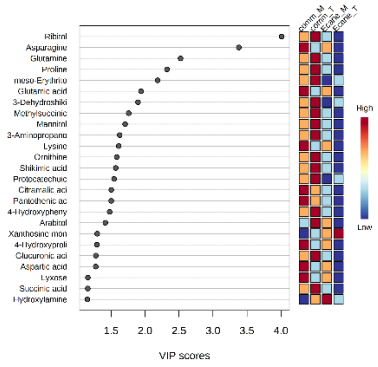
Suitable Biomass for a **Sustainable Sugarcane** Industry



UPDATE 4:
Mid-season sugarcane water-soluble chemical composition

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Introduction

The oil crisis in the 1970's and the foresight of more problems in the future, spark the interest for using sugarcane as a biomass source for energy , rather than just a source of sucrose [1]. The concept of *energycane* was proposed [4] to distinguish primarily between two sugarcane management systems: one for the production of sugar, i.e., sugarcane, and the other for the production of energy, i.e., *energycane*. Alexander argued that a reorientation of the crop management system to utilize the whole aboveground biomass and maximize growth, as opposed to maximizing sucrose, could almost double the bioenergy yield compared to the traditional sugarcane system, even when using the same varieties.

In the past decade, *energycane* has been increasingly used to describe several systems that,
in addition to a changed management system, could involve higher fibre and higher biomass varieties [2]. Although there is no general agreement in describing *energycane* varieties, the term generally refers to varieties containing higher fibre levels and lower sucrose levels than traditional sugar production varieties.

The sugarcane cropping system has been optimised to maximise the levels of sucrose in the stem. A movement away from sucrose as the focus for sugarcane production necessitate a re-evaluation of all aspects of sugarcane production and knowledge regarding the chemical composition of the biomass at different stages in the cropping cycle.

In this project the composition of the sugarcane biomass will be determent at two stages representing immature (6 months) mature cane (12 months) of growth.

Here we report on the composition of the varieties after the first six months of growth.

Primary quality components

In the SRA breeding program Brix, Pol, purity and fibre content are routine measured as the primary quality components of sugarcane [@berding2010].

In this update

- Primary quality components
- Impact of environment on quality
- Variation in most abundant metabolites

In the picture above a Variable Importance in Projection (VIP) score plot of the 25 most important water-soluble metabolites in the 15 genotypes in the Tablelands and Mossman trials.

*"The concept **energycane** is used to distinguish between two sugarcane management systems: one for the production of sugar, i.e., sugarcane, and the other for harnessing biomass and energy, i.e., **energycane**".*

"Sugarcane quality is determined based on its sugar content, known as commercial cane sugar (CCS). CCS is derived from brix (soluble solids content), pol (sucrose content) and fibre content."

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 (see data for details).

Samples were analysed with a modified method [@berding2010. 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.

Strong environmental effect on quality

The relationship between all the quality parameters in mature sugarcane has been well established and published. Less in know about these relationships in immature 6-month-old sugarcane.

There is a wider distribution in values for all the quality parameters at Mossman (Fig 1). This is most probably a direct consequence of the much larger variation in water content at Mossman. The variation in water content of the cane, especially at the Tablelands site, suggest that there is a significant genetic variation between the genotypes for this trait.

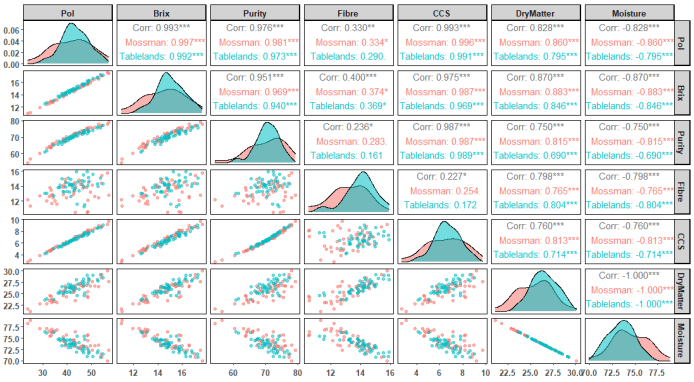

Figure 1: A pairs plot of the cane quality parameters of the 15 sugarcane genotypes at Mossman and in the Tablelands. All variables are quantitative, and the variables are plotted as scatterplots below the diagonal. The diagonal contains density plots reflecting the distribution of values at the two research sites. The correlation between the different quantitative values is presented above the diagonal. (*=$P_{0.05}$), (**=$P_{0.01}$) and (***=$P_{0.001}$).

In a sugarcane processing environment where the emphasis is on sucrose extraction and sucrose quality, the sucrose content (CCS) and high juice purity are two critical parameters. Low juice, especially when there is a high reducing sugar content (glucose and fructose) is a negative factor in the recovery of sucrose [3].

"In mature sugarcane stem tissue sugars represent more than 90% of TSS and hence Brix in this case would be a good reflection of sugar content. However, in immature tissue and leaves other compounds can dominate TSS.".

However, when considering diversification options, and extraction of the maximum value of the water-soluble components sucrose content is of lesser importance. Instead, total soluble solids (TTS) and low juice purity would be of prime importance. Total soluble solids (TSS) include all sugars (monosaccharides, disaccharides, or oligosaccharides, such as sucrose, fructose, etc.), organic acids (citric, malic, tartaric acids etc.), amino acids and other compounds such as soluble lipid, minerals, alcohol, and flavonoids.

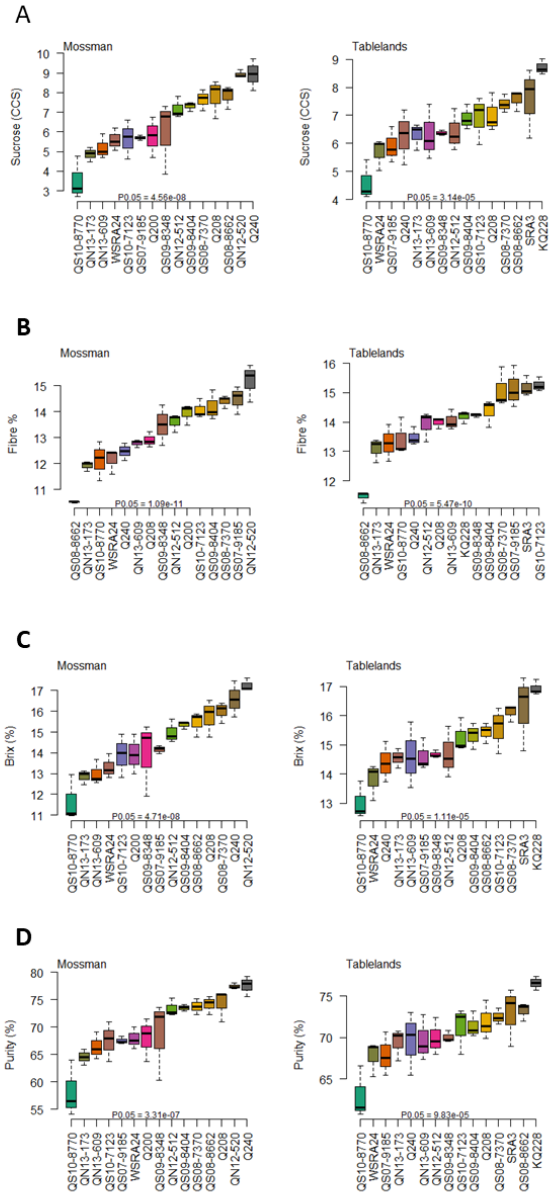

Figure 2: Primary quality components of the 15 genotypes at Mossman and the Tablelands. A. Sucrose content (CCS), (B) fibre, (C) Brix and (D) juice purity. All four parameters were significantly different across the genotypes (P<0.01).

"An analysis of variance (ANOVA) showed that there are significant differences in sucrose content, fibre, Brix and juice purity between the genotypes at both the Mossman and Tableland sites"

Typical of immature sugarcane the sucrose content (CCS) is low in all the genotypes at both locations (Fig 2A.). At Mossman Q240 and QN12-520 had the highest and QS10-8770 the lowest sucrose content. At the Tablelands site KQ228 and SRA3 had the highest and QS10-8770 the lowest sucrose content. The same was true for Brix (Fig.2C.)

Overall, the Energycanes in the trials had the highest fibre content (Fig 2B).

The juice purity of all the clones were low (Fig2.D). This is no surprise as juice purity refers to the percentage of sucrose present in the total solids content in the juice.

"A cane crop is considered fit for harvesting if it has attained a minimum of 16% sucrose and 85% purity"

Metabolic profiles

Untargeted metabolomics detection

Metabolomic profiles of the water-soluble components of the sugarcane genotypes were captured using a high-performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) assay.

For this study, we group the genotypes into two clusters. The first clusters represent the commercial varieties (comm) and the second all the genotypes that are promising high biomass clones not released to the industry. The latter cluster was designate Energycane (Ecane). In addition, each cluster were considered within the environment where the trails were conducted.

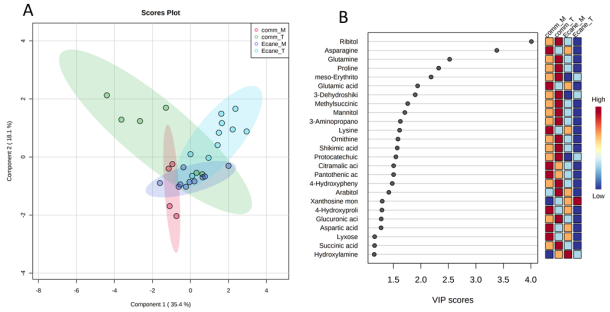

Figure 3: Multivariate data analysis of all the water-soluble metabolites. (A) Partial Least Square-Discriminant Analysis (PLS-DA) scores plot of the genotype clusters in the Mossman and Tableland trials. Shaded areas are the 95% confidence regions of each group. (B) Variable Importance in Projection (VIP) score plot of the 25 metabolites that differed most significantly between the genotype clusters in the Mossman and Tableland trials. comm_M (commercial varieties at Mossman), Ecane_M (Energycane at Mossman), comm_T (commercial varieties at Tablelands) and Ecane_T (Energycane at Tablelands).

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.

A total of 127 metabolites were identified in the sugarcane juice samples. Data was log-transformed and median-

normalised prior to statistical analysis using the software program Metaboanalyst .

These score plots displayed a significant separation between the genotype clusters and the two locations (Fig. 3A). Variable Importance in the Projection (VIP) is a weighted sum of squares of the PLS loadings taking into account the amount of explained Y-variation, in each dimension (https://www.metaboanalyst.ca). The higher VIP scores of the metabolites had, the more important contribution of it in the differences between genotype clusters and two locations. A VIP plot generated from the PLS-DA models ranked individual metabolites for their power to discriminate the genotype clusters and locations (Fig. 3B). It can be seen that ribitol and asparagine mainly contributed to the metabolic differences between genotypes and two locations (VIP > 3.0). However, 25 metabolites significantly contributed to the separation between the genotype clusters and locations (Fig. 3B).

An issue with untargeted metabolite profiling is that it only provides information on the relative abundance of a

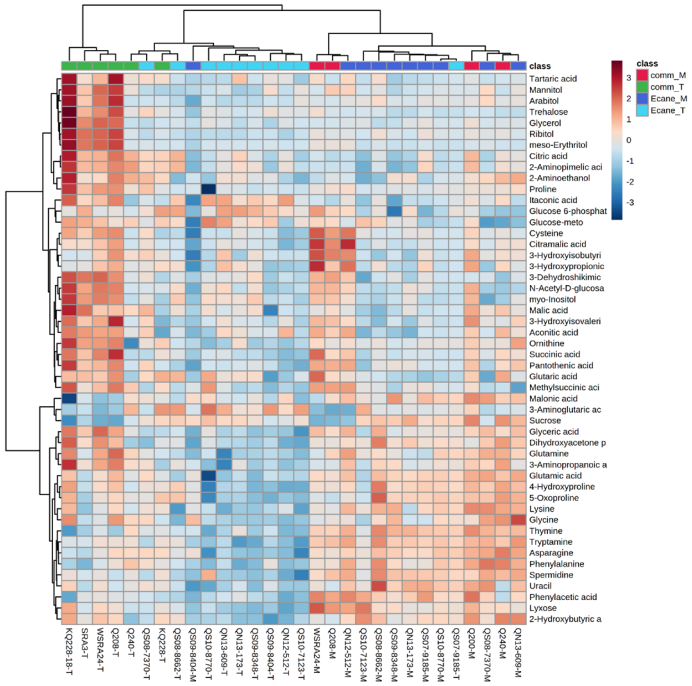

Figure 4 : Heatmap of metabolites that differed in the 15 genotypes at the two trial sites. The heatmap shows abundance of the top 50 metabolites based on VIP scores on VIP scores.

metabolite. This imply that the a single metabolite can be compared between samples and some conclusion drawn about changes in its abundance. However, metabolites cannot be compared against each other nor can conclusions be drawn about its concentration.

To overcome this limitation a targeted analyses must be done.

Targeted metabolite detection

Initial analyses showed that up to 24 metabolites were present at levels that might be adequate for commercial

"The two genotype clusters in the untargeted analyses suggest that environment is a more important factor than the genetic differences between the clones."

exploitation. To quantify these metabolites 24 authenticated chemical standards were used to construct calibration curves. Three-point calibration curve were plotted with low (0.062 mM), medium (0.25 mM) and high (1mM) concentrations to obtain the concentration of the endogenous metabolites.

Concentrations were normalized to the internal standard and weight of the material and reported as moles g^{-1}. Using the molecular weight of the metabolites the concentration in tonnes ha^{-1} were calculated.

Based on the level of the abundant metabolites the genotypes separate into two clusters (Fig. 5). It is interesting to note that cluster one represents the commercial varieties and cluster two the Energycanes. For most of the metabolites there are large significant variation in concentration between the genotypes and the two different environments.

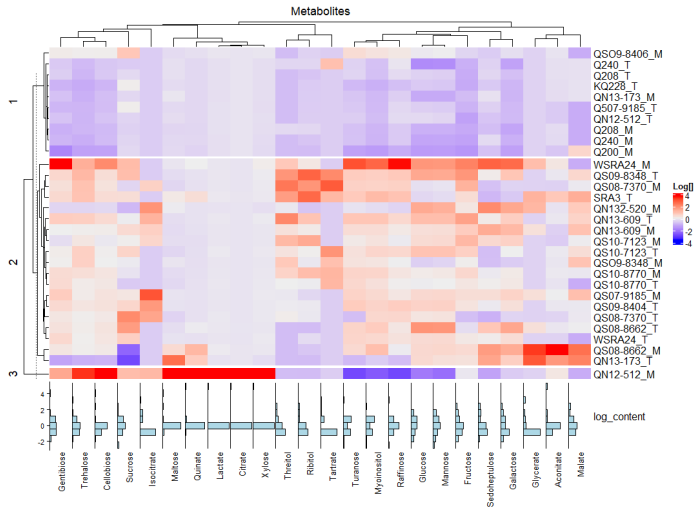

Figure 5: Heatmap of the metabolites that are present in levels higher than 0.05% of total dry mass. The heatmap shows abundance of the metabolites based on VIP scores on VIP scores. The histogram reflects the range in concentration of each metabolite between the genotypes.

'Based on the concentration of the abundant metabolites there is a good separation between the commercial varieties and the Energycanes"

References

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