Investigating the use of sweet sorghum as a model for sugar accumulation in sugarcane

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Abstract

This paper outlines a current investigation of sugar accumulation in sweet sorghum to assist in understanding and simplifying this complex trait in sugarcane. A recombinant inbred line (RIL) sorghum population, between a sweet and a grain sorghum, has been developed and phenotyped for various morphological and agronomic traits related to grain yield, biomass and stem sugar content. A genetic linkage map will be constructed for the sweet sorghum population with the objective of identifying genomic regions associated with sucrose accumulation in sweet sorghum. This will lead to further work, including comparative mapping in sugarcane, to identify the extent to which sweet sorghum can be used as a model for investigating sugar accumulation in sugarcane.

Media summary

Research is hindered in sugarcane due to its complex genetics. We are studying sugar accumulation in sweet sorghum, its genetically simpler relative, to assist in understanding the trait in sugarcane.

Key Words

Sorghum, sweet sorghum, sugarcane, sugar accumulation, sucrose.

Introduction

The Australian sugar industry produces raw and refined sugar from sugarcane (*Saccharum* spp.) mainly grown in the coastal portions of north-eastern Australia. Approximately 85% of raw sugar produced in Australia is exported, and the net income for Australia from sugar sales in 1999/2000 was approximately $1 billion (SRDC 2002). However, even with the economic importance of this crop, the ultimate aim of increasing the concentration of sucrose in the stem remains a great challenge due to the complexity of the sugarcane genome. This is exacerbated by the fact that commercial sugarcane is derived from hybrid crosses between several species: *S. officinarum*, *S. spontaneum* and *S. barberi*. Among the cultivated grasses, the closest relative of sugarcane is sorghum (*Sorghum* spp), from which sugarcane ancestors may have diverged only five million years ago (Al-Janabi et al. 1994). Whilst sugarcane is one of the most genetically complex crop species, characterised by high chromosome numbers, high levels of polyploidy and frequent aneuploidy, sorghum is a simple diploid species. Comparative mapping between sugarcane and sorghum has revealed a high level of syntony between the two species (Grivet et al. 1994; Ming et al. 1998). Like sugarcane, particular varieties of *Sorghum bicolor* L. Moench known as ‘sweet sorghums’, also accumulate large amounts of sugar in their stems; near the time of grain maturity sweet sorghums have 10 to 25% sugar in stalk juice, with sucrose being the predominant disaccharide (Hunter and Anderson 1997). As a result of this relationship between sugarcane and its simple relative sorghum, we are investigating sugar accumulation in sweet sorghum, to assist in understanding and simplifying this complex trait in sugarcane.

Methods

A recombinant inbred line (RIL) population: “R9188” x “403463-2-1” with 189 progeny lines was developed. “R9188” is a sweet sorghum inbred line developed in Texas from the commercial open

pollinated variety, Rio, and “403463-2-1” is a grain sorghum inbred from the QDPI breeding program. To ensure that the population segregated adequately for stem sugar accumulation, a preliminary trial at the University of Queensland Redland Bay Field Station was conducted in March – September 2003 which included the two parental lines of the population and a selection of 48 progeny lines. At physiological maturity a 1.5m^2 area of each genotype was harvested. Leaves and heads were removed from the plants, and the stripped stems, heads and leaves were weighed. The juice was extracted from the stripped stems by a portable mill designed to press sugarcane. A brixometer (Atago, Japan) was used to measure the percentageby weight of total soluble solids (ie sucrose, glucose and fructose) in solution. Each brix reading was immediately taken from the extracted juice before a juice sample was frozen for later analysis of sucrose, fructose and glucose content using HPLC-PAD (high performance liquid chromatography-pulsed amperometric detection) as described in Albertson and Grof (2004b).

As segregation among lines for stem sugar accumulation was noted in the population in the preliminary trial, two further trials were conducted at the Redland Bay Field Station. The first trial was planted on 16 October 2003 and harvesting began on 2 February 2004, while the second was planted on 28 November 2003 and harvesting began on 22 March 2004. Both parents and 187 progeny lines, replicated twice, were included in both trials. Phenotypic data was collected for leaf number, height, awn presence, head shape, grain colour and flowering before harvesting at physiological maturity. Once again, a 1.5m^2 area of each genotype was harvested. The total fresh weight was recorded and a 20-plant subsample was selected for further analysis. The subsample was dissected into leaf, head and stem sections, and a fresh weight of each section was recorded. The stripped stems were milled and a brix reading was taken immediately from the extracted juice. A juice sample was also frozen for later juice analysis. The fresh weight of the remaining milled stem was recorded and a subsample of all components was selected for drying. At the time of writing, juice analysis for sucrose, fructose and sucrose content had not been done.

Results

In the preliminary trial, the juice extracted from the parental line “403463-2-1” had an average brix value of 7.1%, whilst the sweet sorghum parent, “R9188”, had an average brix value of 13.0%. The brix values for the progeny lines varied between 6.6-15.8%. “403463-2-1” had on average 2.4% sucrose, 0.6% fructose and 0.7% glucose, whilst “R9188” had on average 8.6% sucrose, 0.4% fructose and 0.4% glucose. The progeny lines varied between 2.2-10.9% sucrose, 0.4-0.6% fructose and 0.4-0.6% glucose (Figure1).

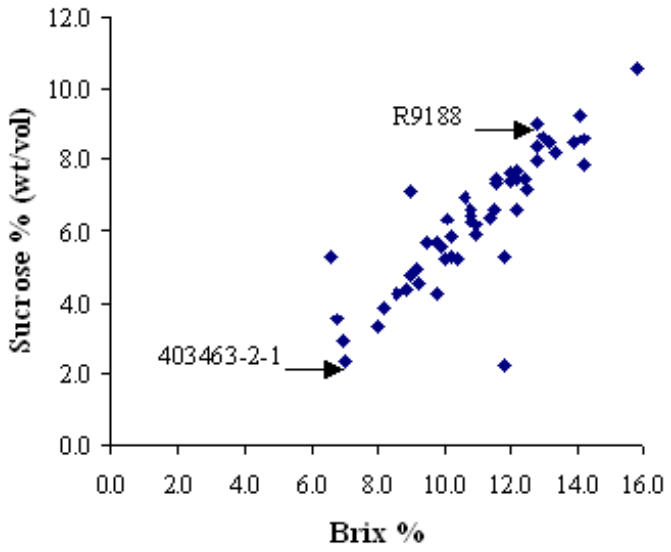

Figure 1: Distribution of the lines involved in the Redland Bay preliminary trial when the sucrose and brix percentages are compared.

In the first trial involving the entire population, “403463-2-1” had an average brix reading of 5.6%, “R9188” had an average value of 11.9% and the population varied between 3.8-15.4%. Slightly higher brix results were shown in the second trial, whereby “403463-2-1” had an average brix of 7.6%, “R9188” had an average reading of 12.5% and the population ranged between 5.0-16.5%. These brix ranges are lower than those displayed in sugarcane, which has brix readings of 20-23% in the more mature part of the stalk, and 8-12% in the younger stalk (Fernandes and Benda 1985). Sugarcane also has approximately 50% sucrose, 1% glucose and 1% fructose in the mature stalk, and 12% sucrose, 46 % glucose and 43% fructose in the younger stalk (Albertson and Grof 2004a). The sucrose, fructose and glucose content determined in the initial sweet sorghum juice analysis is lower than that displayed in sugarcane, however these are preliminary results only, conducted under different environmental conditions and further analysis is required before firm conclusions can be drawn.

Future Directions

A genetic linkage map is being constructed for the sweet sorghum population using a combination of markers, including SSRs, AFLPs and RFLPs. Using the phenotypic measurements collected from the population, linkage analysis will be undertaken, with particular emphasis on identifying genomic regions associated with sucrose accumulation. Markers identified as linked to sucrose accumulation in sweet sorghum will then be comparatively mapped in sugarcane to identify regions of sucrose accumulation in the more complex species.

To indicate the level of genetic diversity in sweet sorghums compared to grain sorghums, and to reveal the relationship between sorghum and sugarcane, a genetic diversity study involving sweet and grain sorghums and sugarcane lines has also begun. Seventy-eight genotypes have currently been selected, and these genotypes are being screened with SSR and AFLP markers.

Finally, a CEF (controlled environment facility) trial is planned to develop a profile of sucrose, fructose and glucose within the stems and temporally of sweet and grain sorghum lines from anthesis to physiological maturity. Within this study, a subset of lines will be “deheaded” at anthesis to compare the differences in the level of sugars accumulated when the panicles are removed from the plant. To complement this glasshouse trial, and previous field trials, enzyme activity in the stems of the sweet sorghum will also be measured. This information can then be compared to previous sugarcane studies to determine which enzymes are more, or less, active in sweet sorghum and contribute to the different sugar concentrations accumulated in sweet sorghum compared to sugarcane.

Conclusion

As a result of this research, it is expected that genomic regions associated with sucrose accumulation in sweet sorghum will be identified. If markers identified in these regions are successfully identified in similar regions in sugarcane, useful markers for marker-assisted selection in sugarcane will have been obtained. A better understanding of the sugar accumulation processes and the relationship between sugar accumulation and other morphological and agronomic traits in sorghum will be ascertained. Knowledge about the similarities and differences in the sugar accumulation processes, and the respective enzymes involved, in sorghum and sugarcane will assist in determining the extent to which sorghum can be used as a model for investigating sugar accumulation in sugarcane.

Acknowledgements

Appreciation is extended to the Sugar Research and Development Corporation (SRDC) for providing funding for this project.

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