. The mass yield of glucose per tonne of cellulose is 1100 kg (a), and for pentosans 1136 kg (d) due to conversion from polymeric to monomeric forms.

There are several potential technologies that can be used for ethanol production from lignocellulosic feedstock. Here only dilute acid hydrolysis of biomass in two plug-flow reactors is considered. This can be considered to representative of a near-commercial dilute-acid hydrolysis facility. Important to note that this is the most inefficient system but closest to commercial exploitation. The yield of hexoses is 555kg tonne^{-1} cellulose and for pentoses 966 kg tonne^{-1} feedstock.

Table 8: Biogas and biomethane production from mature stalks from different sugarcane genotypes.

Genotype	Volatile Solids (% Dry Mass)	Biogas Production		Biomethane Potential (BMP)		Final Weighted Biogas Composition			
		Volatile Solids	Dry Mass Basis	Volatile Solids	Dry Mass Basis	CH_4	CO_2	O_2	H_2S
	% DW	litre kg^{-1}				%			ppm
KQ228	98.60	428.8	422.8	312.7	308.3	72.9	27.1	0.0	8
Q208	98.90	339.7	335.9	247.0	244.3	72.7	27.3	0.0	30
Q240	98.95	424.3	419.8	302.7	299.5	71.3	28.7	0.0	18
QN12-512	98.70	393.0	387.9	273.1	269.5	69.5	30.5	0.0	10
QN13-173	98.80	419.6	414.5	286.6	283.2	68.3	31.7	0.0	7
QN13-609	98.68	428.8	423.1	312.7	308.6	72.9	27.1	0.0	11
QS07-9185	98.69	403.8	398.5	289.5	285.7	71.7	28.3	0.0	38
QS08-7370	98.93	374.3	370.3	281.2	278.2	75.1	24.9	0.0	15
QS08-8662	98.98	468.4	463.6	351.3	347.7	75.0	25.0	0.0	13
QS09-8348	99.06	418.2	414.3	307.7	304.8	73.6	26.4	0.0	16
QS10-7123	98.88	459.9	454.7	317.6	314.1	69.1	30.9	0.0	3
QS10-8770	98.90	366.6	362.6	271.7	268.8	74.1	25.9	0.0	16
SRA3	98.38	451.6	444.2	333.8	328.3	73.9	26.1	0.0	26
SRA32	99.09	435.6	431.7	307.5	304.7	70.6	29.4	0.0	15
WSRA24	98.69	526.8	519.9	383.1	378.1	72.7	27.3	0.0	9

Water solubles (juice)

Sugarcane juice obtained from mature sugarcane stalks contains high sugar levels and is an excellent raw material for bioprocessing. Sucrose, the primary sugar in mature sugarcane juice, can be easily converted into biofuels and other products through fermentation. With its simple composition and established processing techniques, sugarcane juice from mature stalks is economically feasible. However, it is unclear what the composition of juice from immature stalks, green leaves, or energy canes is.

Since this project aimed to generate more revenue from the entire sugarcane plant, it was necessary first to determine how much biomass, besides sucrose, glucose, and fructose, is present in sugarcane tissues. Second, we needed to identify other potential chemicals that could be extracted from the juice.

Sugars contributed more than 75% of the total biomass of stalk-derived juice, and sucrose represented the bulk of the sugar (Figure 9). In contrast, the sugar content of the leaves was low and contributed maximally 5% to leaf biomass.

Sugarcane juice (SCJ) is a highly valuable starting material that can be used to make a variety of products. Depending on the region and traditional demand, different products can be created from SCJ. The ingredients in SCJ have many health benefits, including immunological, anti-toxicity, cytoprotective, anticarcinogenic, and diabetes and hypertension regulation effects (Jaffé 2012; Rajendran et al. 2021). With the help of modern high-resolution technologies like GC-MS, the potential list of valuable products that can be made from SCJ continues to expand (Zidan and Azlan 2022).

When extracting specialized compounds and metabolic intermediates, it is important to take special precautions. This is because some metabolites have an extremely short half-life of just a few seconds. In order to prevent changes in the levels of these metabolites, liquid nitrogen or non-aqueous media are typically used to stop metabolism.

In this project the emphasis were on stable metabolites that will be present after harvest and transportation before the material is processed at the mill.

For this project, it is essential to note that other solutes refer to substances, not sucrose, glucose, or fructose. This component is crucial as any other potential value extracted from sugarcane will be in this juice fraction. In the stalk juice, this fraction accounts for approximately 5%-8% of the total biomass, depending on the maturity of the cane. However, in the leaves, this fraction represents around 15% of the total biomass (Figure 9).

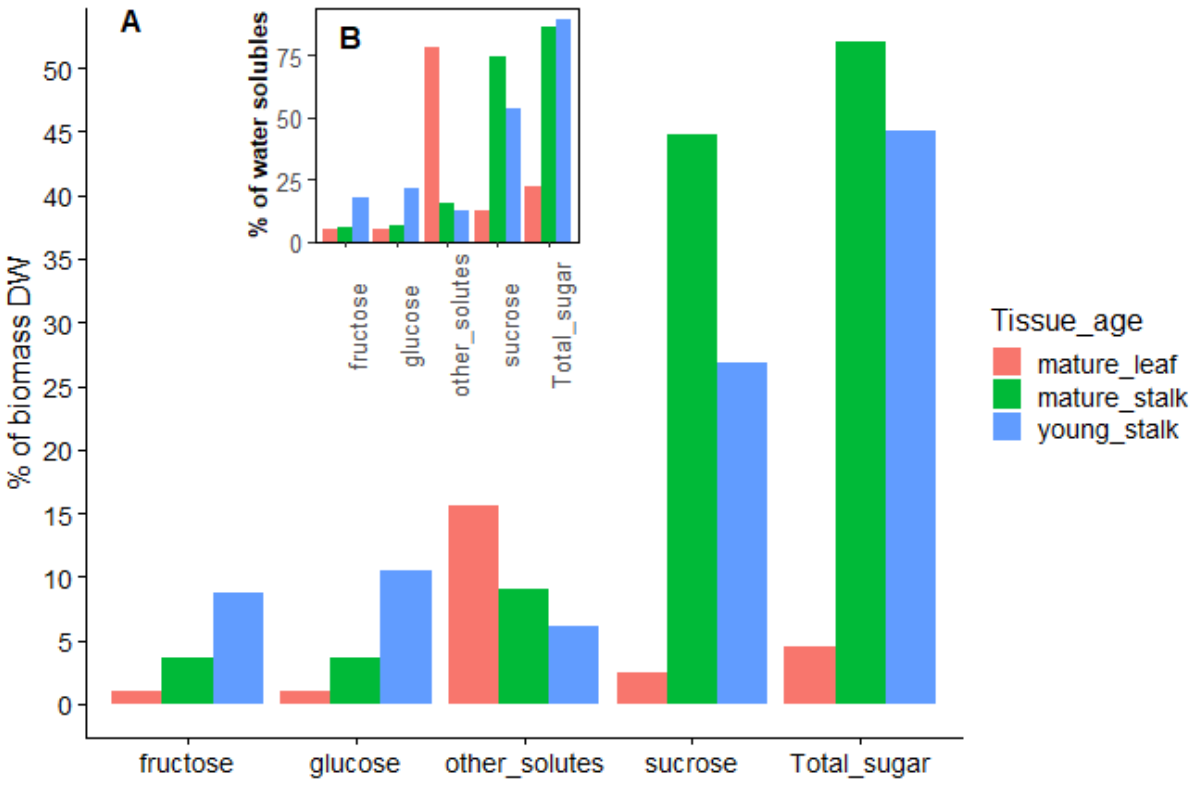

Figure 9: Composition of water-soluble fraction from Q208 at different stages in the cropping cycle. The composition as a percentage of total dry mass (A). The contribution of each fraction to the total soluble content (B).

The other substances in the leaves varied from 140kg to 166kg per tonne of dry weight. This component contributed between 80 and 130kg per tonne of cane in immature sugarcane stalk juice. The variance in immature cane is strongly related to its growth rate and early ripening. The other soluble components decrease as the cane matures, and more sucrose accumulates. In mature sugarcane juice, the other substances only contributed 50 to 87kg per tonne of biomass.

It is essential to recognise that although the leaves contained more solutes per unit biomass, the biomass contribution of leaves to the total aerial biomass is less than 20%.

There were 199 polar metabolites in the sugarcane leaf juice. In the stalk juice, there were significantly fewer metabolites; depending on the maturity, it varied between 87 and 96 metabolites {Table A5.1].

Apart from sucrose, only nineteen other metabolites were present at levels greater than 0.05% of the total dry mass and could be extracted from the tissue. The levels of these metabolites vary significantly between the leaves, young cane, and mature cane-derived juice.

There are significant variations in the levels of metabolites found in leaves and stalks, and these levels also differ among stalks harvested during different stages of the cropping cycle (Table 10). The most variable metabolites between the different tissues are aconitic acid, quinic acid, glyceric acid, mannose, sedoheptulose and maltose (Table 13)

This data emphasises the challenges that would be faced to exploit these other chemicals commercially. The 18 compounds listed in Table 10, 7 and 8 represent 80% and 90% of the other water-soluble compounds. This implies that in leaves, the other 181 compounds together represent only 10 % of the total biomass in the juice, or 5% of total biomass. For the leaves, more than 80 compounds only make up 10% of the juice or less than 1kg per tonne of DW

Table 9 lists high-value metabolites that have a small international market size, meaning they don't provide opportunities for diversification among multiple sugarcane mills. Furthermore, these metabolites can also be produced through controlled fermentation technology, which makes recovery easier and production costs lower. Before pursuing these high-value product opportunities, it's crucial to assess market demand, production scalability, regulatory requirements, and quality standards. Additionally, it's essential to align production processes with sustainability principles and environmentally friendly practices to appeal to conscious consumers.

Table 9: Contribution of the non-sugar water solubles to total DW yield of leaves and stalks from the sugarcane genotypes. One-way ANOVA followed by HSD. nova other solutes. Values followed by the same letters are not significantly different (p=0.05).

Genotype	Leaf		Genotype	Young stalk		Genotype	Mature stalk	
	%DW			%DW			%DW	
QS10-8770	16.62	a	QS08-7370	12.95	a	Q240	8.62	a
QS10-7123	16.29	ab	QN13-609	12.67	a	Q208	8.40	ab
QN13-173	15.77	bc	WSRA24	12.33	ab	QS08-8662	7.96	abc
Q240	15.60	cd	QS08-8662	11.58	abc	WSRA24	7.50	abcd
QN13-609	15.55	cde	SRA3	11.36	abc	QS09-8348	7.23	abcd
QN12-512	15.44	cde	QS10-8770	10.77	abc	QN13-173	7.04	abcd
QS08-8662	15.42	cde	QS09-8348	10.64	abc	QS07-9185	6.93	abcd
Q208	15.35	cde	Q240	10.30	abc	QN13-609	6.07	abcd
QS09-8348	15.26	cde	QN12-512	10.03	abc	SRA32	5.97	bcd
QS07-9185	15.25	cde	QN13-173	9.23	abc	QS10-7123	5.85	bcd
KQ228	15.03	def	QSl0-7123	8.61	bc	QS08-7370	5.76	bcd
QS08-7370	14.89	efg	Q208	8.56	bc	QN12-512	5.52	cd
SRA32	14.42	fg	SRA32	8.35	c	KQ228	5.48	cd
SRA3	14.37	fg	KQ228	8.24	c	QS10-8770	5.10	d
WSRA24	14.31	g	QS07-9185	8.22	c	SRA3	5.10	d

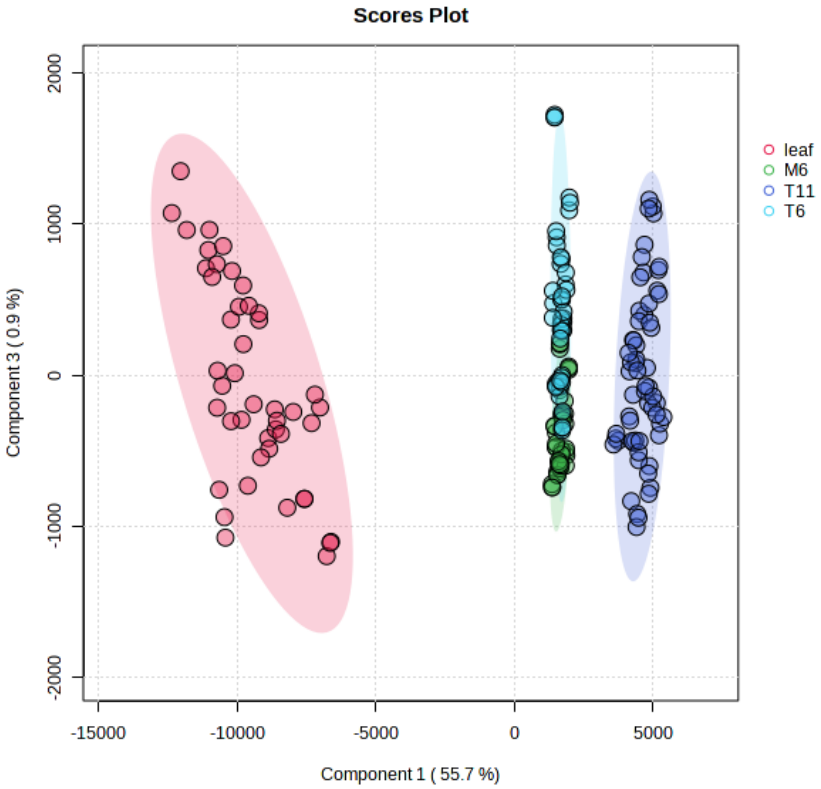

Figure 10: Partial Least Square-Discriminant Analysis (PLS-DA) scores plot of the abundant metabolites present in the leaf, mid-season stalk from (M6,T6), and mature stalk juice (T11). Shaded areas are the 95% confidence regions of each group. M=Mossman, T=Tablelands.

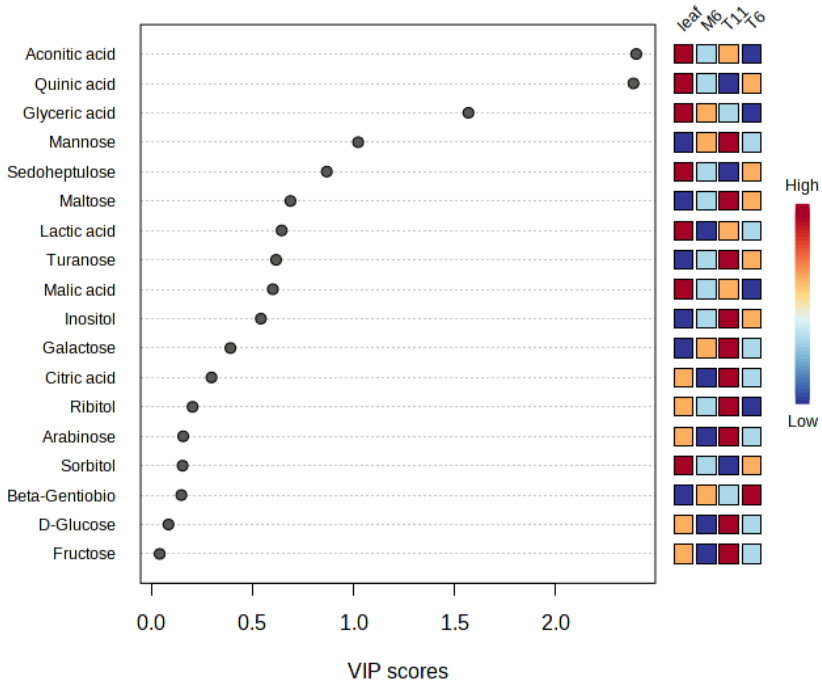

Figure 11: Variable Importance in Projection (VIP) score plot of the 18 abundant metabolites in the leaf, mid-season stalk, and mature stalk juice that vary most between the samples.

Table 10: Abundant water-soluble (polar) metabolites in the leaves of four sugarcane genotypes.

Metabolite	Leaf								
	Q208			QS10-8770			QS10-7123			WSRA24		
	kg Tonne^{-1}								
aconitate	1.04	±	0.074	0.84	±	0.013	0.88	±	0.036	0.73	±	0.028
cellobiose	3.43	±	0.125	2.90	±	0.085	3.11	±	0.055	2.50	±	0.044
citrate	0.00	±	0.000	0.00	±	0.000	0.00	±	0.000	0.00	±	0.000
fructose	30.73	±	3.731	34.05	±	4.604	36.80	±	6.258	29.57	±	5.028
galactose	14.27	±	1.860	13.79	±	1.692	15.71	±	2.720	12.62	±	2.186
gentibiose	0.34	±	1.629	13.66	±	0.306	14.85	±	0.364	11.93	±	0.292
glucose	20.08	±	3.742	21.98	±	2.483	24.30	±	4.681	19.53	±	3.761
glycerate	0.39	±	0.020	0.32	±	0.005	0.33	±	0.021	0.27	±	0.017
lactate	0.82	±	0.230	0.56	±	0.030	0.53	±	0.043	0.43	±	0.035
malate	0.33	±	0.017	0.27	±	0.011	0.29	±	0.011	0.24	±	0.009
maltose	1.86	±	0.121	1.51	±	0.058	1.65	±	0.057	1.32	±	0.045
mannose	23.73	±	2.941	22.39	±	2.213	24.27	±	3.041	19.50	±	2.443
myoinisitol	1.01	±	0.042	0.88	±	0.028	0.96	±	0.036	0.77	±	0.029
quinate	0.36	±	0.012	0.28	±	0.012	0.32	±	0.010	0.25	±	0.008
raffinose	1.65	±	0.118	1.39	±	0.087	1.48	±	0.033	1.19	±	0.027
ribitol	1.01	±	0.061	0.82	±	0.022	0.88	±	0.030	0.71	±	0.024
seduheptulose	0.67	±	0.028	0.57	±	0.039	0.67	±	0.004	0.54	±	0.003
turanose	3.68	±	0.195	2.99	±	0.077	3.35	±	0.162	2.70	±	0.130

Table 11: Abundant water-soluble (polar) metabolites in the immature (mid-season) stalks of four sugarcane genotypes.

Metabolite	Immature stalk								
	Q208			QS10-8770			QS10-7123			WSRA24		
	kg Tonne^{-1}								
aconitate	0.48	±	0.034	0.66	±	0.011	0.42	±	0.017	0.58	±	0.029
cellobiose	1.43	±	0.052	2.05	±	0.060	1.34	±	0.024	1.80	±	0.019
citrate	0.00	±	0.000	0.00	±	0.000	0.00	±	0.000	0.34	±	0.386
fructose	15.99	±	1.941	30.05	±	4.064	19.78	±	3.364	26.40	±	1.590
galactose	11.14	±	1.452	18.25	±	2.240	12.67	±	2.194	18.66	±	1.821
gentibiose	0.89	±	0.084	1.19	±	0.027	0.79	±	0.019	1.03	±	0.060
glucose	13.62	±	2.537	25.28	±	2.856	17.02	±	3.279	23.70	±	1.899
glycerate	0.20	±	0.010	0.28	±	0.004	0.18	±	0.011	0.08	±	0.189
lactate	0.35	±	0.099	0.41	±	0.022	0.24	±	0.019	0.32	±	0.034
malate	0.19	±	0.010	0.27	±	0.011	0.18	±	0.007	0.23	±	0.012
maltose	1.08	±	0.071	1.49	±	0.058	0.99	±	0.034	1.38	±	0.085
mannose	19.06	±	2.363	30.51	±	3.669	20.15	±	2.524	31.56	±	2.847
myoinisitol	0.43	±	0.018	0.64	±	0.020	0.42	±	0.016	0.56	±	0.023
quinate	0.31	±	0.010	0.41	±	0.017	0.28	±	0.009	0.36	±	0.011
raffinose	0.85	±	0.060	1.21	±	0.076	0.79	±	0.018	1.04	±	0.020
ribitol	0.45	±	0.027	0.62	±	0.016	0.41	±	0.014	0.55	±	0.015
seduheptulose	0.30	±	0.012	0.43	±	0.029	0.31	±	0.002	0.36	±	0.026
turanose	1.63	±	0.087	2.25	±	0.058	1.54	±	0.074	2.02	±	0.064

Table 12: Abundant water-soluble (polar) metabolites in the mature stalks of four sugarcane genotypes.

Metabolite	Mature stalk			
	Q208	QS10-8770	QS10-7123	WSRA24
	kg Tonne^{-1}			
aconitate	0.53 ± 0.038	0.26 ± 0.004	0.29 ± 0.012	0.35 ± 0.014
cellobiose	1.59 ± 0.058	0.80 ± 0.023	0.92 ± 0.016	1.10 ± 0.009
citrate	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000	0.21 ± 0.181
fructose	17.76 ± 2.157	11.66 ± 1.576	13.53 ± 2.301	16.17 ± 0.744
galactose	12.38 ± 1.613	7.08 ± 0.869	8.66 ± 1.500	11.43 ± 0.852
gentibiose	0.99 ± 0.093	0.46 ± 0.010	0.54 ± 0.013	0.63 ± 0.028
glucose	15.13 ± 2.819	9.81 ± 1.108	11.64 ± 2.243	14.52 ± 0.888
glycerate	0.23 ± 0.012	0.11 ± 0.002	0.12 ± 0.008	0.05 ± 0.089
lactate	0.39 ± 0.110	0.16 ± 0.008	0.16 ± 0.013	0.20 ± 0.016
malate	0.21 ± 0.011	0.11 ± 0.004	0.12 ± 0.005	0.14 ± 0.006
maltose	1.20 ± 0.079	0.58 ± 0.022	0.68 ± 0.023	0.84 ± 0.040
mannose	21.18 ± 2.625	11.83 ± 1.423	13.78 ± 1.726	19.34 ± 1.332
myoinisitol	0.48 ± 0.020	0.25 ± 0.008	0.29 ± 0.011	0.34 ± 0.011
quinate	0.34 ± 0.011	0.16 ± 0.007	0.19 ± 0.006	0.22 ± 0.005
raffinose	0.94 ± 0.067	0.47 ± 0.029	0.54 ± 0.012	0.64 ± 0.010
ribitol	0.50 ± 0.030	0.24 ± 0.006	0.28 ± 0.009	0.34 ± 0.007
seduheptulose	0.33 ± 0.014	0.17 ± 0.011	0.21 ± 0.001	0.22 ± 0.012
turanose	1.82 ± 0.096	0.87 ± 0.023	1.05 ± 0.051	1.24 ± 0.030

Table 13: Potential economic value of the compounds that are present in sugarcane juice derived from immature and mature cane.

Metabolite	Leaf				Culm			
	Q208	QS10-8770	QS10-7123	WSRA24	Q208	QS10-8770	QS10-7123	WSRA24
	USD Tonne^{-1} cane							
aconitate	2	2	2	2	1	1	1	1
cellobiose	7505	6355	6808	5471	3298	3114	2466	3182
citrate	0	0	0	0	0	0	0	39
fructose	17	19	21	16	9	12	9	12
galactose	2257	2180	2484	1996	1860	2003	1687	2380
gentibiose	5	193	210	169	13	12	9	12
glucose	361	395	437	351	259	316	258	344
glycerate	0	0	0	0	0	0	0	0
lactate	2	2	1	1	1	1	1	1
malate	1	1	1	1	0	0	0	0
maltose	8	6	7	6	5	4	4	5
mannose	1119	1055	1144	920	948	998	800	1200
myoinisitol	36	32	34	28	16	16	13	16
quinate	10	8	9	7	9	8	7	8
raffinose	1178	990	1060	851	638	598	473	598
ribitol	1488	1206	1302	1046	697	630	502	653
seduheptulose	866	743	873	701	405	388	337	381
turanose	105602	85784	96191	77294	49440	44784	37113	46772

Sweet Sorghum as a Supplementary Crop

Background

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 because there is already a shortfall in available biomass during the short crushing season.

The length of the cropping cycle is primarily 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.

Two options should be considered for year-round operation and to address the current shortfall in total biomass availability. Firstly, if sucrose is no longer the main emphasis, 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.

Sweet sorghum (*Sorghum bicolor* L. Moench) is a versatile, drought-tolerant crop known for its sugar-rich stalks. Unlike traditional grain sorghum, sweet sorghum is cultivated primarily for its sugary stalks, which can be processed into ethanol, syrup, and other products. Its ability to thrive in diverse environments and its efficient water use make it an attractive option for sustainable agriculture and bioenergy production (Mathur et al., 2017).

Sweet sorghum has gained attention as a bioenergy and biofuel source due to its high sugar content and suitability for marginal lands. Understanding sweet sorghum's growth stages and sugar accumulation patterns is crucial for optimising its cultivation and utilisation. There are at least seven clearly defined stages in the sweet sorghum growth cycle, including germination, seedling stage, tillering stage, stem elongation, panicle initiation, boot stage, flowering and grain filling.

Harvesting time is dependent on the intended use of the material. However, it is essential to recognise that the stem's total sugar and sucrose content is highly variable (Lingle, 1987; Teetor et al., 2011). The sugar content of the stalk is lowest at the boot stage and highest during grain filling. As with sugarcane, sucrose accumulation in the stalk is tightly linked to a reduction in growth.

Sweet sorghum can supplement the biomass supply at the Mossman mill. It can be grown worldwide in tropical, semi-tropical, and semiarid regions due to its high photosynthesis rate, water resistance, and nutrient efficiency.

Like sugarcane, sweet sorghum produces a high sugar concentration in its culm/stalk. The juice contains a mixture of sugars, including sucrose, glucose, and fructose, which can be directly fermented into a first-generation biofuel. The bagasse can be used as fodder, as heat generation through burning, or as a raw material for second-generation biofuels after pretreatment (Bihmidine et al., 2015; Mathur et al., 2017)).

A study showed that sweet sorghum and energy cane, which have different harvest times from sugarcane, have similar chemical compositions and structures and can be processed by traditional sugarcane harvest and processing systems (Kim and Day, 2011; Viator et al., 2009). This presents an opportunity to increase ethanol production and expand the feedstock supply outside the sugarcane season in Louisiana. However, incorporating new crops into the existing sugarcane infrastructure and partitioning feedstocks for both fuel and sugar during standard sugarcane processing remain challenging (Mathur et al., 2017).

The data presented in this report emphasise the challenges in growing sweet sorghum in tropical conditions in Australia. In addition, the chemical composition of the sweet sorghum under tropical conditions would impose additional challenges for the processing of the material in a “normal sugarcane processing system”.

Biomass production

At Singh Farming Pty Ltd ATF Singh Farming Business Enterprise Trust in the Atherton Tablelands, 18 sorghum genotypes were tested in four trials (see material and methods).

The germination process for the crop is fast, and within 25 days, it becomes well-established. However, there are significant variations in the yield among the different sorghum genotypes (Table 1). From this trial, three sorghums (SE45, SE19, and Megasweet), along with two others (Dynasweet and SK106), were chosen for further evaluation in fully replicated trials.

Based on the data, it is evident that Megasweet and SK106 exhibit the highest yields and stalk populations (Figure 12: 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.(Table 14). Planting in autumn leads to significantly lower yields than summer planting);. Earlier research has also highlighted that the planting time significantly impacts the quality of juice and levels of soluble sucrose (Rao et al., 2013).

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

Genotype	Fresh mass Yield (TH)	Plant population	Flowered
SE35	82.3	135844	N
SE23	78.4	172797	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 the transition to flowering

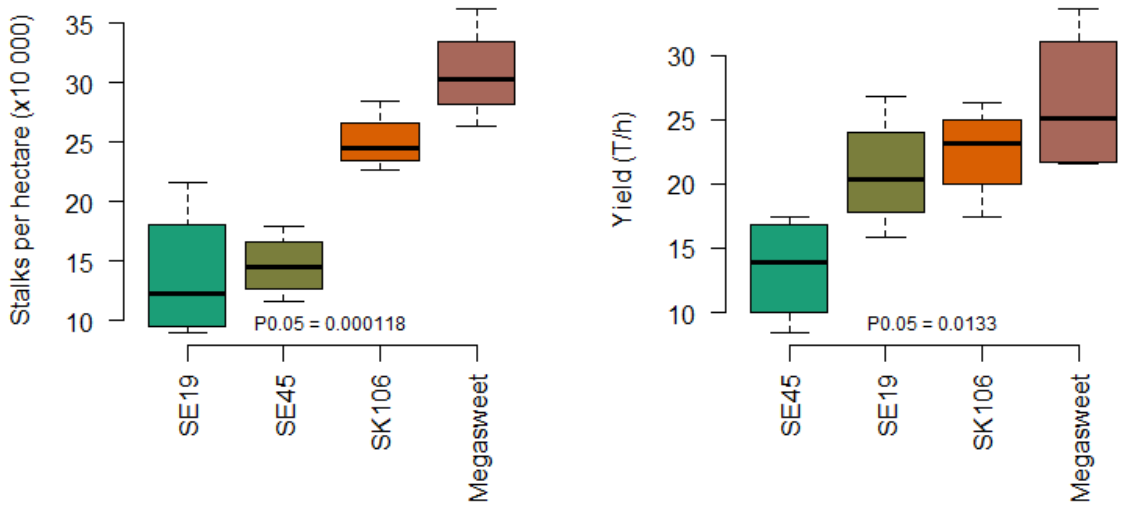

Figure 12: Stalk population (A) and yield (B) of four sweet sorghum genotypes in the Tablelands. Plants were planted in April and harvested 58 days after planting. All the varieties flowered more than 90% at this stage.

Flowering

During the initial screening trial, all 15 genotypes at 62 DAP have flowered
Table 14). It's worth noting that genotypes SE1, SE5, SE20, SE23, and SE35 may require more time to reach the flowering stage compared to other varieties.

To successfully integrate sorghum into a sugarcane-based production system, finding genotypes with a more extended vegetative growth period or allowing the processing facility to handle both vegetative material and flowers/seeds is necessary. In sorghum, delaying flowering can increase the size of stems and the potential for sucrose accumulation. This trait is associated with high biomass yield and nitrogen use efficiency, so delayed flowering and long vegetative growth duration are essential [7, 9, 10].

During our search, we identified a new hybrid (SK106)[1] with a late flowering response, moderate sugar content, and high biomass production (as shown in Fig 4). However, when we conducted fully replicated trials, including SE19, SE45, Megasweet and SK106, all the genotypes either started flowering or showed signs of wilting 59 DAP.

What controls flowering

Photoperiod sensitivity and flowering time in sorghum are controlled through the maturity alleles Ma_1 through Ma_6 (Murphy et al., 2014, 2011). Genotypes dominant for Ma_1 and Ma_6 will flower very late under long-day conditions. A genotype such as SK106 falls into this category.

The amount of daylight hours during different seasons decreases as you move closer to the equator. This means there is less variation in day length in tropical areas compared to places with temperate climates. The

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warm temperature and consistent day length in the tropics are ideal for plant growth, leading to early flowering. However, most plant types will mature too soon in the tropics to produce enough biomass.

Pest and disease challenges for sorghum production

In all the trials, armyworm was a significant problem 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 (Das et al., 2015). The first report on the occurrence of PBD in sorghum dates back to 1941 (Ramakrishnan, 1941). In the first season of this project and all the initial screening trials, PBD was not observed.

During the replicated trials in the second half of 2021, PDB became a significant problem. The varieties with milder symptoms have recovered, but the ones with top rot have been severely affected, resulting in the abandonment of trials (**Figure 13**).

One of the main symptoms of PBD is the presence of deformed or discoloured leaves near the top of the plant. These leaves may become wrinkled, twisted, and fail to unfold correctly, giving a ladder-like appearance. Other noticeable symptoms include wrinkling of leaf bases, minor transverse cuts in the leaf margin, stem bending, and twisting of nodes and internodes. In severe cases, the infection may spread from the leaves and sheath into the stem, causing top rot (Das et al., 2015 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 the damage caused by Pokkah boeng (**Figure 13**B).

A).

Figure 13: Pokkah boeng disease (PBD) in the Atherton Tablelands (A) sorghum trials. Damage caused by fall armyworm (*Spodoptera frugiperda*) to the spindle leaves (B).

Biomass composition

During harvest, the majority of the biomass in sweet sorghum is found in the stalks (around 70%), followed by the leaves (20%), and the remaining 10% in the flowers and seeds (Zhao et al. 2009; Rao et al. 2013;

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

Mathur et al. 2017). Determining the composition of any supplementary crop introduced into a sugarcane processing facility is crucial. Additionally, it is important to comprehend the composition of each component of the supplementary crop.

More than 60% of leaf and stalk tissue biomass is in water-insoluble or bagasse components (Figure 14). There is a statistical difference in the total cell wall and water-soluble fractions ($p<0.001$). However, it is unlikely that the leaves and stalks will be separated before they enter the sugarcane mill; hence, we only present the composition of the total aerial biomass.

There was a statistically significant variation in the dry weight composition of the four sorghum varieties that have been analysed in detail. The cell wall sugar component, cellulose and pentoses, varied between 42 and 50% (Figure 15A). The total cell wall sugar of SE19 was significantly higher ($P<0.001$) than that of the other three genotypes. The cellulose and pentan component of SE19 is considerably higher than that of the other varieties (Figure 15B&C). The lignin content of SE19 and SK106 was significantly higher than that of SE45 (Figure 15D).

Lignin provides structural support and protection to plant cell walls, contributing to the recalcitrance of lignocellulosic biomass. It hinders access to cellulose and hemicellulose by enzymes and microorganisms during fermentation processes to produce biofuels and bioproducts from biomass (Chundawat et al., 2011; Himmel et al., 2007). Following this, a variety like SE45 is more suited for processing the lignocellulosic fraction.

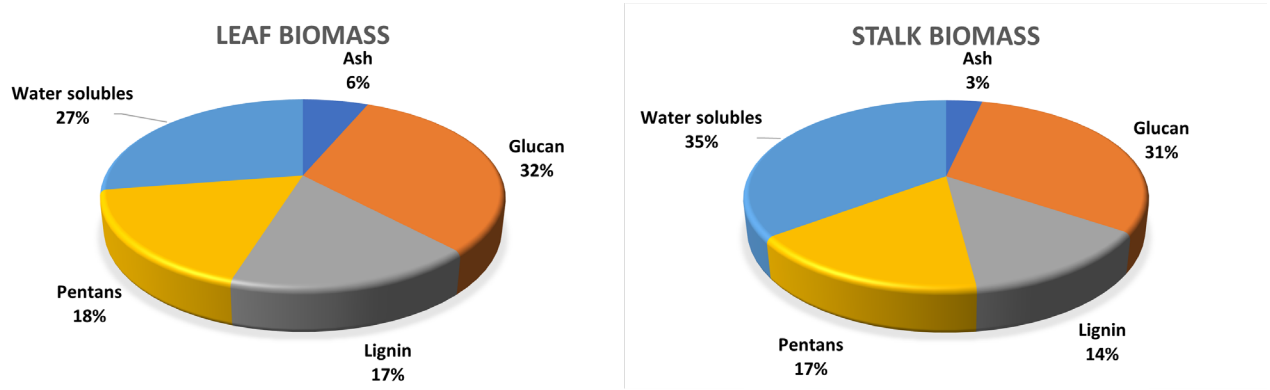

Figure 14: Pie chart of the average biomass composition of the leaves and stalks of sweet sorghum grown for 70 DAP under tropical conditions.

The total soluble solids content (Brix) of Megasweet and SE45 were significantly higher than that of SK106 and SE19 (A). These two varieties also had the highest soluble sugar content. The total soluble sugars (

Figure 16B) only represent up to 50% of the total brix. The reducing sugar (glucose and fructose) content was higher than the sucrose content in all the genotypes. Interestingly, Megasweet and SK106 were the two varieties with the highest sucrose content. Although SE45 was a high soluble sugar variety, its sucrose content was only 25% of the total soluble sugars.

Based on the significant amount of reducing sugars found in sorghum juice, there are better sources for sucrose production. Combining it with sugarcane during processing could result in a decrease in juice quality and sucrose recovery. Nevertheless, the high level of reducing sugars in sorghum juice could make it a better option than sugarcane for use in fermentation technologies.

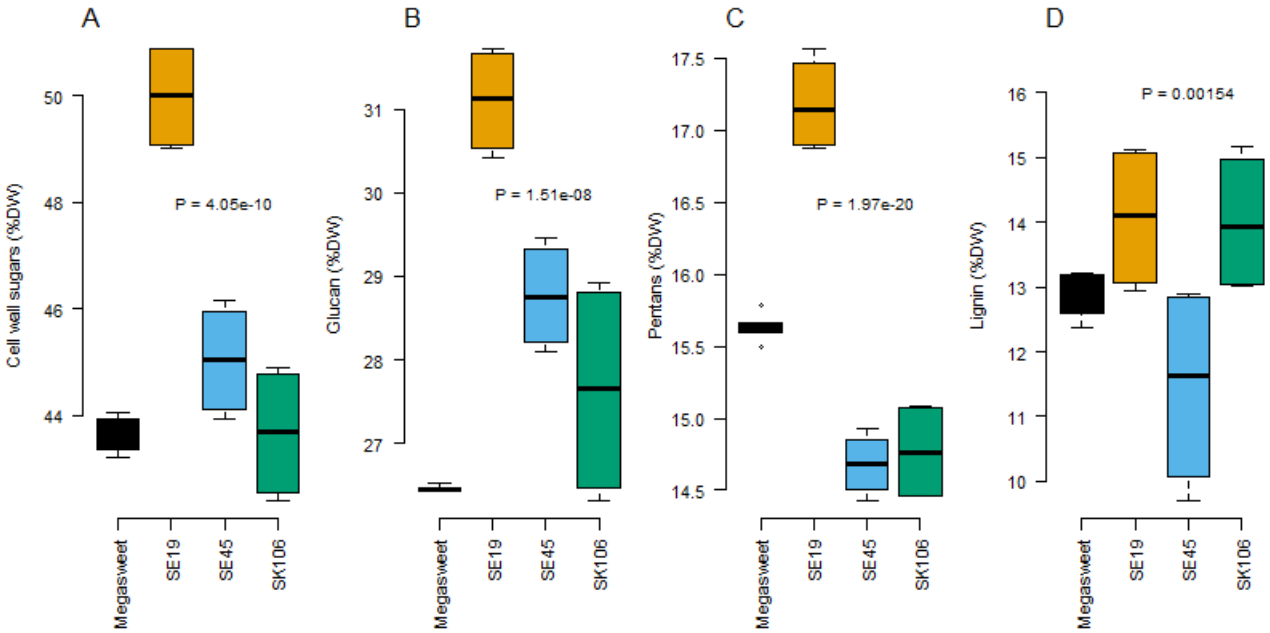

Figure 15: Cell wall components of four sorghum genotypes on the Tablelands 70DAP. Cell wall sugars (A), glucan (B), pentan (C), and lignin (D).

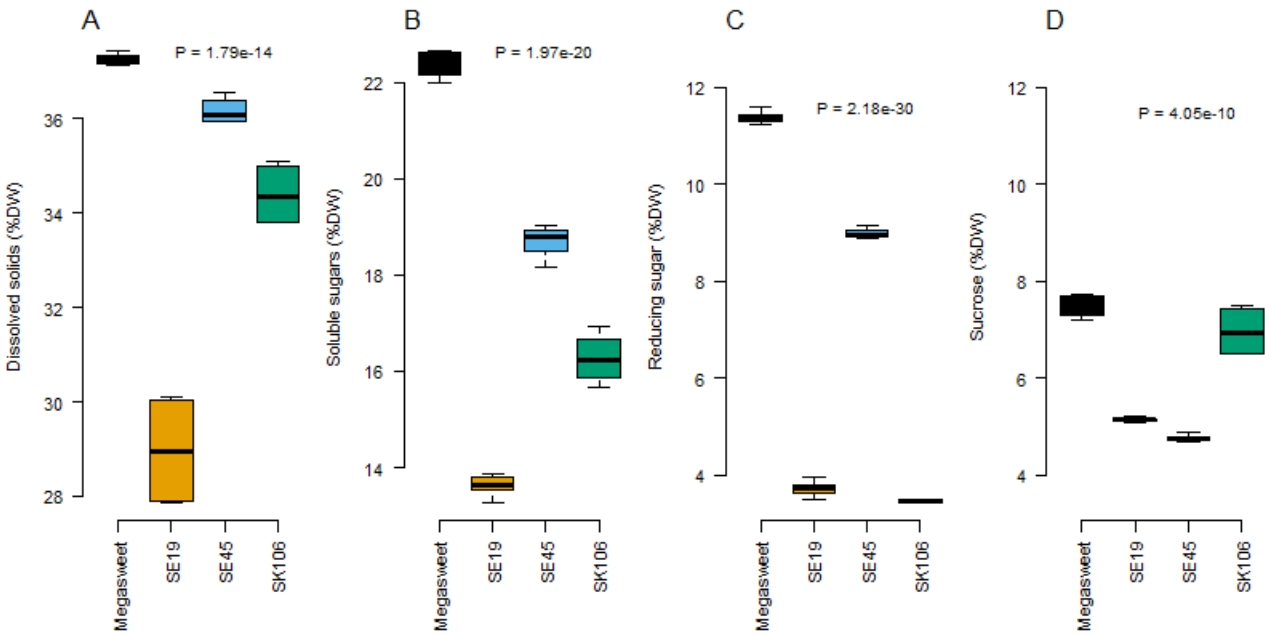

Figure 16:Water soluble fractions of sorghum 70DAP. Dissolved solids (Brix) (A), total water-soluble sugars (B), reducing sugars (C) and sucrose (D) of four sorghum genotypes on the Tablelands.

Other water-soluble metabolites

Metabolomic profiles of the water-soluble components of the sorghum genera were captured using a high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) assay. GC-MS analysis was used to determine the sorghum genera's polar metabolite profile. More than 200 polar metabolites were in the sorghum juice (Table A5.1). [Only nineteen of these metabolites are present at levels > 0.05% of total dry mass and could be recovered from the tissue.

The metabolome of leaf samples was completely different from that of stalk samples (Figure 17). The separation between the two tissue types is largely linked to the dominance of metabolites from secondary metabolism in the leaf and metabolites associated with sugar metabolism in the stalk. There are two main clusters within the leaf samples. Sk106 was very different from the other three genotypes in particular due to the very high levels of the secondary metabolites. There are two clusters in the stalk samples. SK106 and SE19 in one cluster, and Megasweet and SE45 in a second. The dominance of high sugar levels in Megasweet and SE45 is evident (Figure 17).

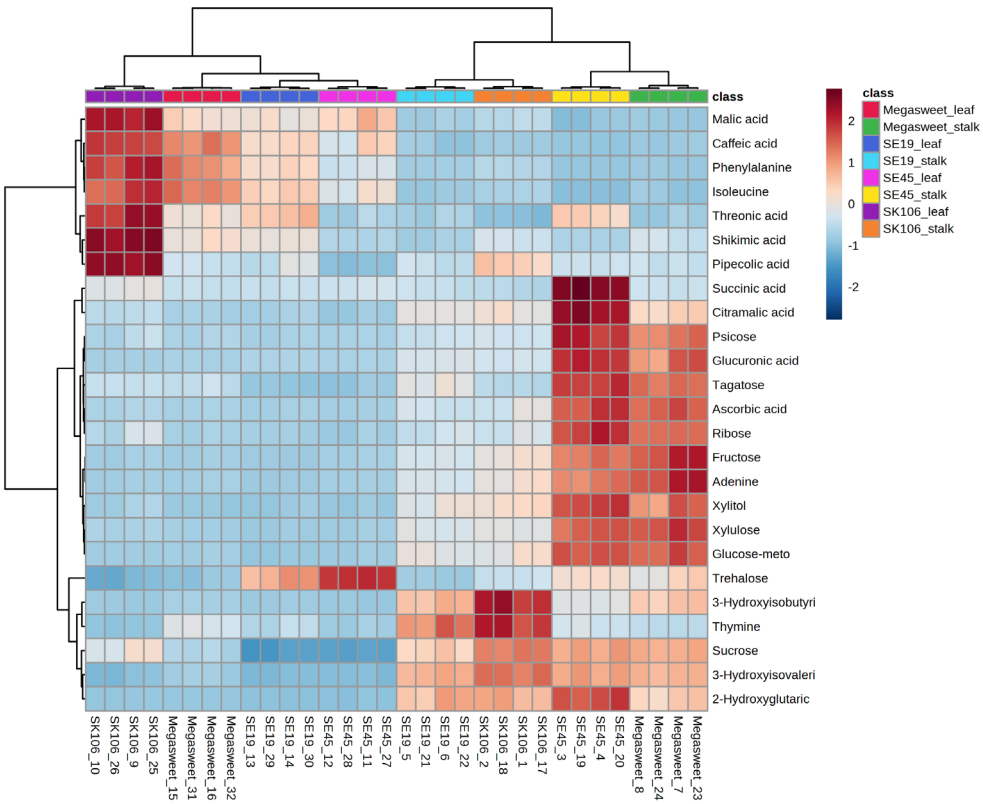

Figure 17: Heat map of metabolites in sorghum leaf and stalk samples of four genotypes. differentially expressed genes. Blue colour represents a lower and red colour represents a higher concentration level.

The completely different metabolic profiles between the varieties suggest that juice derived from these samples will have different flavour and fermentation properties.

The metabolic profile of the abundant metabolites between the four varieties was also different (Table 17). Megasweet and SE45 would be more suited for the recovery of most of the abundant metabolites. Other than sucrose and aconitate, SK106 would not be a good source to recover some of these alternative metabolites.

Interesting to note that in contrast to sugarcane, in sorghum some of these other metabolites have significantly higher values than sucrose.

Utilising the lignocellulosic fraction

Ethanol production potential

Table 15: The cellulose and hemicellulose content, and theoretical ethanol production potential of four sorghum varieties

Genotype	Cellulose		Hemicellulose		Ethanol	
	kg tonne^{-1}				litre tonne^{-1}	
SE45	450.6739124	a	229.56	a	230.8849	a
SE19	437.7738733	ab	241.87	b	231.098	a
Megasweet	421.6080347	b	249.239	c	228.57	a
SK106	421.5211234	b	225.1351	d	219.647	b

Biogas potential

In Table 3, you can see the biogas and BMP results of various sorghum samples. The leaf samples had an average moisture content of 8.49%, with total solids making up 91.51% of the samples. On a dry mass basis, the ash percentage was 7.49%. The C/N ratio of the samples was 23:1, which indicates that they are highly suitable for mono-digestion if other trace elements are added. The C/S ratio was 307:1, and the low sulphur content in the sample suggests that there is a very low possibility of hydrogen sulphide accumulation in the digester.

The analysis revealed notable variations in methane production between leaves, stalks, and different genotypes. The cultivar Megasweet generated the most significant levels of methane production. Earlier studies have reported genotypic variations in BMP between sorghum genotypes (Sambusiti et al., 2013; Thomas et al., 2019).

The leaf digestates are not suitable for a standalone fertiliser as the average K concentration of 44517 ppm (DW) is considerably higher than the N levels (31900 ppm DW).

Table 16: Biogas, biomethane and hydrogen sulphite production from sorghum bagasse. Yields were determined over a 28-day period.

Genotype	Leaf			Stalks		
	Biogas	BMP	H_2S	Biogas	BMP	H_2S
	litre kg^{-1} DW		ppm	litre kg^{-1} DW		ppm
Megasweet	452.0	300.8	21	496.9	445.0	17
SK106	442.0	313.8	25	500.0	360.0	19
SE19	430.5	292.7	19	487.0	360.4	12
SE45	475.7	356.8	22	468.0	322.9	15

Challenges to integrating sorghum as a supplementary crop

Incorporating sweet sorghum into a sugarcane-based farming operation in the wet tropics will pose numerous obstacles. For optimal results, planting should take place as soon as possible after the wet season ends, with an April/May planting maximising plant crop yield (refer to Fig. 3). However, it is essential to acknowledge that unfavourable weather conditions may cause a delay in planting until the start of September, particularly in Mossman.

One of the main challenges growing sorghum in tropical areas is its tendency to flower early due to minimal variation in day-length. Our trials revealed that even sorghum genotypes like SK106, which are selected for late flowering, still flower extensively in Northern Queensland. For instance, at a latitude of 27.5606'S (Toowoomba), SK106 did not flower within the first 130 days after planting (DAP), but it reached 100% flowering at a latitude of 17.268 'S (Atherton Tablelands).

The impact of flowering on biomass has two main effects [3]. Firstly, there is competition between grain filling and the accumulation of soluble sugars in stalks. Removing the inflorescences as they form leads to an increase in soluble sugar accumulation in the stalks. However, this effect is only moderate, indicating that the carbon needed to support flowering and grain fill does not come from the re-mobilisation of stalk sugars. The second and more significant impact of flowering is creating a new major sink for the photosynthate from the leaves, which strongly suppresses vegetative biomass accumulation. In sorghum, delaying flowering increases stem size and the potential for sucrose accumulation [4, 2, 8, 6]. Delayed flowering and prolonged vegetative growth are crucial traits associated with high biomass yield and nitrogen use efficiency.

The crop is harvested between June and November (the driest months of the year) at both locations. Blocks scheduled for plough out are cut at the end of the harvest season. Ideally, the fallow period is from December to March. However, if the wet season lingers, it might be December to May or June (Figure 18).

To effectively incorporate sorghum into a sugarcane-based production system, it is imperative to select genotypes with a more extended vegetative growth period in tropical regions than those tested in the project. Planting sorghum from spring to
early summer results in higher biomass production. However, given the short growth cycle, harvesting must be completed by the end of December or early January.

Planting must occur by January using the currently tested germplasm for a successful crop between March and May. A range of maturation groups that can grow within 90 to 120 days would be highly beneficial. A study conducted in Brazil has shown that the highest biomass production is achieved when planted in the early spring and summer, and early-maturing varieties yield less biomass when planted outside the ideal planting period (Viator et al., 2009).

Two issues lack sufficient data. Firstly, planting sorghum during the period intended for a fallow or break-crop can severely affect pest and disease cycles. Sugarcane and sweet sorghum are susceptible to similar diseases and pests. Secondly, planting sorghum coincides with the beginning of the wet season, and harvesting during this time would be less than ideal.

Sugar is typically obtained from sweet sorghum by squeezing its stalks through a roller mill. However, this process is ineffective as a significant amount of fermentable sugar remains after just one crushing. Sugarcane mills use multi-stage extraction technologies, which result in more excellent recovery but require more energy. A more efficient method is diffusion extraction, which is similar to the process used for sugar beets and sugarcane. However, Australian sugarcane mills do not use this method.

This report assumes that all soluble sugars and other metabolites can be recovered from the material, but even with full recovery, the juice purity will be low due to the high reducing sugar content. In fact, in most cases, the reducing sugar content exceeds the sucrose content, making sucrose recovery impossible. A similar problem will arise when sugarcane and sorghum are significantly blended into the milling train.

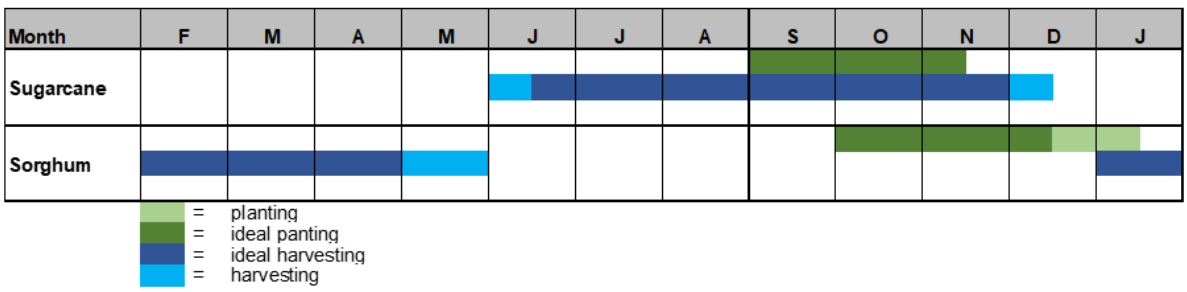

Figure 18: Potential cropping cycles of sugarcane and sorghum tropical Northern Queensland.

Table 17: Abundant water-soluble (polar) metabolites in the leaves and stalks of sweet sorghum grown on the Tablelands. These metabolites are present at levels >0.05% of total water solubles.

Metabolite	Leaves				Stalks			
	min	max			min	max		
	Kg. tonne^{-1} Dry weight							
Aconitic acid	2.93	10.88	±	2.71	2.37	4.22	±	0.58
Cellobiose	5.74	6.42	±	0.23	5.97	6.83	±	0.27
Citric acid	2.03	3.03	±	0.31	1.92	2.11	±	0.06
Fructose	4.85	9.58	±	1.42	17.23	49.41	±	9.84
Galactose	5.12	11.93	±	2.18	28.88	137.48	±	47.33
Gentibiose	3.44	3.75	±	0.11	3.88	5	±	0.38
Glucose	1.02	1.12	±	0.04	5.72	6.94	±	0.46
Glyceric acid	0.89	1.03	±	0.04	0.85	0.94	±	0.03
Lactic acid	1.09	1.51	±	0.12	1.04	1.63	±	0.18
Malic acid	1.43	3.24	±	0.57	0.89	1.31	±	0.13
Maltose	7.33	8.34	±	0.29	7.67	8.79	±	0.4
Mannose	1.8	5.18	±	1.09	13.55	61.48	±	21.16
Myoinositol	1.31	1.69	±	0.11	1.17	1.33	±	0.05
Quinic acid	1.21	1.49	±	0.09	1.2	1.44	±	0.08
Raffinose	5.8	6.35	±	0.18	5.85	6.5	±	0.23
Ribitol	1.08	3.32	±	0.8	1.06	1.2	±	0.04
Sedoheptulose	2.49	5.89	±	1.06	15.16	44.02	±	8.83
Sucrose	3.75	16.75	±	4.83	29.24	67.66	±	12.6
Turanose	4.49	7.15	±	0.81	6.6	7.46	±	0.29

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Firstly, I would like to acknowledge Donald Murday, Jason Salvetti and Rajinder Singh, who generously made their land and resources available for the field trials.

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References

Alalwan, H.A., Alminshid, A.H., Aljaafari, H.A.S., 2019. Promising evolution of biofuel generations. Subject review. Renew. Energy Focus 28, 127–139.

Alexander, A.G., 1988. Sugarcane as a source of biomass. FAO Anim. Prod. Health Pap. FAO.

Alexander, A.G., 1985. The energy cane alternative. Elsevier Science Publishers,New York, NY, United States.

Arif, S., Batool, A., Nazir, W., Khan, R.S., Khalid, N., 2019. 8 - Physiochemical Characteristics Nutritional Properties and Health Benefits of Sugarcane Juice, in: Grumezescu, A.M., Holban, A.M. (Eds.), Non-Alcoholic Beverages. Woodhead Publishing, pp. 227–257. https://doi.org/10.1016/B978-0-12-815270-6.00008-6

Benetto, E., Gericke, K., Guiton, M., 2018. Designing sustainable technologies, products and policies: from science to innovation. Springer Nature.

Berding, N., Marston, D.H., 2010. Operational validation of the efficacy of spectracanetm, a high-speed analytical system for sugarcane quality components. Proc. 2010 Conf. Aust. Soc. Sugar Cane Technol. Held Bundaberg Qld. Aust. 11-14 May 2010 445–459.

Biely, P., 2012. Microbial carbohydrate esterases deacetylating plant polysaccharides. Biotechnol. Adv. 30, 1575–1588.

Bihmidine, S., Baker, R.F., Hoffner, C., Braun, D.M., 2015. Sucrose accumulation in sweet sorghum stems occurs by apoplasmic phloem unloading and does not involve differential Sucrose transporter expression. BMC Plant Biol 15, 186. https://doi.org/10.1186/s12870-015-0572-8

Bihmidine, S., Hunter, C.T., Johns, C.E., Koch, K.E., Braun, D.M., 2013. Regulation of assimilate import into sink organs: update on molecular drivers of sink strength. Front. Plant Sci. 4, 177. https://doi.org/10.3389/fpls.2013.00177

Bonnett, G.D., Hewitt, M.L., Glassop, D., 2006. Effects of high temperature on the growth and composition of sugarcane internodes. Aust. J. Agric. Res. 57, 1087–1095. https://doi.org/10.1071/ar06042

Botha, F.C., 2019. A Research model for carbon partitioning in sugarcane. Res. Outreach.

Botha, F.C., Moore, P.H., 2013. Biomass and Bioenergy, in: Sugarcane: Physiology, Biochemistry, and Functional Biology. pp. 521–540. https://doi.org/10.1002/9781118771280.ch19

Botha, F.C., Scalia, G., Marquardt, A., Wathen-Dunn, K., 2023. Sink Strength During Sugarcane Culm Growth: Size Matters. Sugar Tech. https://doi.org/10.1007/s12355-023-01273-0

Burton, R.A., Fincher, G.B., 2014. Plant cell wall engineering: applications in biofuel production and improved human health. Food Biotechnol. ⬢ Plant Biotechnol. 26, 79–84. https://doi.org/10.1016/j.copbio.2013.10.007

Carvalho-Netto, O.V., Bressiani, J.A., Soriano, H.L., Fiori, C.S., Santos, J.M., Barbosa, G.V.S., Xavier, M.A., Landell, M.G.A., Pereira, G.A.G., 2014. The potential of the energy cane as the main biomass crop for the cellulosic industry. Chem. Biol. Technol. Agric. 1, 20. https://doi.org/10.1186/s40538-014-0020-2

Cherubini, F., 2010. The biorefinery concept: Using biomass instead of oil for producing energy and chemicals. Energy Convers. Manag. 51, 1412–1421.

Chundawat, S.P.S., Beckham, G.T., Himmel, M.E., Dale, B.E., 2011. Deconstruction of Lignocellulosic Biomass to Fuels and Chemicals. Annu. Rev. Chem. Biomol. Eng. 2, 121–145. https://doi.org/10.1146/annurev-chembioeng-061010-114205

Das, I.K., Rakshit, S., Patil, J.V., 2015. Assessment of artificial inoculation methods for development of sorghum pokkah boeng caused by Fusarium subglutinans. Crop Prot. https://doi.org/10.1016/j.cropro.2015.07.020

De Mendiburu, F., Reinhard, S., 2015. Agricolae - ten years of an open source statistical tool for experiments in breeding, agriculture and biology. PeerJ Prepr.

de Souza Barbosa, G.V., dos Santos, J.M., Diniz, C.A., Cursi, D.E., Hoffmann, H.P., 2020. Chapter 6 - Energy cane breeding, in: Santos, F., Rabelo, S.C., De Matos, M., Eichler, P. (Eds.), Sugarcane Biorefinery, Technology and Perspectives. Academic Press, pp. 103–116. https://doi.org/10.1016/B978-0-12-814236-3.00006-8

Doehlert, D.C., 1993. Sink strength: Dynamic with source strength. Plant Cell Environ. 16, 1027–1028.

Evans, M.W., 1940. Developmental Morphology of the Growing Point of the Shoot and the Inflorescence in Grasses. J. Agricutural Res. 61, 481-520.

Gille, S., Pauly, M., 2012. O-acetylation of plant cell wall polysaccharides. Front. Plant Sci. 3, 12.

Herbers, K., Sonnewald, U., 1998. Molecular determinants of sink strength. Curr. Opin. Plant Biol. 1, 207–216. https://doi.org/10.1016/S1369-5266(98)80106-4
Himmel, M.E., Ding, S.-Y., Johnson, D.K., Adney, W.S., Nimlos, M.R., Brady, J.W., Foust, T.D., 2007. Biomass Recalcitrance: Engineering Plants and Enzymes for Biofuels Production. Science 315, 804–807. https://doi.org/10.1126/science.1137016
Ho, L.C., 1988. Metabolism and Compartmentation of Imported Sugars in Sink Organs in Relation to Sink Strength. Annu. Rev. Plant Physiol. Plant Mol. Biol. 39, 355–378. https://doi.org/10.1146/annurev.pp.39.060188.002035
Inman-Bamber, G., 2014. Sugarcane Yields and Yield-Limiting Processes, in: Sugarcane: Physiology, Biochemistry, and Functional Biology. John Wiley & Sons Ltd, pp. 579–600. https://doi.org/10.1002/9781118771280.ch21
Inman-Bamber, G., 2013. Sugarcane yields and yield-limiting processes, in: Moore, P.H., BOTHA, F.C. (Eds.), Sugarcane: Physiology, Biochemistry, and Functional Biology. Wiley Online Library, pp. 579–600.
Inman-Bamber, N., Muchow, R., Robertson, M., 2002. Dry matter partitioning of sugarcane in Australia and South Africa. Field Crops Res. 76, 71–84.
Inman-Bamber, N.G., 1994. Temperature and seasonal effects on canopy development and light interception of sugarcane. Field Crops Res. 36, 41–51. https://doi.org/10.1016/0378-4290(94)90051-5
Jaffé, W.R., 2012. Health Effects of Non-Centrifugal Sugar (NCS): A Review. Sugar Tech 14, 87–94. https://doi.org/10.1007/s12355-012-0145-1
Jatoi, A.S., Abbasi, S.A., Hashmi, Z., Shah, A.K., Alam, M.S., Bhatti, Z.A., Maitlo, G., Hussain, S., Khandro, G.A., Usto, M.A., Iqbal, A., 2023. Recent trends and future perspectives of lignocellulose biomass for biofuel production: a comprehensive review. Biomass Convers. Biorefinery 13, 6457–6469. https://doi.org/10.1007/s13399-021-01853-8
Jeffrey, S.J., Carter, J.O., Moodie, K.B., Beswick, A.R., 2001. Using spatial interpolation to construct a comprehensive archive of Australian climate data. Environ. Model. Softw. 16, 309–330. https://doi.org/10.1016/S1364-8152(01)00008-1
Kebrom, T.H., McKinley, B., Mullet, J.E., 2017. Dynamics of gene expression during development and expansion of vegetative stem internodes of bioenergy sorghum. Biotechnol. Biofuels 10, 159. https://doi.org/10.1186/s13068-017-0848-3
Kim, M., Day, D.F., 2011. Composition of sugar cane, energy cane, and sweet sorghum suitable for ethanol production at Louisiana sugar mills. J. Ind. Microbiol. Biotechnol. https://doi.org/10.1007/s10295-010-0812-8
Lingle, S.E., 1999. Sugar metabolism during growth and development in sugarcane internodes. Crop Sci. 39, 480–486.
Lingle, S.E., 1987. Sucrose Metabolism in the Primary Culm of Sweet Sorghum During Development1. Crop Sci. 27, cropsci1987.0011183X002700060025x. https://doi.org/10.2135/cropsci1987.0011183X002700060025x
Lingle, S.E., Thomson, J.L., 2012. Sugarcane internode composition during crop development. BioEnergy Res. 5, 168–178.
Mathur, S., Umakanth, A.V., Tonapi, V.A., Sharma, R., Sharma, M.K., 2017. Sweet sorghum as biofuel feedstock: recent advances and available resources. Biotechnol. Biofuels. https://doi.org/10.1186/s13068-017-0834-9
McMaster, G.S., 2005. Phytomers, phyllochrons, phenology and temperate cereal development. Phytomers Phyllochrons Phenol. Temp. Cereal Dev. 143, 137–150.
Meek, D.W., Hatfield, J.L., Howell, T.A., Idso, S.B., Reginato, R.J., 1984. A Generalized Relationship between Photosynthetically Active Radiation and Solar Radiation1. Agron. J. 76, 939–945.
Murphy, R.L., Klein, R.R., Morishige, D.T., Brady, J.A., Rooney, W.L., Miller, F.R., Dugas, D.V., Klein, P.E., Mullet, J.E., 2011. Coincident light and clock regulation of pseudoresponse regulator protein 37 (PRR37) controls photoperiodic lowering in sorghum. Proc. Natl. Acad. Sci. USA. https://doi.org/doi:10.1073/pnas.1106212108
Murphy, R.L., Morishige, D.T., Brady, J.A., Rooney, W.L., Yang, S., Klein, P.E., Mullet, J.E., 2014. Ghd7 (ma6) represses sorghum flowering in long days: Ghd7 alleles enhance biomass accumulation and grain production. Plant Genome. https://doi.org/10.3835/plantgenome2013.11.0040
Natarajan, S., Basnayake, J., Wei, X., Lakshmanan, P., 2019. High-throughput phenotyping of indirect traits for early-stage selection in sugarcane breeding. Remote Sens. 11, 2952. https://doi.org/10.3390/rs11242952

Rajendran, I., Alagarsamy, V., Palaniswami, C., 2021. Value added products from clarified sugarcane juice. J. Sugarcane Res. 11, 1. https://doi.org/10.37580/JSR.2021.1.11.1-5
Ramakrishnan, T.S., 1941. Top rot (Twisted top or pakkah bong) of sugarcane, sorghum and cumbu. Curr. Sci. India.
Rao, P.S., Kumar, C.G., Reddy, B.V.S., 2013. Sweet sorghum: From theory to practice. Charact. Improv. Sweet Sorghum Cultiv. https://doi.org/10.1007/978-81-322-0783-2_1
Riajaya, P., Hariyono, B., Cholid, M., Kadarwati, F., Santoso, B., Djumali, Subiyakto, 2022. Growth and Yield Potential of New Sugarcane Varieties during Plant and First Ratoon Crops. Sustainability 14, 14396. https://doi.org/10.3390/su142114396
Robertson, M.J., Wood, A.W., Muchow, R.C., 1996. Growth of sugarcane under high input conditions in tropical Australia. I. Radiation use, biomass accumulation and partitioning. Field Crops Res. http://dx.doi.org/10.1016/0378-4290(96)00041-X
Sambusiti, C., Ficara, E., Malpei, F., Steyer, J., Carrère, H., 2013. Effect of sodium hydroxide pretreatment on physical, chemical characteristics and methane production of five varieties of sorghum. Energy 55, 449–456.
Santchurn, D., Ramdoyal, K., Badaloo, M.G.H., Labuschagne, M.T., 2014. From sugar industry to cane industry: Evaluation and simultaneous selection of different types of high biomass canes. Biomass Bioenergy 61, 82–92. https://doi.org/10.1016/j.biombioe.2013.11.023
Singels, A., Inman-Bamber, N.G., 2011. Modelling genetic and environmental control of biomass partitioning at plant and phytomer level of sugarcane grown in controlled environments. Crop Pasture Sci. 62, 66–81.
Singh, A., Jasso, R.M.R., Gonzalez-Gloria, K.D., Rosales, M., Cerda, R.B., Aguilar, C.N., Singhania, R.R., Ruiz, H.A., 2019. The enzyme biorefinery platform for advanced biofuels production. Bioresour. Technol. Rep. 7, 100257.
Slewinski, T.L., 2012. Non-structural carbohydrate partitioning in grass stems: a target to increase yield stability, stress tolerance, and biofuel production. J. Exp. Bot. 63, 4647–4670. https://doi.org/10.1093/jxb/ers124
Steel, R.G.D., Torrie, J.H., Dickey, D.A., 1997. Principles and procedures of statistics: A biometrical approach.
Teetor, V.H., Duclos, D.V., Wittenberg, E.T., Young, K.M., Chawhuaymak, J., Riley, M.R., Ray, D.T., 2011. Effects of planting date on sugar and ethanol yield of sweet sorghum grown in Arizona. Ind. Crops Prod. 34, 1293–1300.
Tew, T.L., Cobill, R.M., 2008. Genetic improvement of sugarcane (Saccharum spp.) as an energy crop. Genet. Improv. Bioenergy Crops 249–272.
Thomas, H.L., Pot, D., Latrille, E., Trouche, G., Bonnal, L., Bastianelli, D., Carrère, H., 2019. Sorghum Biomethane Potential Varies with the Genotype and the Cultivation Site. Waste Biomass Valorization 10, 783–788. https://doi.org/10.1007/s12649-017-0099-3
Thompson, G.D., 1988. Comparisons of the growth of plant and first ratoon crops of sugarcane at Pongola. Proc. South Afr. Sugar Technol. Assoc. June.
Tian, T., Lee, T.S., 2017. Advanced Biodiesel and Biojet Fuels from Lignocellulosic Biomass, in: Lee, S.Y. (Ed.), Consequences of Microbial Interactions with Hydrocarbons, Oils, and Lipids: Production of Fuels and Chemicals. Springer International Publishing, Cham, pp. 1–25. https://doi.org/10.1007/978-3-319-31421-1_372-1
Ubando, A.T., Felix, C.B., Chen, W.-H., 2020. Biorefineries in circular bioeconomy: A comprehensive review. Bioresour. Technol. 299, 122585.
van Dillewijn, C., 1952. Botany of sugarcane. Chronica Botanica Co, Waltham, USA.
Viator, H.P., Alison, M., Gravois, K., Han, K.J., Harrell, D., Hogan, A., Pittman, W., Salassi, M., Whatley, J., 2009. Sweet sorghum for biofuel production in Louisiana. La. Agric.
Yao, G., Staples, M.D., Malina, R., Tyner, W.E., 2017. Stochastic techno-economic analysis of alcohol-to-jet fuel production. Biotechnol. Biofuels 10, 18. https://doi.org/10.1186/s13068-017-0702-7
Zhang, B., Zhang, L., Li, F., Zhang, D., Liu, X., Wang, H., Xu, Z., Chu, C., Zhou, Y., 2017. Control of secondary cell wall patterning involves xylan deacetylation by a GDSL esterase. Nat. Plants 3, 17017. https://doi.org/10.1038/nplants.2017.17
Zhang, Q., Xiao, J., Hao, J., 2023. Cumulative exergy analysis of lignocellulosic biomass to bio-jet fuel through aqueous-phase conversion with different lignin conversion pathways. Energy 265, 126301. https://doi.org/10.1016/j.energy.2022.126301
Zidan, D., Azlan, A., 2022. Non-Centrifugal Sugar (NCS) and Health: A Review on Functional Components and Health Benefits. Appl. Sci. 12. https://doi.org/10.3390/app12010460

Appendix 1: Genotypes

Seventeen sugarcane genotypes obtained from Sugar Research Australia (Table 1) are included in the trials at Mossman and Atherton Tablelands in Northern Queensland. These included six current commercial varieties and nine non-commercial genotypes. At the Tablelands site SRA26 is included in the genotype mix. SRA 26, which was released to the industry in 2019, forms part of the trials.

Table A. 1 The sugarcane genotypes used in this study.

Genotype	Code used	TYPE[1]	Tested at [3]	Parents		TCH[2]
				Female	Male	
KQ228	KQ228	Comm	T	QN80-3425	CP74-2005	*
Q200	Q200	Comm	M	QN63-1700	QN66-2008	*
Q208	Q208	Comm	T&M	Q135	QN61-1232	*
Q240	Q240	Comm	T&M	QN81-289	SP78-3137	*
QN12-512	Exp1	Exp	T&M	QN84-2969	QC90-353	8.7
QN12-520	Exp2	Exp	M	QC83-625	CP88-1540	6.0
QN13-173	Exp3	Exp	T&M	Q183	Q232	6.7
QN13-609	Exp4	Exp	T&M	Q256	SRA14	29.8
QS07-9185	Exp5	Exp	T&M	QN96-1017	QC83-627	9.5
QS08-7370	Exp6	Exp	T&M	QC84-620	QN91-3322	5.7
QS08-8662	Exp7	Exp	T&M	QC90-289	Q205	6.0
QS09-8348	Exp8	Exp	T&M	QN80-3425	Q170	7.1
QS09-8404	SRA32	Exp	T&M	QN80-3425	QN86-2168	16.0
QS10-7123	Exp10	Exp	T&M	Q170	Q232	13.5
QS10-8770	Exp11	Exp	T&M	QN95-288	QN89-109	13.1
SRA3	SRA3	Exp	T	QN86-2214	Q200	*
WSRA24	WSRA24	Exp	T&M	QN80-3425	BN61-1123	30.9

[1] Comm= commercial variety, Exp = clones and varieties tested for performance against commercial standards

[2] Difference in tonne cane ha^{-1} (TCH) versus commercial standards (data from SRA variety trials)

[3] Location of trial sites, T=Tablelands and M=Mossman

Table A. 1: The sugarcane genotypes used in this study.

Appendix 2: Environmental conditions

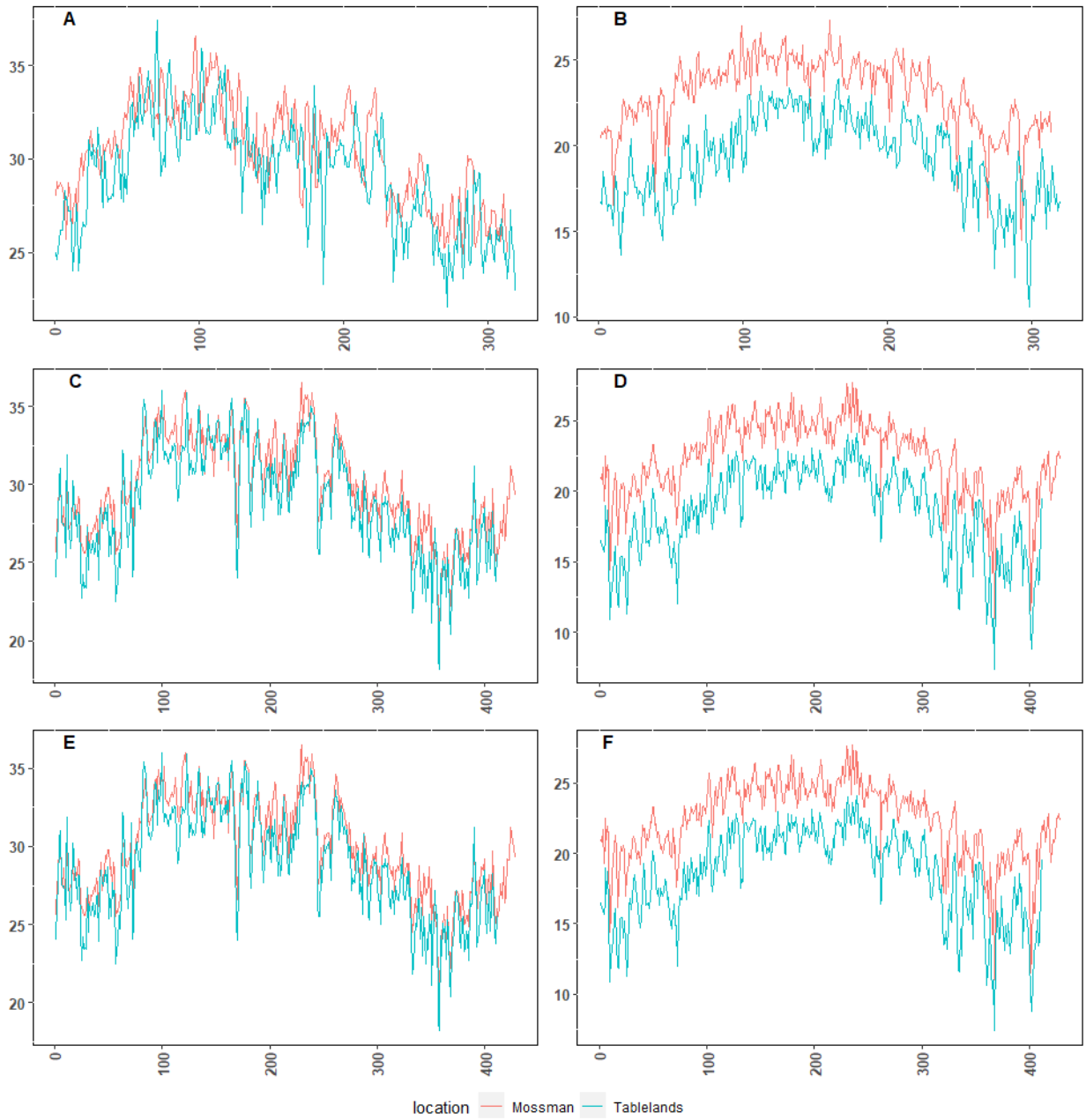

Figure A2. 1: Daily maximum and minimum temperatures at the Mossman (A,C,E) and Tablelands (B,D.F) trial sites in 2020-2021 (A,B),2021-2022 (C,D), and 2022-2023 (E,F) seasons.

.

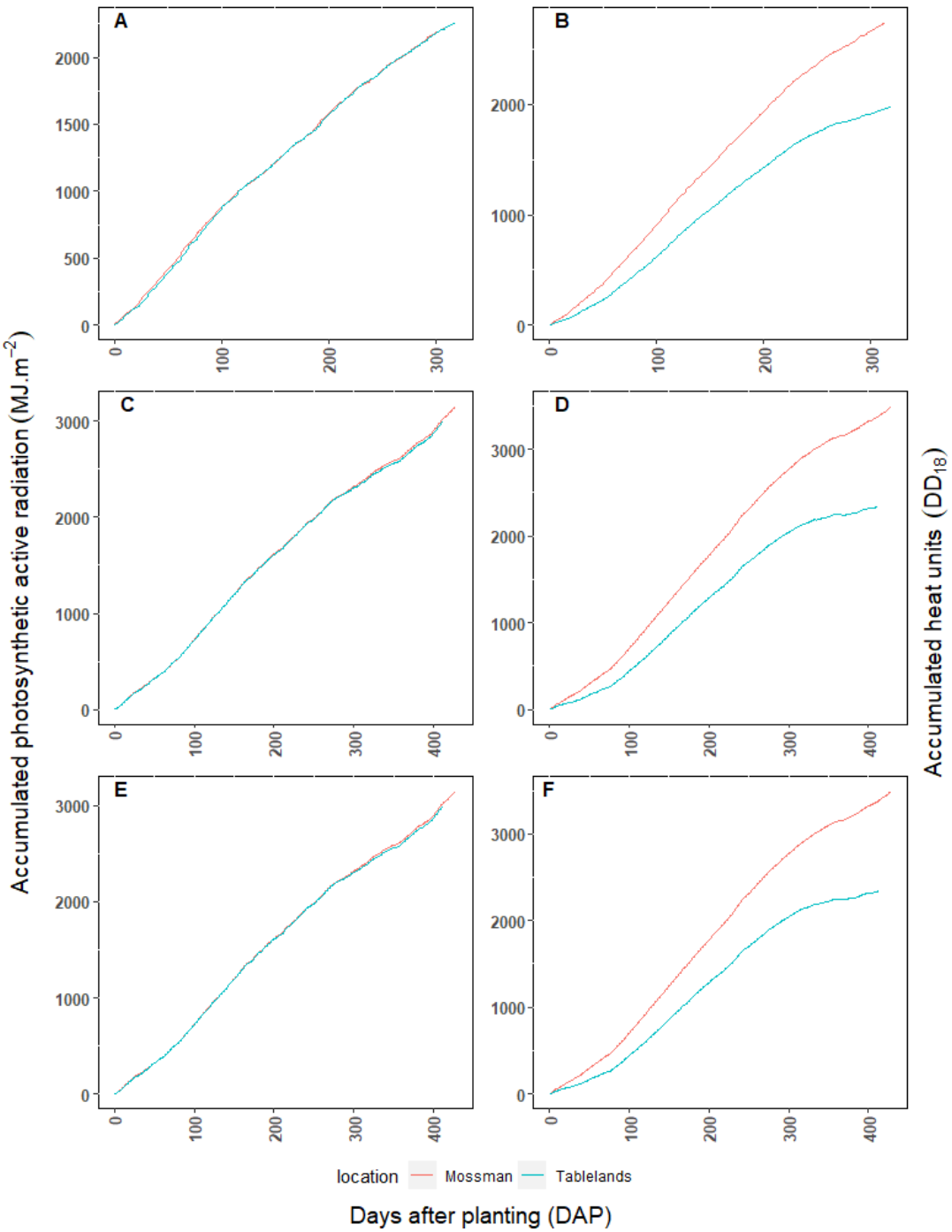

Figure A2. 2:Accumulation of photosynthetic active radiation (A,C,E) and heat units (DD18) (B,D,F) at the Tablelands and Mossman trial sites over three seasons (2020-2023).

Appendix 3: Biomass production

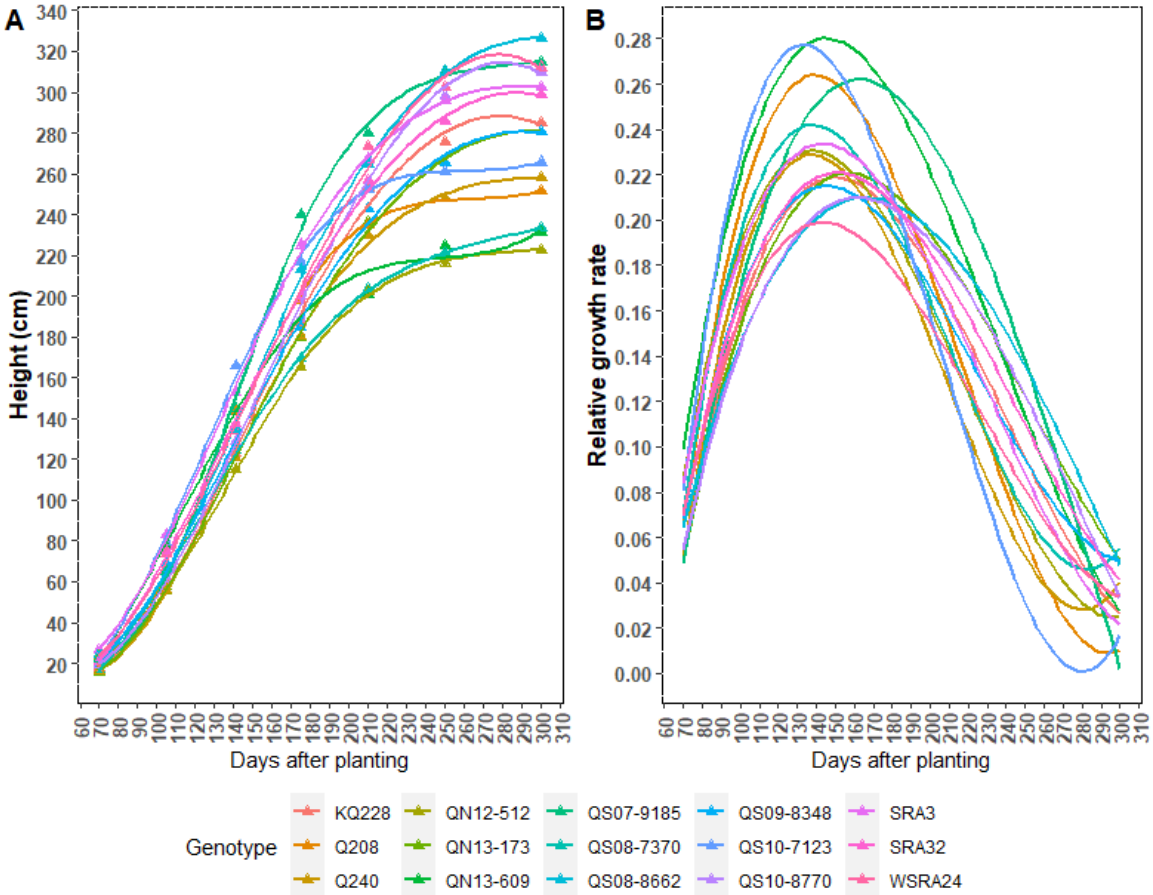

Figure A3 1: Increase in culm height (A) and changes in growth rate (B) during the growth cycle of different sugarcane genotypes.

Table A3 1: Parameters derived from fitting a logistic growth model to the measured crop height of the different genotypes. The data is sorted according to the t_{mid} values.

Genotype	Vmax (cm)			t_{mid} (days after planting)			scal		
	mean	sd	Pvalue	mean	sd	Pvalue	mean	sd	Pvalue
KQ228	282	6.8	2.E-06	152	3.2	1.E-06	36	2.7	2.E-04
Q208	242	7.7	6.E-06	128	4.4	8.E-06	29	3.9	2.E-03
Q240	259	3.0	1.E-07	147	1.5	7.E-08	32	1.3	2.E-05
QN12-512	220	4.4	1.E-06	127	2.9	2.E-06	31	2.5	3.E-04
QN13-173	283	4.9	5.E-07	154	2.3	3.E-07	35	1.9	5.E-05
QN13-609	225	6.0	3.E-06	125	3.7	5.E-06	28	3.3	1.E-03
QS07-9185	283	6.6	2.E-06	140	3.3	2.E-06	35	2.8	2.E-04
QS08-7370	231	5.2	2.E-06	139	3.1	1.E-06	33	2.7	2.E-04
QS08-8662	331	4.5	2.E-07	155	1.8	1.E-07	36	1.5	2.E-05
QS09-8348	284	5.5	9.E-07	150	2.7	6.E-07	36	2.2	9.E-05
QS09-8404	264	3.2	1.E-07	129	1.7	2.E-07	27	1.4	5.E-05
QS10-7123	318	7.2	2.E-06	157	3.0	8.E-07	35	2.4	1.E-04
QS10-8770	300	4.1	2.E-07	139	1.9	2.E-07	33	1.6	3.E-05
SRA3	302	6.0	9.E-07	149	2.7	7.E-07	36	2.3	9.E-05
WSRA24	321	7.7	2.E-06	153	3.2	1.E-06	36	2.7	2.E-04

Table A3. 2: Simulated fresh stalk biomass production using the logistic growth equation. The assumptions were that there is a tight correlation between crop height and biomass for each genotype. Parameters were derived from fitting a logistic growth model to the measured crop height of the different genotypes. The data is sorted according to the t_{mid} values.

Genotype	Vmax (TCH)			t_{mid} (days after planting)			scal		
	mean	sd	Pvalue	mean	sd	Pvalue	mean	sd	Pvalue
KQ228	65	4.5	1.E-04	151	11.1	2.E-04	40	10.0	2.E-02
Q208	57	2.2	1.E-05	119	5.9	4.E-05	28	5.4	7.E-03
Q240	61	4.3	1.E-04	155	11.3	2.E-04	39	9.9	2.E-02
QN12-512	61	1.7	3.E-06	135	4.3	6.E-06	35	4.1	1.E-03
QN13-173	57	0.8	3.E-07	154	2.2	3.E-07	32	2.0	1.E-04
QN13-609	70	6.3	4.E-04	155	16.1	7.E-04	56	13.7	2.E-02
QS07-9185	67	1.7	2.E-06	163	4.1	2.E-06	42	3.4	2.E-04
QS08-7370	59	1.0	4.E-07	153	2.6	5.E-07	38	2.3	9.E-05
QS08-8662	69	1.5	1.E-06	162	3.6	1.E-06	44	2.9	1.E-04
QS09-8348	58	1.0	4.E-07	152	2.6	5.E-07	38	2.3	9.E-05
QS09-8404	71	5.3	2.E-04	123	11.7	5.E-04	32	11.0	4.E-02
QS10-7123	81	1.7	1.E-06	159	3.3	1.E-06	39	2.9	2.E-04
QS10-8770	84	2.0	2.E-06	154	3.8	2.E-06	39	3.3	3.E-04
SRA3	45	2.2	3.E-05	116	6.8	7.E-05	25	6.0	1.E-02
WSRA24	53	3.5	1.E-04	128	9.7	2.E-04	38	9.0	1.E-02

Table A3. 3: Cane yield (TCH) at the Tablelands and Mossman trial sites.

	Mossman					Tablelands				
Crop	Genotype	Rank	TCH	sd	TUKEY	Genotype	Rank	TCH	sd	TUKEY
Plant	QS09_8348	1	85	16.6	a	QS08_8662	1	88	12.4	a
	QS10_8770	2	84	8.9	a	WSRA24	2	84	6.2	a
	QS10_7123	3	79	4.4	a	QS10_7123	3	81	9.6	a
	Q200	4	76	3.0	a	QS10_8770	4	79	25.8	a
	WSRA24	5	74	4.8	a	SRA3	5	76	10.2	a
	QS08_8662	6	69	5.6	ab	QS07_9185	6	71	4.7	a
	QS07_9185	7	67	4.9	ab	KQ228	7	70	4.5	a
	SRA32	8	65	15.8	ab	QN13_173	8	69	29.8	a
	QN13_173	9	60	13.5	abc	QS09_8348	9	69	22.9	a
	Q208	10	59	8.0	abc	SRA32	10	66	4.4	a
	QN13_609	11	58	4.3	abc	Q240	11	64	25.9	a
	QS08_7370	12	46	7.7	bcd	Q208	12	62	10.5	a
	Q240	13	44	9.1	bcd	QS08_7370	13	58	5.2	a
	QN12_512	14	36	6.7	cd	QN13_609	14	57	11.6	a
	QN12_520	15	29	10.6	d	QN12_512	15	55	6.6	a
Ratoon	QN13_173	1	121	8.5	a	SRA32	1	115	13.8	a
	WSRA24	2	118	30.8	a	QS10_8770	2	113	17.8	a
	QS08_7370	3	118	16.8	a	QS07_9185	3	109	10.1	a
	Q208	4	113	7.9	a	WSRA24	4	98	4.8	a
	SRA32	5	111	11.7	a	QS08_8662	5	94	17.2	a
	QS10_7123	6	109	11.8	a	KQ228	6	94	8.2	a
	QS10_8770	7	108	17.5	a	Q240	7	94	25.0	a
	QS07_9185	8	107	20.3	a	QN13_609	8	93	15.9	a
	QN13_609	9	99	23.9	ab	Q208	9	88	26.9	a
	QN12_512	10	97	12.1	ab	QN12_512	10	84	8.0	a
	QS08_8662	11	96	13.8	ab	QS10_7123	11	83	13.7	a
	Q240	12	95	12.3	ab	QN13_173	12	83	15.4	a
	Q200	13	92	8.3	ab	SRA3	13	82	24.9	a
	QS09_8348	14	84	22.1	ab	QS09_8348	14	80	15.3	a
	QN12_520	15	55	11.9	b	QS08_7370	15	77	19.0	a
Crop	QS10_8770	1	98	7.9	a	QS10_7123	1	97	13.0	a
	WSRA24	2	96	17.8	ab	SRA3	2	95	10.7	a
	QS10_7123	3	94	6.6	ab	QS08_8662	3	91	12.8	ab
	QN13_173	4	90	2.5	ab	WSRA24	4	91	4.5	ab
	SRA32	5	87	13.8	ab	QS07_9185	5	90	4.0	ab
	QS07_9185	6	87	10.4	ab	KQ228	6	82	1.9	abc
	Q208	7	86	3.2	ab	QS10_8770	7	80	25.2	abc
	QS09_8348	8	85	18.2	ab	Q240	8	79	25.4	abc
	Q200	9	84	4.2	ab	QN13_173	9	76	18.5	bc
	QS08_8662	10	83	4.8	ab	Q208	10	75	18.7	bc
	QS08_7370	11	82	10.5	ab	QN13_609	11	75	13.6	bc
	QN13_609	12	78	9.8	ab	QS09_8348	12	75	17.8	bc
	Q240	13	70	9.3	abc	SRA32	13	74	9.0	bc
	QN12_512	14	66	9.3	bc	QN12_512	14	69	7.2	c
	QN12_520	15	42	10.4	c	QS08_7370	15	67	7.2	c

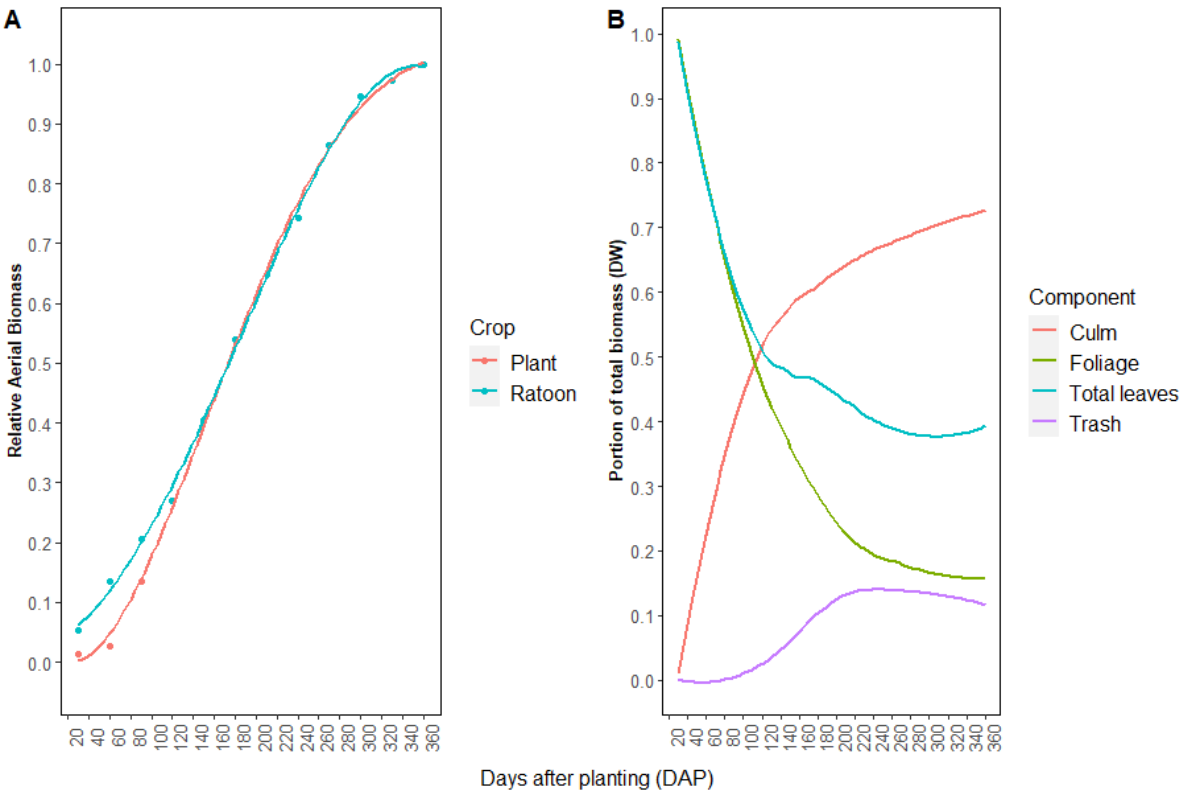

Figure A3 2: Relative aerial biomass development of a plant and ratoon crops (A). Relative biomass is a fraction of the biomass after 360 days of growth. Partitioning of aerial biomass into foliage (green leaves), culm, total leaf mass and trash attached to the crop during a 12-month growth cycle (B). Each biomass fraction is expressed as a fraction of the total biomass.

Ratoon crop development is faster than a plant crop during the first 3-4 months of growth.

Appendix4: Sugarcane biomass composition

Table A4 1: Biomass composition of young and mature stalks of sugarcane varieties grown at Mossman. Values are representative of a plant and two ratoon crops.

Components of total biomass (% of DW)																
	Young (6 months)								Mature (11 months)							
Genotype	Insoluble	sd	Soluble	sd	Lignin	sd	Ash	sd	Insoluble	sd	Soluble	sd	Lignin	sd	Ash	sd
Q200	54.05	2.24	47.92	3.03	10.99	0.90	1.49	0.15	46.76	1.44	53.24	1.44	10.04	0.15	0.97	0.11
Q208	53.64	3.86	46.36	3.35	11.56	0.83	1.47	0.15	45.22	1.10	54.79	1.10	11.00	0.30	1.33	0.05
Q240	50.81	2.75	50.39	2.51	10.79	0.90	1.45	0.10	43.61	2.57	56.39	2.57	10.53	0.42	1.03	0.02
QN12-512	55.73	3.00	45.26	2.01	11.02	0.68	1.64	0.11	46.53	2.51	53.47	2.51	9.94	0.68	1.27	0.10
QN12-520	52.19	3.02	42.73	2.99	10.62	0.63	1.49	0.14	46.67	1.54	53.33	1.54	11.11	0.20	1.16	0.07
QN13-173	55.19	2.29	43.66	2.46	11.80	0.55	1.60	0.17	46.19	2.19	53.81	2.19	11.37	0.53	1.27	0.15
QN13-609	52.97	3.47	47.20	5.27	11.54	0.71	1.48	0.14	44.58	4.45	55.42	4.45	10.79	0.63	1.02	0.09
QS07-9185	57.64	2.99	43.19	2.65	11.98	0.95	1.77	0.24	46.80	2.55	53.20	2.55	10.81	0.44	1.38	0.12
QS08-7370	54.13	3.71	46.63	2.75	12.58	1.83	1.96	0.76	53.07	3.75	46.94	3.75	12.14	0.80	1.56	0.29
QS08-8662	46.11	4.04	53.83	3.90	10.12	0.93	1.61	0.29	41.80	3.81	58.20	3.81	9.47	0.48	1.45	0.11
QS09-8348	55.29	2.76	42.25	2.04	11.65	0.83	1.37	0.08	52.06	1.55	47.94	1.55	11.92	0.18	1.47	0.15
QS10-8770	55.41	2.78	43.97	3.40	12.21	0.82	1.41	0.16	51.46	3.33	48.54	3.33	11.84	0.28	1.09	0.08
QSI0-7123	55.72	1.92	43.97	1.14	11.66	1.23	1.31	0.15	50.30	0.77	49.70	0.77	10.54	0.09	0.87	0.05
SRA32	52.91	2.81	43.76	2.39	10.37	0.63	1.31	0.09	43.37	1.74	56.63	1.74	11.43	0.65	1.03	0.09
WSRA24	53.71	5.30	45.30	4.04	11.71	1.34	1.86	0.41	52.84	1.94	47.17	1.94	11.04	0.64	0.99	0.07
Components of insoluble fraction (% of DW)																
	Young (6 months)								Mature (11 months)							
Genotype	Hexosans	sd	Glucan	sd	Pentosans	sd	Lignin	sd	Hexosans	sd	Glucan	sd	Pentosans	sd	Lignin	sd
Q200	21.82	1.07	21.21	0.95	13.16	0.62	10.99	0.90	21.72	0.96	21.81	0.52	12.72	0.26	10.04	0.15
Q208	21.67	1.54	21.23	1.52	12.82	0.93	11.56	0.83	19.82	1.24	18.98	0.65	12.43	0.33	11.00	0.30
Q240	19.02	1.18	18.86	1.17	12.70	0.73	10.79	0.90	16.58	0.44	15.62	0.71	12.43	0.43	10.53	0.42
QN12-512	21.75	1.44	21.44	1.09	12.44	0.57	11.02	0.68	20.13	0.65	18.27	0.70	12.07	0.41	9.94	0.68
QN12-520	21.38	0.88	20.77	1.19	13.96	1.09	10.62	0.63	18.30	1.13	17.59	0.47	12.65	0.61	11.11	0.20
QN13-173	22.84	1.16	22.39	1.03	14.12	0.82	11.80	0.55	18.96	0.94	18.66	0.86	11.34	1.02	11.37	0.53
QN13-609	20.45	1.13	20.28	1.36	13.25	0.84	11.54	0.71	19.06	1.49	19.10	0.72	11.40	0.54	10.79	0.63
QS07-9185	23.10	1.30	22.70	1.29	14.19	0.76	11.98	0.95	17.61	1.33	16.57	0.28	10.81	0.22	10.81	0.44
QS08-7370	21.47	1.22	21.28	1.49	13.36	1.03	12.58	1.83	22.36	1.82	22.12	1.49	15.08	0.63	12.14	0.80
QS08-8662	19.11	1.51	18.37	1.71	11.76	0.90	10.12	0.93	16.74	1.34	15.65	0.30	11.24	0.54	9.47	0.48
QS09-8348	23.32	1.73	22.55	1.79	14.77	0.93	11.65	0.83	20.07	1.68	19.34	1.41	14.56	0.27	11.92	0.18
QS10-8770	23.77	1.25	23.41	1.30	14.42	0.73	12.21	0.82	19.78	0.59	19.86	1.47	13.06	0.09	11.84	0.28
QSI0-7123	23.02	1.75	22.95	1.42	14.55	1.06	11.66	1.23	19.77	0.83	20.70	1.27	13.09	0.54	10.54	0.09
SRA32	20.59	1.39	20.50	1.69	13.21	0.95	10.37	0.63	18.48	0.66	17.83	1.31	10.42	0.21	11.43	0.65
WSRA24	20.89	2.25	20.13	2.39	13.18	1.70	11.71	1.34	19.09	0.91	18.84	0.59	12.54	0.82	11.04	0.64
Components of soluble fraction (% of DW)																
	Young (6 months)								Mature (11 months)							
Genotype	Sugars	sd	Sucrose	sd	Glucose	sd	Fructose	sd	Sugars	sd	Sucrose	sd	Glucose	sd	Fructose	sd
Q200	39.34	2.13	20.48	1.96	10.09	0.55	8.56	0.44	44.40	1.30	33.35	2.09	5.44	0.30	2.58	0.08
Q208	42.28	2.99	23.04	1.64	10.44	0.73	8.66	0.61	41.47	1.04	26.81	0.38	6.87	0.32	4.02	0.29
Q240	40.67	2.52	26.36	1.43	7.13	0.29	7.39	0.35	39.38	2.34	28.01	1.21	6.19	0.43	2.00	0.10
QN12-512	36.25	5.65	18.68	3.38	8.73	0.76	8.82	1.28	43.03	1.09	34.40	1.98	5.44	0.35	4.05	0.18
QN12-520	37.39	3.02	21.53	3.29	7.70	0.65	7.63	0.67	41.42	2.38	28.81	1.06	7.57	0.28	3.67	0.18
QN13-173	36.55	1.59	16.06	0.87	10.29	0.62	9.31	0.62	46.90	1.12	32.01	1.43	6.53	0.53	4.15	0.21
QN13-609	38.21	1.81	18.62	1.61	10.47	0.72	9.30	0.64	45.83	0.99	32.64	2.32	5.64	0.14	4.79	0.21
QS07-9185	35.22	1.74	16.09	2.33	9.77	1.02	9.11	0.86	40.82	1.86	24.01	1.12	6.47	0.24	3.17	0.15
QS08-7370	38.93	5.24	20.17	4.63	9.45	0.71	9.20	0.71	36.20	1.18	23.56	1.15	5.65	0.19	3.21	0.09
QS08-8662	40.95	3.94	21.94	2.35	9.70	0.81	9.33	0.58	45.26	2.22	28.01	0.87	7.28	0.59	3.37	0.16
QS09-8348	36.01	2.23	19.21	1.43	8.58	1.11	7.86	0.89	39.16	1.72	25.34	1.17	5.56	0.34	3.38	0.13
QS10-8770	37.78	2.50	19.01	2.47	9.95	0.46	9.11	0.61	40.18	0.81	25.70	1.01	5.37	0.24	3.79	0.04
QSI0-7123	38.43	1.50	16.92	0.88	11.28	0.42	9.92	0.48	45.55	2.49	22.07	1.36	8.81	0.15	6.88	0.16
SRA32	38.40	1.56	19.07	1.28	10.59	0.65	9.29	0.57	46.66	2.07	27.48	0.39	6.00	0.35	3.72	0.32
WSRA24	37.61	3.59	19.09	2.05	9.67	0.73	9.16	0.94	43.66	1.94	30.65	1.17	6.77	0.53	3.18	0.10

Table A4 2: Biomass composition of young and mature stalks of sugarcane varieties grown at Tablelands. Values are representative of a plant and two ratoon crops.

Components of total biomass (% of DW)																
	Young (6 months)								Mature (11 months)							
Genotype	Insoluble	sd	Soluble	sd	Lignin	sd	Ash	sd	Insoluble	sd	Soluble	sd	Lignin	sd	Ash	sd
KQ228	51.71	2.70	49.60	1.80	11.41	0.61	1.54	0.06	42.19	2.47	57.81	2.47	9.82	1.69	1.53	0.17
Q208	49.75	3.69	48.25	3.52	9.36	0.67	1.62	0.12	44.51	6.54	55.49	6.54	8.89	0.39	1.33	0.25
Q240	49.04	4.20	49.45	3.17	10.44	1.33	1.40	0.12	45.88	4.22	54.12	4.22	10.20	1.28	1.17	0.12
QN12-512	52.89	2.76	45.44	2.79	11.48	0.81	1.55	0.07	50.69	1.75	49.31	1.75	12.25	0.97	1.52	0.21
QN13-173	51.18	1.91	44.49	1.88	10.24	0.54	1.36	0.04	44.00	4.23	56.00	4.23	10.04	1.16	1.36	0.20
QN13-609	55.28	2.17	44.39	1.69	11.78	0.44	1.59	0.21	41.72	5.51	58.28	5.51	10.34	1.54	1.66	0.35
QS07-9185	49.68	3.01	48.21	3.30	10.74	0.72	1.56	0.10	45.28	3.25	54.72	3.25	10.62	0.70	1.28	0.12
QS08-7370	56.42	2.18	44.08	2.00	12.52	1.39	1.93	0.13	47.20	3.17	52.80	3.17	10.71	0.41	1.27	0.22
QS08-8662	53.72	2.80	44.78	2.83	11.01	0.55	2.12	0.61	40.80	5.11	59.20	5.11	9.31	0.80	1.37	0.39
QS09-8348	43.78	1.77	53.22	3.23	9.63	0.45	1.42	0.11	45.22	4.71	54.79	4.71	11.14	1.11	1.30	0.44
SRA32	54.49	3.35	43.34	2.99	11.34	0.81	1.39	0.12	44.79	0.43	55.21	0.43	9.90	0.51	1.44	0.22
QS10-7123	51.04	2.04	44.63	2.08	11.41	0.53	1.57	0.08	45.12	3.88	54.88	3.88	10.84	1.11	1.31	0.20
QS10-8770	54.52	1.28	47.98	3.81	10.93	0.46	1.39	0.11	46.31	1.81	53.69	1.81	11.29	0.64	1.14	0.05
SRA3	56.21	3.48	45.35	3.37	12.37	0.59	1.60	0.03	46.14	3.16	53.86	3.16	10.54	0.66	1.50	0.14
SRA32	52.30	3.14	51.03	3.29	12.74	0.97	1.76	0.13	44.80	0.96	55.20	0.96	9.79	0.41	0.92	0.04
WSRA24	51.81	3.30	48.35	4.58	10.58	0.93	1.20	0.07	46.66	3.53	53.34	3.53	11.17	1.22	1.56	0.26
Components of insoluble fraction (% of DW)																
	Young (6 months)								Mature (11 months)							
Genotype	Hexosans	sd	Glucan	sd	Pentosans	sd	Lignin	sd	Hexosans	sd	Glucan	sd	Pentosans	sd	Lignin	sd
KQ228	19.70	0.81	19.30	0.75	12.37	0.84	11.41	0.61	17.45	2.10	16.94	1.52	11.33	1.71	9.82	1.69
Q208	18.81	1.41	18.41	1.38	11.51	0.85	9.36	0.67	17.14	1.55	16.97	0.98	10.38	0.40	8.89	0.39
Q240	17.87	1.70	17.45	1.54	12.25	1.27	10.44	1.33	17.28	2.65	16.45	2.33	10.91	0.87	10.20	1.28
QN12-512	20.66	1.00	20.29	0.96	12.09	0.55	11.48	0.81	18.78	0.92	18.11	1.10	11.09	0.66	12.25	0.97
QN13-173	20.99	0.87	20.53	0.87	12.89	0.51	10.24	0.54	17.34	1.43	16.98	1.33	11.47	1.44	10.04	1.16
QN13-609	23.25	1.07	22.79	1.09	14.48	0.58	11.78	0.44	15.57	2.85	15.08	2.42	10.39	0.46	10.34	1.54
QS07-9185	19.06	1.38	18.85	1.40	12.86	0.64	10.74	0.72	17.37	2.00	16.39	2.23	11.78	1.79	10.62	0.70
QS08-7370	23.14	1.06	22.72	1.07	14.51	0.67	12.52	1.39	19.12	0.88	18.77	1.25	12.27	0.60	10.71	0.41
QS08-8662	21.83	1.25	21.49	1.24	13.41	0.78	11.01	0.55	16.12	1.21	15.21	1.11	9.70	0.88	9.31	0.80
QS09-8348	16.94	1.71	16.44	1.59	10.78	0.65	9.63	0.45	17.44	0.60	16.91	0.94	12.80	1.69	11.14	1.11
SRA32	21.55	1.43	21.08	1.37	14.31	0.93	11.34	0.81	18.31	0.62	18.28	0.59	11.57	0.53	9.90	0.51
QS10-7123	21.99	1.90	21.84	1.84	13.55	1.42	11.89	0.73	18.34	0.88	18.04	0.93	11.83	1.24	10.84	1.11
QS10-8770	21.93	0.45	21.53	0.46	14.08	0.45	10.93	0.46	19.15	0.74	18.20	0.89	11.07	0.59	11.29	0.64
SRA3	19.70	1.21	19.31	1.21	11.53	0.63	12.74	0.97	18.95	2.33	18.60	2.12	12.16	0.73	10.54	0.66
SRA32	21.32	1.25	21.00	1.24	13.63	0.77	10.58	0.93	17.04	0.40	16.22	0.67	10.97	0.59	9.79	0.41
WSRA24	19.13	1.45	18.68	1.31	12.37	1.08	11.43	0.99	18.28	3.07	17.29	1.80	11.88	1.37	11.17	1.22
Components of soluble fraction (% of DW)																
	Young (6 months)								Mature (11 months)							
Genotype	Sugars	sd	Sucrose	sd	Glucose	sd	Fructose	sd	Sugars	sd	Sucrose	sd	Glucose	sd	Fructose	sd
KQ228	41.25	2.96	24.67	3.42	8.70	0.59	8.01	0.71	45.58	2.92	32.79	7.37	7.17	1.90	3.32	0.34
Q208	43.67	3.37	26.05	2.07	8.98	0.66	8.50	0.65	44.97	1.77	31.87	4.16	8.06	0.57	4.15	0.68
Q240	40.17	3.14	24.99	1.71	7.77	1.32	7.42	0.77	42.73	2.33	31.09	3.69	6.91	0.62	3.57	0.64
QN12-512	34.98	4.54	17.95	2.68	8.61	1.02	8.19	1.03	40.68	1.83	28.90	4.61	6.68	1.14	4.08	0.79
QN13-173	39.21	1.19	24.80	0.73	7.28	0.24	6.98	0.22	45.01	2.80	33.30	6.40	7.82	1.20	4.32	1.06
QN13-609	37.45	3.30	17.70	2.47	10.53	0.72	9.00	0.35	47.90	1.64	37.02	6.70	6.63	1.29	4.14	0.77
QS07-9185	35.94	3.49	19.14	0.96	8.93	1.69	7.93	1.06	42.78	3.50	32.15	4.72	6.95	1.30	3.44	0.71
QS08-7370	36.72	2.45	16.64	2.84	10.24	0.53	9.63	0.35	41.36	3.55	29.20	6.90	6.96	1.91	4.28	1.42
QS08-8662	33.55	2.52	16.20	0.91	8.99	0.92	8.16	0.95	50.94	3.31	33.80	5.59	7.66	1.06	4.30	0.71
QS09-8348	40.82	2.35	22.13	2.39	9.54	0.30	8.97	0.58	43.80	2.36	27.94	5.35	7.23	1.34	4.42	1.08
SRA32	35.62	2.51	19.27	1.33	8.55	1.12	7.66	0.79	43.76	1.62	25.93	0.59	9.14	0.32	6.24	0.28
QS10-7123	38.00	3.32	18.03	2.88	10.15	0.41	9.24	0.42	43.26	4.02	31.48	6.81	7.55	1.39	4.59	0.73
QS10-8770	41.51	2.89	19.77	2.17	11.49	0.45	10.07	0.39	42.95	3.68	29.57	6.43	7.06	1.53	4.43	0.91
SRA3	40.60	2.54	21.15	1.25	10.21	0.68	9.08	0.59	44.53	2.86	28.76	6.89	7.41	1.49	5.00	1.69
SRA32	43.41	3.94	21.25	2.07	11.81	1.06	10.20	0.85	44.86	0.82	36.89	1.06	5.59	0.24	3.32	0.14
WSRA24	37.89	2.95	19.46	1.32	9.55	0.82	8.65	0.74	40.24	3.84	28.79	6.06	6.52	0.45	3.47	0.31

Table A4 3: Biomass composition of the leaves of sugarcane varieties grown at Tablelands. Values are representative of a plant and two ratoon crops.

Components of total biomass (% of DW)																
Genotype	Insol	sd	Hexosans	sd	Glucan	sd	Pentosans	sd	Xylan	sd	Uronic acic	sd	Lignin	sd	Ash	sd
KQ228	81.34	0.43	32.59	1.15	32.73	0.38	21.69	0.12	19.14	0.33	0.79	0.02	18.71	0.15	0.36	0.08
Q208	80.00	0.12	31.33	0.10	30.70	0.14	20.93	0.07	17.92	0.09	0.97	0.00	17.88	0.10	0.52	0.02
Q240	80.20	0.09	33.56	0.32	32.78	0.31	22.49	0.05	19.53	0.04	0.89	0.01	16.39	0.15	0.43	0.17
QN12-512	81.42	0.24	32.21	0.14	31.43	0.16	21.75	0.12	19.01	0.10	0.89	0.00	17.81	0.18	0.73	0.06
QN13-173	80.76	0.03	33.66	0.01	32.79	0.01	20.39	0.08	17.66	0.08	1.17	0.01	16.75	0.11	0.30	0.30
QN13-609	82.32	0.60	32.78	0.20	32.05	0.21	22.79	0.21	20.01	0.21	0.85	0.03	17.23	0.29	0.55	0.15
QS07-9185	81.43	0.34	32.97	0.01	32.21	0.02	22.18	0.01	19.08	0.01	1.24	0.01	18.78	0.21	0.54	0.05
QS08-7370	81.14	0.21	32.82	0.06	32.17	0.06	22.00	0.05	19.39	0.05	0.85	0.01	16.68	0.05	0.17	0.17
QS08-8662	80.25	0.33	31.26	0.06	30.59	0.06	21.33	0.09	18.46	0.11	1.05	0.06	18.21	0.04	0.52	0.12
QS09-8348	78.04	0.09	32.50	0.11	31.66	0.09	20.34	0.02	17.53	0.00	0.84	0.03	15.09	0.22	0.00	0.00
QS10-7123	79.94	0.34	31.68	0.21	30.91	0.20	21.36	0.05	18.72	0.05	0.91	0.00	16.40	0.15	0.73	0.12
QS10-8770	80.53	0.11	31.42	0.09	30.73	0.09	21.78	0.01	19.23	0.01	0.87	0.00	18.39	0.19	0.48	0.18
SRA3	81.77	0.17	31.82	0.03	31.16	0.04	22.28	0.02	19.30	0.03	0.86	0.00	17.21	0.29	0.21	0.22
SRA32	81.52	0.13	34.13	0.13	33.43	0.16	21.41	0.02	18.81	0.05	0.86	0.01	18.00	0.21	0.33	0.01
WSRA24	80.91	0.37	33.41	0.12	32.68	0.11	21.18	0.05	18.56	0.03	0.78	0.01	17.23	0.13	0.40	0.03
Q200	80.51	0.21	32.50	0.52	32.07	0.27	21.70	0.08	18.86	0.15	0.88	0.01	17.66	0.13	0.43	0.09

Components of total biomass (% of DW)																
Genotype	Soluble	sd	Sol_sugar	sd	Sucrose	sd	Glucose	sd	Fructose	sd	Other	sd	Alcohols	sd	Starch	sd
KQ228	18.66	0.43	3.28	0.12	2.30	0.04	0.54	0.03	0.40	0.03	0.07	0.01	0.01	0.00	0.45	0.01
Q208	20.00	0.12	4.45	0.05	2.47	0.01	0.95	0.03	0.93	0.01	0.09	0.00	0.01	0.00	1.07	0.01
Q240	19.80	0.09	4.36	0.12	2.62	0.08	0.81	0.03	0.84	0.01	0.08	0.00	0.00	0.00	0.41	0.01
QN12-512	18.58	0.24	3.20	0.01	2.09	0.03	0.56	0.02	0.47	0.02	0.06	0.01	0.01	0.00	0.46	0.03
QN13-173	19.24	0.03	3.63	0.02	1.68	0.03	0.89	0.01	0.95	0.00	0.09	0.00	0.01	0.00	0.35	0.00
QN13-609	17.68	0.60	2.34	0.04	1.67	0.03	0.32	0.01	0.30	0.01	0.04	0.00	0.01	0.00	0.04	0.01
QS07-9185	18.57	0.34	3.17	0.05	1.80	0.03	0.65	0.01	0.64	0.01	0.08	0.00	0.01	0.00	0.71	0.01
QS08-7370	18.86	0.21	4.02	0.01	2.50	0.00	0.69	0.00	0.76	0.01	0.06	0.00	0.01	0.00	0.90	0.02
QS08-8662	19.75	0.33	4.34	0.09	3.16	0.07	0.57	0.01	0.53	0.01	0.06	0.00	0.01	0.00	0.59	0.01
QS09-8348	21.96	0.09	6.76	0.09	4.80	0.12	0.95	0.02	0.91	0.01	0.08	0.00	0.00	0.00	1.49	0.27
QS10-7123	20.06	0.34	3.77	0.00	2.03	0.00	0.80	0.00	0.83	0.00	0.09	0.00	0.01	0.00	0.51	0.00
QS10-8770	19.47	0.11	3.01	0.03	1.60	0.00	0.65	0.02	0.70	0.01	0.06	0.00	0.01	0.00	0.30	0.00
SRA3	18.23	0.17	3.86	0.00	2.10	0.00	0.80	0.00	0.86	0.00	0.08	0.00	0.00	0.00	0.79	0.01
SRA32	18.48	0.13	4.25	0.02	2.36	0.03	0.94	0.01	0.86	0.00	0.08	0.00	0.01	0.00	0.40	0.02
WSRA24	19.09	0.37	4.74	0.02	3.59	0.01	0.55	0.00	0.54	0.01	0.05	0.00	0.00	0.00	0.58	0.02
Q200	19.49	0.21	4.03	0.10	2.47	0.04	0.76	0.03	0.72	0.02	0.08	0.00	0.01	0.00	0.65	0.01

Appendix 5: Metabolite profiles

Table A5 1: Polar metabolites present in the sugarcane and sorghum leaf and internode tissues. There are 199 metabolites in the leaf tissues and 96 metabolites in the internodal tissue. Metabolites indicated with blue are those that at least at some.

Name	HMDB	L	I	Name	HMDB	L	I
Gamma-tocopherol	HMDB0001492	✓	✖	Malonic acid	HMDB0000691	✓	✖
Deoxyuridine	HMDB0000012	✓	✖	2-Hydroxyglutaric acid	HMDB0000694	✓	✓
p-Hydroxyphenylacetic acid	HMDB0000020	✓	✖	Methionine	HMDB0000696	✓	✖
(S)-3-Hydroxyisobutyric acid	HMDB0000023	✓	✓	Hydroxypropionic acid	HMDB0000700	✓	✓
Adenine	HMDB0000034	✓	✓	Homoserine	HMDB0000719	✓	✖
Adenosine	HMDB0000050	✓	✖	4-Hydroxyproline	HMDB0000725	✓	✖
Cellobiose	HMDB0000055	✓	✓	Alpha-Hydroxyisobutyric acid	HMDB0000729	✓	✓
Beta-Alanine	HMDB0000056	✓	✓	Malic acid	HMDB0000744	✓	✓
Acetoacetic acid	HMDB0000060	✓	✖	Mesaconic acid	HMDB0000749	✓	✖
Cholesterol	HMDB0000067	✓	✖	3-Hydroxyisovaleric acid	HMDB0000754	✓	✓
Pipecolic acid	HMDB0000070	✓	✓	Mannitol	HMDB0000765	✓	✓
cis-Aconitic acid	HMDB0000072	✓	✓	Azelaic acid	HMDB0000784	✓	✓
Dopamine	HMDB0000073	✓	✖	Myristic acid	HMDB0000806	✓	✖
Dihydrouracil	HMDB0000076	✓	✖	3-Phosphoglyceric acid	HMDB0000807	✓	✖
Citric acid	HMDB0000094	✓	✓	Stearic acid	HMDB0000827	✓	✖
xylose	HMDB0000098	✓	✓	Salicyluric_acid	HMDB0000840	✖	✓
Galactitol	HMDB0000107	✓	✖	Pelargonic acid	HMDB0000847	✓	✖
Gamma-Aminobutyric acid	HMDB0000112	✓	✓	Rhamnose	HMDB0000849	✓	✓
Glycolic acid	HMDB0000115	✓	✓	N-Acetyl-L-tyrosine	HMDB0000866	✓	✖
Homovanillic acid	HMDB0000118	✓	✖	Ribonic acid	HMDB0000867	✓	✓
Glyoxylic_acid	HMDB0000119	✖	✓	Valine	HMDB0000883	✓	✓
Glucose	HMDB0000122	✓	✓	Suberic acid	HMDB0000893	✓	✖
Glycine	HMDB0000123	✓	✓	Tryptophan	HMDB0000929	✓	✖
Glycerol 3-phosphate	HMDB0000126	✓	✖	Stigmasterol	HMDB0000937	✓	✖
Homogentisic acid	HMDB0000130	✓	✖	Threonic acid	HMDB0000943	✓	✖
Glycerol	HMDB0000131	✓	✓	Ferulic acid	HMDB0000954	✓	✖
Guanine	HMDB0000132	✓	✖	Pyrocatechol	HMDB0000957	✓	✖
Guanosine	HMDB0000133	✓	✖	Trehalose	HMDB0000975	✓	✖
Fumaric acid	HMDB0000134	✓	✓	3-Sulfinoalanine	HMDB0000996	✓	✓
Glyceric acid	HMDB0000139	✓	✓	Glyceraldehyde	HMDB0001051	✖	✖
Galactose	HMDB0000143	✓	✓	Fructose 1-phosphate	HMDB0001076	✓	✖
Glutamic acid	HMDB0000148	✓	✓	N-Acetylmannosamine	HMDB0001129	✓	✖
Ethanolamine	HMDB0000149	✓	✓	N-Acetyl-L-glutamic acid	HMDB0001138	✓	✖
Gluconolactone	HMDB0000150	✓	✖	Spermidine	HMDB0001257	✖	✓
Tyrosine	HMDB0000158	✓	✖	Sorbose	HMDB0001266	✓	✓
Phenylalanine	HMDB0000159	✓	✓	Glucose 6-phosphate	HMDB0001401	✓	✖
Alanine	HMDB0000161	✓	✓	Putrescine	HMDB0001414	✓	✓
Proline	HMDB0000162	✓	✓	DHAP	HMDB0001473	✖	✓
Maltose	HMDB0000163	✓	✓	Nicotinic acid	HMDB0001488	✓	✓
Threonine	HMDB0000167	✓	✓	Glucosamine	HMDB0001514	✓	✖
Asparagine	HMDB0000168	✓	✓	Ribose 5-phosphate	HMDB0001548	✓	✖

Mannose	HMDB0000169	✖	✓	xanthylic acid	HMDB0001554	✓	✖
Isoleucine	HMDB0000172	✓	✓	xylulose	HMDB0001644	✓	✖
Maleic acid	HMDB0000176	✓	✖	4-Coumaric acid-2TMS	HMDB0001713	✓	✖
Lysine	HMDB0000182	✓	✖	Methylsuccinic acid	HMDB0001844	✓	✓
Serine	HMDB0000187	✓	✖	Protocatechuic acid	HMDB0001856	✓	✓
Lactic acid	HMDB0000190	✓	✓	Benzoic acid	HMDB0001870	✓	✓
Aspartic acid	HMDB0000191	✓	✖	Dihydroxyacetone	HMDB0001882	✓	✓
Isocitric acid	HMDB0000193	✓	✖	Salicylic acid	HMDB0001895	✓	✖
Indoleacetic acid	HMDB0000197	✓	✖	Ribonolactone	HMDB0001900	✓	✖
Methylmalonic acid	HMDB0000202	✓	✖	2_Aminoisobutyric_acid	HMDB0001906	✖	✓
Oleic acid	HMDB0000207	✓	✓	Caffeic acid	HMDB0001964	✓	✖
Oxoglutaric acid	HMDB0000208	✓	✖	Eicosapentaenoic acid	HMDB0001999	✓	✖
Phenylacetic acid	HMDB0000209	✓	✓	Phytol	HMDB0002019	✓	✖
Pantothenic acid	HMDB0000210	✓	✓	Acetylputrescine	HMDB0002064	✖	✓
Inositol	HMDB0000211	✖	✓	Itaconic acid	HMDB0002092	✓	✖
N-Acetylgalactosamine	HMDB0000212	✓	✖	Phosphoric acid	HMDB0002142	✓	✖
Ornithine	HMDB0000214	✓	✖	Oxalic acid	HMDB0002329	✓	✖
N-Acetyl-D-glucosamine	HMDB0000215	✓	✖	Sumiki's acid	HMDB0002432	✓	✓
Palmitic acid	HMDB0000220	✓	✓	Hydroquinone	HMDB0002434	✓	✖
O-Phosphoethanolamine	HMDB0000224	✓	✖	Beta-Glycerophosphoric acid	HMDB0002520	✓	✖
Orotic acid	HMDB0000226	✓	✖	Galacturonic acid	HMDB0002545	✓	✓
Pyridoxine	HMDB0000239	✓	✖	xylitol	HMDB0002917	✓	✖
Pyruvic acid	HMDB0000243	✓	✓	N-Acetylserine	HMDB0002931	✓	✓
Sorbitol	HMDB0000247	✓	✓	Inositol phosphate	HMDB0002985	✓	✖
Succinic acid	HMDB0000254	✓	✓	Erythritol	HMDB0002994	✓	✓
Sucrose	HMDB0000258	✓	✓	O-Acetylserine	HMDB0003011	✓	✖
Serotonin	HMDB0000259	✓	✖	Shikimic acid	HMDB0003070	✓	✓
Thymine	HMDB0000262	✓	✓	Quinic acid	HMDB0003072	✓	✓
Pyroglutamic acid	HMDB0000267	✓	✓	2,3-Butanediol	HMDB0003156	✓	✖
Ribose	HMDB0000283	✓	✓	Chlorogenic acid	HMDB0003164	✓	✖
Vanillylmandelic acid	HMDB0000291	✓	✖	Sedoheptulose	HMDB0003219	✓	✓
xanthine	HMDB0000292	✓	✖	Palmitoleic acid	HMDB0003229	✓	✖
Urea	HMDB0000294	✓	✖	Hydroxylamine	HMDB0003338	✓	✖
Uridine	HMDB0000296	✓	✖	5-Aminopentanoic acid	HMDB0003355	✓	✓
Uracil	HMDB0000300	✓	✓	Lyxose	HMDB0003402	✖	✓
Tryptamine	HMDB0000303	✖	✓	Tagatose	HMDB0003418	✓	✖
Tyramine	HMDB0000306	✓	✖	1-Hexadecanol	HMDB0003424	✓	✖
3-Hydroxymethylglutaric acid	HMDB0000355	✓	✓	Threitol	HMDB0004136	✓	✖
3-Hydroxybutyric acid	HMDB0000357	✓	✓	p-Octopamine	HMDB0004825	✓	✖
Citramalic acid	HMDB0000426	✓	✓	Gallic acid	HMDB0005807	✓	✖
3-Hydroxyglutaric acid	HMDB0000428	✓	✖	Galactinol	HMDB0005826	✓	✖
Adipic acid	HMDB0000448	✓	✓	N-Acetylglutamine	HMDB0006029	✓	✖
Caprylic acid	HMDB0000482	✓	✓	Erythrulose	HMDB0006293	✓	✓
Vanillic acid	HMDB0000484	✓	✓	MG(18:0e/0:0/0:0)	HMDB0011143	✓	✖
3-Methyl-2-oxovaleric acid	HMDB0000491	✓	✖	Turanose	HMDB0011740	✓	✓
4-Hydroxybenzoic acid	HMDB0000500	✓	✓	Coniferyl alcohol	HMDB0012915	✓	✖
Ribitol	HMDB0000508	✓	✓	1,2,3-Trihydroxybenzene	HMDB0013674	✓	✖
Capric acid	HMDB0000511	✓	✓	Norvaline	HMDB0013716	✓	✖

N-Acetyl-L-phenylalanine	HMDB0000512	✓	✖	Psicose	HMDB0250793	✖	✓
Arginine	HMDB0000517	✓	✖	Fucose	HMDB0029196	✓	✖
Caproic acid	HMDB0000535	✓	✖	Arabinose	HMDB0029942	✓	✖
Arabitol	HMDB0000568	✓	✓	Triethanolamine	HMDB0032538	✓	✖
Elaidic acid	HMDB0000573	✓	✖	Sinapic acid	HMDB0032616	✓	✖
Cysteine	HMDB0000574	✓	✓	2-Aminoheptanedioic acid	HMDB0034252	✓	✖
Leucic_acid	HMDB0000624	✖	✓	Theanine	HMDB0034365	✓	✖
Gluconic acid	HMDB0000625	✓	✓	2,3-Dihydroxybutanedioic acid	HMDB0059916	✓	✖
Cytosine	HMDB0000630	✓	✖	Glutamate, gamma-methyl ester	HMDB0061715	✓	✖
Dodecanoic acid	HMDB0000638	✓	✖	2-Deoxyglucose	HMDB0062477	✓	✖
Levoglucosan	HMDB0000640	✓	✓	2-Deoxy-D-ribose	HMDB0245099	✓	✖
Glutamine	HMDB0000641	✓	✓	Isobutylamine-2TMS		✓	✖
Alpha-aminobutyric acid	HMDB0000650	✓	✖	Cystamine	HMDB0250701	✓	✖
Fructose	HMDB0000660	✓	✓	3-Dehydroshikimic acid	HMDB12710	✓	✓
Glutaric acid	HMDB0000661	✓	✓	Psicose	HMDB0250793	✓	✓
Glucaric acid	HMDB0000663	✓	✓	3-Dehydroshikimic acid	HMDB12710	✓	✓
Linoleic acid	HMDB0000673	✓	✓	Psicose	HMDB0250793	✓	✓
Leucine	HMDB0000687	✓	✓	Galactose	HMDB0000143	✓	✓

✓ = present and ✖ = absence of the metabolite in the particular tissue

Table A5 2: The market process for the most abundant chemicals present in sugarcane and sweet sorghum juice.

Compound	Unit	Price (USA$)	Source
Aconitate	kg	2	cell.com/cell-reports-physical-science
Cellobiose	kg	2,189	calpaclab.com
Citrate	kg	143	echemi.com
Fructose	kg	1	selinawamucii.com
Galactose	kg	158	biosynth.com
Gentibiose	kg	14	chemicalbook.com
Glucose	kg	18	bevtech.de
Glycerate	kg	1	selinawamucii.com/glycerol
Isocitrate	kg	3	made-in-china.com/citric_acid
Lactate	kg	2	pharmacompass.com/lactic-acid
Malate	kg	4	pharmacompass.com/malic-acid
Maltose	kg	47	pharmacompass.com/maltose
Mannose	kg	36	made-in-china.com/D-mannose
Myo-inositol	kg	29	pharmacompass.com/myo-inositol
Quinate	kg	714	pharmacompass.com
Raffinose	kg	1,473	rpicorp.com/d-raffinose
Ribitol	kg	1,300	pharmacompass.com
Sedoheptulose	5 mg	2,028	sigmaaldrich.com/sigma
Sucrose	kg	2	globalproductprices.com
Tartrate	kg	173	biosynth.com/147-71-7-d-tartaric-acid
Threitol	kg	4	ahelite.en.made-in-china.com
Trehalose	kg	23	trehalose.co.uk/trehalose-1kg
Turanose	kg	28,671	biosynth.com/547-25-1-d-turanose
Xylose	kg	133	chemicalbook.com
Ethanol	litre	1	iea.org/ethanol-and-gasoline-prices-2019-to-April-2022
Methane gas	cubic meter	1	globalpetrolprices.com/India/methane_prices
Molasses	tonne	409	selinawamucii.com/molasses