Issues of starch in sugarcane processing and prospects of breeding for low starch content in sugarcane

by M.M. Zhou,¹ C.A. Kimbeng,¹* G. Eggleston,² R.P. Viator,³ A.L. Hale,³ and K.A. Gravois⁴

¹School of Plant, Environmental and Soil Sciences, LSU AgCenter, Baton Rouge, LA 70803, USA
²Commodity Utilization Unit, Southern Regional Research Center; ARS, USDA, New Orleans, LA 70124, USA
³Sugar Research Station, LSU AgCenter, Baton Rouge, LA 70776, USA
⁴Sugar Research Laboratory, SRRC, ARS, USDA, Houma, LA 70360, USA

*Corresponding author: E-mail: ckimbeng@agcenter.lsu.edu

ABSTRACT

Starch is a sugarcane impurity that adversely affects the quantity and quality of sugar processes and products. The increased production of combine and green harvested sugarcane has increased delivery of starch to sugarcane factories. Starch occurs as granules composed of amylase and amylopectin polysaccharides. Starch can reduce crystallization and centrifugation rates, occlude into the sucrose crystal, increase molasses production, reduce filterability and affiliation of raw sugars, and impede refinery decolorization processes. The behavior of starch granules on hydration and heating directly influences processing. The enzyme α-amylase used to hydrolyze starch in the factory is expensive and not always efficient. Low starch cultivars would be a more preventative, economical, and efficient solution. This paper reviews issues of starch in sugarcane processing, reports on the variations in starch among wild species germplasm, and prospects of breeding for low starch. Significant differences exist in starch levels among Saccharum and allied species and clones within these species. Saccharum species can be grouped into high (S. bengalense, Erianthus and S. spontaneum), medium (S. barberi, S. sinense and S. robustum) and low (S. officinarum and Miscanthus) starch. Cultivated species produce less starch than their wild relatives; thus low starch in sugarcane may be advantageous for sucrose production. Normal distribution in starch for S. spontaneum, a high starch species, suggests that low starch clones can be selected for introgression. When cultivars were crossed to S. spontaneum and the F1's backcrossed to cultivars, the starch content ranked as cultivars < BC1 < F1 clones. Environmental conditions such as freezing temperature tend to decrease starch content in sugarcane. From a breeding standpoint, cultivars developed or selected for low levels of starch are likely to produce relatively low and stable starch content over a wide range of conditions. To avoid increasing selection traits for breeding programs, future research to lower starch in cultivars should focus on selecting parents with low starch in introgression and crossing programs.

Keywords: α-amylases, Saccharum species, breeding, basic germplasm, wild relatives

Introduction

Sucrose yield, which is both a function of sucrose accumulation in the plant and extraction during processing, is the most important trait in a commercial sugarcane breeding program. Industry profitability can be enhanced by increasing the yield of extractable sucrose from the sugarcane crop. However, juice quality can affect the amount of extractable sucrose during milling.

Starch (α-1→4-glucan), is a sugarcane juice impurity that adversely affects factory and refinery processes and subsequently the quantity and quality of sugar products (Eggleston et al., 2006). Unfortunately, the delivery of sugarcane starch to U.S. and other countries’ factories has risen markedly in recent times because of the increased production of combine and green (unburnt) harvested sugarcane (Godshall et al., 2000). Inefficient harvesting techniques drastically increase the amount of extraneous matter, including leaves delivered to mills (Viator et al., 2006).

Starch is found in all factory and refinery sugar products including raw and refined sugars. Starch concentrations in a particular product vary widely depending on country, season, cultivar, diurnal cycle, occurrence of sugarcane disease (Grisham et al., 2006), sugarcane maturity, process conditions, effectiveness of starch removal techniques, and method of starch analysis (Imrie & Tilbury 1972). Currently, starch problems in processing are alleviated in many countries, including the U.S., with the application of α-amylase enzyme. However, such α-amylase applications are expensive, complicated, and sometimes inefficient (Eggleston et al., 2006). A more preventative, economical, and efficient long-term solution would be the development of sugarcane varieties with low starch content.

Starch may inadvertently affect selection progress during introgression breeding. The cultivated sugarcane (Saccharum spp. hybrid) is a crop for which interspecific hybridization provided a major breakthrough in its improvement. Modern sugarcane cultivars were derived in the early 1900s from interspecific hybridization between two major Saccharum species: S.
officinarum and S. spontaneum (Price, 1963). Saccharum officinarum was the primary source of genes for sucrose accumulation, whereas S. spontaneum contributed genes for general adaptability but also contributed unfavorable attributes relating to sugar quality (Roach, 1986). Only a few clones were used in the initial hybridization, so attempts are now being made in several breeding programs to utilize S. spontaneum and germplasm of other Saccharum species and related genera to improve sugarcane. These newly acquired germplasm need to be characterized for several important traits including starch. If starch is present at high levels within this germplasm, this may ultimately lead to elevated levels of starch in their progeny which would adversely impact progress from utilizing them (germplasm) to enhance sugarcane.

In Louisiana, for example, S. spontaneum is still the most important species used for germplasm enhancement. However, starch is not currently considered when deciding which S. spontaneum clones to use for germplasm enhancement, whereas F1 (commercial x S. spontaneum) clones are severely penalized for low sucrose content. Starch content in the S. spontaneum parent may inadvertently influence sucrose yield in the F1 and subsequent generations, and maybe one of the factors responsible for the slow progress in improving sucrose content during germplasm enhancement. Knowledge about the starch content of S. spontaneum and other Saccharum species and allied genera may be of interest to breeders seeking to use these germplasm. Clones with low starch content, when used as parents, may minimize the negative influence of the unfavorable juice quality of this germplasm. In commercial breeding programs, screening of cultivars to determine their relative starch content may offer Louisiana breeders a choice of which parents to use in future crossing to lower overall starch levels in cultivars or F1s of cultivar x S. spontaneum crosses.

This review has been written to highlight the issues of starch in sugarcane processing and the prospects of breeding for low starch content in sugarcane. We also present data from studies undertaken to characterize starch levels in several diverse sugarcane populations. Potential strategies to alleviate starch through germplasm enhancement without encumbering the commercial breeding program with a new selection criterion are also discussed.

**Issues of starch in sugarcane processing**

In recent years there have been warnings by some U.S. raw sugar refineries that they may impose a penalty for high starch levels in raw sugar if starch control is not improved (Eggleston et al., 2006). Processing costs increase not only in terms of additional processing aids but also from increased viscosity of massecuites, reduction of crystallization and centrifugation rates, occlusion of starch into the sucrose crystal, increased molasses production (Kampen et al., 1998), reduced filterability and affination of raw sugars, and impediment of refinery decolorization processes (Eggleston et al., 2006). Mud filtration is particularly impeded when a carbonatation refinery processes raw sugar containing >250 ppm/°Brix starch. For these reasons, Louisiana U.S. factories are being encouraged to deliver raw sugar containing >250 ppm/°Brix starch, with <200 ppm/°Brix preferred for Louisiana carbonatation refinery. In comparison, in South Africa raw sugar containing starch >130 ppm/°Brix is penalized (P. Schorn, Tongaat-Hulett Sugar Ltd., personal communication). In the U.S., however, there is no current penalty for high starch concentrations in raw sugar. Instead, an informal policy of encouragement and cooperation exists between the carbonatation refinery and fac-
tory, which refinery staff considered to have been successful in the past 3 to 4 years (F. Goodrow, Domino Sugar, personal communication). Cooperation includes the application of α-amylase in the factory to hydrolyze starch (Eggleston et al., 2006). However, not all U.S. raw sugar factories apply α-amylase and some just apply it intermittently.

**Starch composition**

Starch exists as semi-crystalline granules (1 to 10 μm) in sugarcane tissue and extracted sugarcane juice. These granules are smaller than those for corn (5 to 25 μm) and potato (15 to 100 μm) (Imrie and Tilbury, 1972). Sugarcane starch granules contain two glucose polysaccharides: ~19% amylose and 81% amyllopectin (Vignes 1974). Amylose is linear with the glucose molecules α-D-(1→4) linked (Figure 1). Amylopectin, in addition to the α-D-(1→4) linked glucose found in amylose has α-D-(1→6) linked branch points (Figure 1). Amylose forms a blue color in the presence of iodine while amyllopectin forms a red-violet color (Honig, 1953; Imrie and Tilbury, 1972).

**Starch occurrence and physiology in the sugarcane plant**

Starch is produced in the sugarcane plant as a storage polysaccharide (carbohydrate reserve), and utilized during periods of rapid growth, e.g., sprouting of roots and buds, seedling germination and emergence (Imrie and Tilbury 1972; Bewley and Black 1998). Starch granules are present in stalks, green and brown leaves (Eggleston et al., 2007b), and roots of the sugarcane plant (Alexander 1973) but are most abundant in the green leaves and growing point region (Table 1). There is strong varietal effect on starch content in the global juice (Eggleston et al., 2007b; Muangmontri et al., 2007) and for starch tissue distribution (Eggleston et al., 2008a) (Table 1). Starch decreases with sugarcane maturity.

Stalks of sugarcane have variable amounts of starch depending on the area of the stalk (Table 1). Starch is generally lower at the bottom of the stalk, and starch granules are deposited mainly at the nodes and disappear during rapid growth. Growing conditions such as soil type, nutrients and agronomic practices, water supply and temperature have been reported to affect the levels of starch found in sugarcane stalks (Imrie and Tilbury, 1972). Although starch levels in stalks are relatively low compared to other tissues (Table 1), when calculated on a percent tissue weight basis it is observed that stalks actually deliver a considerable amount of starch to the factory just because of their much higher weight (Eggleston et al., 2008a). Therefore, factory delivery of starch by stalks should **not be underestimated**.

Starch in green leaves varies considerably with the diurnal cycle because the products of photosynthesis are temporarily stored in the leaves as starch (Lu et al., 2005). Sugarcane is a C4 photosynthetic plant with high photosynthetic productivity (Du et al., 2000a). Starch synthesis is controlled by sucrose synthesis but not vice versa (Stitt and Quick 1989). Unlike many other crops, the final photosynthetic product in sugarcane is sucrose not starch. Green leaf transitory starch accumulates between 0800 h and 1000 h. Maximum starch content usually occurs between 1600 and 2000 h (Du et al., 2000b). As night after 2000 h, starch levels decrease rapidly until about 2400 h, and then slowly until about 0800 h of the following morning. Starch is hydrolyzed by several plant enzymes (Lu et al., 2005) and converted into soluble sugars that are exported from the leaves to the rest of the plant. Du et al., (2000b) observed that 82% of the carbon fixed during the day was exported immediately, while 17% was accumulated as leaf starch and exported at night. Similar trends in starch accumulation and fluctuation have been observed in sugar beet leaves (Li et al., 1992; Fondy and Geiger 1985). Transitory starch may act as an overflow for newly assimilated carbon when assimilation exceeds the demand for sucrose (Stitt and Quick 1989) and provide a source of carbon for growth during the following night (Trethewy and Smith 2000). The regulation of transitory starch accumulation in leaves depends on the photosynthesis rate and photoperiod (Li et al., 1992). The relative amount of recently assimilated carbon that is allocated to transitory starch for subsequent export is inversely proportional to the duration of the daily photosynthetic period (Chatterton and Silvius 1979; Jablonski and Geiger 1987).

**Starch at the factory**

Starch granules are extracted into juice during factory tandem milling or diffusion. The behaviour of starch granules on hydration and heating in the factory influences their effects on sugarcane processing. In cold juice, starch is not soluble. If hot maceration or imbibition water is added at the factory, the granules are washed out of the shredded plant tissue into the juice, and the heat causes the granules to swell, become partially soluble and gelatinized (Godshall et al., 1991). Solubilization and gelatinization of the granules is completed during clarification and evaporation at the factory (Figure 2). During clarification and evaporation, starch granules are further heated, progressively swell, and rupture with release of amylose and amyllopectin and are transformed into an amorphous viscous solution (Figure 2). Linear amylose molecules are capable of forming helices and can readily associate in water by hydrogen bonding, and these entangled amylose chains increase viscosity. On cooling they can re-associate to form a gel network, whereas branched amyllopectin cannot (Tester et al., 2004). This explains why the amylose fraction is responsible for the deleterious effect of starch in the factory (Godshall et al., 1991). Amylose molecular association phenomenon is known as retrogradation, and influences the distribution of starch in factory and refinery products. Due to the starch physical transformation and concentration effects taking place across the factory, the viscosity of products in the boiling house increase (Kampen et al., 1998). High starch in mixed juice can, therefore, reduce boiling house efficiency and increase molasses formation.

<table>
<thead>
<tr>
<th>Sugarcane Tissue</th>
<th>Mean starch concentration (ppm*/°Brix)</th>
<th>LCP 85-J84</th>
<th>HoCP 96-S40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Green Leaves</td>
<td>1244</td>
<td>1246</td>
<td>1139</td>
</tr>
<tr>
<td>Growing Point Region</td>
<td>2263</td>
<td>1780</td>
<td>2528</td>
</tr>
<tr>
<td>Middle Stalk</td>
<td>502</td>
<td>495</td>
<td>1831</td>
</tr>
<tr>
<td>Lower Stalk</td>
<td>458</td>
<td>336</td>
<td>973</td>
</tr>
</tbody>
</table>

Table 1: The distribution of starch in different tissues of the sugarcane plant from two Louisiana commercial varieties. From Eggleston et al., 2007b.
Factory processes that contribute to the removal of starch

In some countries such as Australia, natural amylases have been used to hydrolyze starch in juice. In Louisiana, U.S., there is only one factory that has a large enough incubation tank to allow the natural amylases to work. Filtrate from the clarifier is recycled into the incubation tank to reduce the sugarcane juice acidity, enabling the natural amylases to hydrolyze starch (Eggleston et al., 2003). Hydrolysis is more efficient when starch granules have been partially solubilized and gelatinized by hot maceration or imbibition water. Unfortunately, there is a possibility that some unwanted acid degradation of sucrose may occur in the tank because of the retention time. Furthermore, the naturally occurring amylases occur at concentrations that are too low to degrade all the starch. Eggleston et al. (2003) reported ~10 to 20% degradation of starch by natural amylases in a 12-min retention time incubation tank.

If intermediate or hot lime clarification is operated in the factory some starch will be removed by precipitation just by preheating the juice before liming and clarification (Eggleston et al., 2003); this will not occur if cold lime clarification is operated. Some starch is also removed in factory clarification process through precipitation. However, the heating, incubation, or clarification of juice to remove or degrade starch, usually does not reduce starch enough to alleviate starch process problems in the boiling house and, therefore, treatment of evaporator syrup with α-amylase to hydrolyze starch is regularly used.

Factory application of α-amylase to hydrolyze starch

α-Amylase is usually added to the penultimate or last evaporator body because starch is in a completely solubilized and gelatinized form that is much more conducive to α-amylase hydrolysis (Tester et al., 2004). The pH, temperature, and retention time are also more conducive to α-amylase action, but are still not optimal (Eggleston et al., 2006).

One of the primary reasons for adding α-amylases in the factory is to stop amylose from retrograding and forming very viscous solutions after the evaporator station. α-Amylases (endo-1→4-α-D-glucan glucohydrolases; EC 3.2.1.1) are endo-hydrolases that, in the presence of water, randomly cleave 1→4-α-D-glucosidic linkages between adjacent glucose molecules in the amylose chain of the solubilized/gelatinized starch. The viscous solution is progressively “thinned” into lower molecular weight (MW) dextrans, and finally maltodextrins (oligosaccharides) of smaller chains (often in the 2 to 7 dp range) (Figure 3).

α-Amylases are classified according to their action and properties (Pandey et al., 2000) and derived from several bacteria, yeasts, and fungi. Bacterial α-amylases, particularly from Bacillus sp., are generally preferred for commercial production and widely used in numerous industries because they have the most diverse biochemical properties and are generally recognized as safe. Most commercial α-amylases used by the U.S. sugar industry to control starch have intermediate temperature stability (up to 85°C with an optimum ~70°C) and are produced from Bacillus subtilis. They are calcium dependent α-amylases but this is not a concern for sugar industry applications because lime is added during the clarification process and, therefore, free calcium concentrations are adequate. However, some factories in the U.S. and world-wide have applied bio-engineered high temperature (HT) stable (up to 115°C) α-amylases from Bacillus licheniformis and B. stearothermophilus, which were developed for much larger markets than the sugar industry. They are not specifically tailored to sugar industry conditions (Eggleston et al., 2006) and, for example, are still less active in high °Brix syrups. HT α-amylases can also be too temperature stable in the sugar industry, and may not denature or inactivate after application, resulting in carry-over activity into raw sugar and molasses. α-Amylase activity in the raw sugar can even carry through subsequent refinery processes and
eventually reside in refined sugar, molasses, and food products. Two U.S. refineries sold final molasses that contained residual \( \alpha \)-amylase activity to barbeque sauce manufacturers, which caused barbeque sauce to detrimentally “liquefy” (Eggleston et al., 2006).

To avoid this, high volume customers of refineries have requested that HT stable \( \alpha \)-amylases are not applied at the refinery. Concomitantly, refineries in Louisiana have requested factories not to apply HT stable \( \alpha \)-amylases.

Another added complication in the application of \( \alpha \)-amylases in sugarcane factories is the existence of a wide variation in the activities and activity per unit cost of HT \( B.\ subtilis \) and HT \( B.\ licheniformis \) and \( B.\ stearothermophilus \) \( \alpha \)-amylases (Eggleston et al., 2007a). This is compounded by there being no uniform or standard method to measure the \( \alpha \)-amylase activity in the sugar industry or a regulatory body to issue or regulate standard activity methods and units for the commercial enzyme. The efficiency of \( \alpha \)-amylase action to hydrolyze starch in syrups is related to the activity of the \( \alpha \)-amylase used (Eggleston et al., 2006). Application of relatively high activity \( \alpha \)-amylase as a working solution diluted 3-fold in water at the factory to the penultimate evaporator body, can improve contact between the starch and \( \alpha \)-amylase and improve starch hydrolysis and is cost-effective (Eggleston et al., 2008b). Eggleston et al. (2008b) recently also observed that it is more difficult to hydrolyze starch with \( \alpha \)-amylase at the factory when starch levels are low (~1000ppm/Brix). This is because of lower contact between the starch (substrate) and \( \alpha \)-amylase (enzyme). This problem can be mitigated by increasing the dose of working solution of high activity \( \alpha \)-amylase added to the penultimate evaporator (Eggleston et al., 2008b).

**Prospects of breeding for low starch content in sugarcane**

Modern sugarcane cultivars were derived from interspecific hybridization between two major *Saccharum* species, namely *S. officinarum* and *S. spontaneum*, in the early 1900s (Price, 1963). Introgression with *S. spontaneum* increased hardness of sugarcane but unfortunately also brought some undesirable traits including high starch. Evaluation of a wide range of the germplasm would result in those clones with less negative traits being used for introgression and, therefore, result in more use of the germplasm in enhancement programs.

**Variation among Saccharum species for starch content**

Among the relatives of cultivated sugarcane, starch was originally thought to occur only in *Saccharum* species other than *S. officinarum* (Van Dillewijn, 1952). However appreciable levels of starch have since been reported in *Erianthus*, *S. barberi*, and *S. sinense* (Dutt and Narasimhan, 1951). Recent studies have shown that the little or no starch recorded in *S. officinarum* was because of the less sensitive analytical methods used (Godshall et al., 2004). In several of our studies, juice starch was analyzed using the Sugar Processing Research Institute (SPRI) method (Godshall et al., 2004) modified by Eggleston et al. (2006). Simple correlation coefficients among pairs of sub-samples were highly significant (r >0.90; P<0.001) indicating that the starch analysis method was reliable and can be used for screening large populations (Zhou et al., 2007). This method detected even very low (134.2 ppm/°Brix) levels of starch in sugarcane juice.

**Variation among Saccharum species for starch content**

Among 49 clones comprising *S. spontaneum*, 13 *S. Barberi*, 11 *S. robustum*, 8 *S. sinense*, 9 *S. officinarum* and 1 each of *Erianthus*, *Miscanthus* and *S. bengalense* (Zhou et al., 2007). Differences in starch content were apparent among the *Saccharum* species (Table 2). There were significant differences (P = 0.01) between species, and among clones within species, suggesting a wide variation for starch accumulation across and within species. Among entries that had at least 5 clones, *S. officinarum* had the lowest mean starch content followed by *S. robustum*, *S. barberi* and *S. sinense*, whereas *S. spontaneum* had the highest mean starch content (Table 2). *Saccharum bengalense* (76%), *Erianthus* (68%) and *S. spontaneum* (60%) produced significantly (P = 0.01) more starch than *S. officinarum*. Among the “so-called” cultivated *Saccharum* species, *S. barberi* (31%), *S. robustum* (19%), and *S. sinense* (31%) produced significantly (P = 0.05) more starch than *S. officinarum*. From these studies, the *Saccharum* and allied species can be grouped into three categories based on their starch content: high starch (*S. bengalense, Erianthus* and *S. spontaneum*), medium starch (*S. barberi, S. sinense and S. robustum*) and low starch (*S. officinarum and Miscanthus*) species. Generally, the cultivated *Saccharum* species produced less starch than their wild relatives, supporting observations in Figure 4 which suggest that accumulating low levels of starch is advantageous for sucrose production in the *Saccharum* species. These results agree with Dutt and Narasimhan (1951) who tested starch accumulation among 215 wild species and cultivars and found that *S. officinarum* and *S. robustum* (cultivated species) had, at most, traces of starch, where-
as *S. spontaneum*, *S. barberi* and *S. sinense* (wild species) accumulated much greater amounts of starch.

The mean value of the *S. barberi* clones was associated with the least standard deviation (Table 2) when compared with other species that had at least five clones. Each of the 13 *S. barberi* clones produced more starch than the mean starch value of the *S. officinarum* clones (data not shown). However, for most other species, even when the mean starch content for the species was high, such as in *S. spontaneum* (Tables 2 and 3), starch content varied widely among the clones. This suggests the potential of selecting for low starch clones even from among high starch producing species.

In a study aimed specifically at identifying *S. spontaneum* clones with low starch content, 52 *S. spontaneum* and one *S. officinarum* (control) clones were evaluated for starch (Zhou et al., 2007). The *S. officinarum* control produced significantly (*P ≤ 0.01*) less starch than the *S. spontaneum* clones (Table 3). Starch content in these germplasm influences starch among the progeny, and presumably total recoverable sugar (TRS) during introgression remains to be elucidated. TRS generally decreases with increasing starch levels (Figure 4). Also, when using *S. spontaneum* germplasm for introgression, backcrossing to a low starch parent (in this case a cultivar) reduces the overall starch levels among the resulting progeny (Table 4). Overall, the results highlight the importance of screening for low starch content among sugarcane germplasm, and further suggest that using germplasm low in starch could lead to lower starch content and most likely higher TRS among the progeny.

### Starch content among cultivars

High levels of starch in sugarcane cultivars have provided major difficulties during processing. Noteworthy examples were report-

| Table 2. Starch content (ppm/°Brix) among Saccharum species. |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Species         | Number of clones | Starch (ppm/°Brix) | Standard Deviation | % of Saccharum officinarum |
| *Saccharum barberi* | 13              | 1914             | 121             | 131             |
| *Saccharum bengalense* | 1        | 2581             | 53              | 176             |
| *Erianthus species*         | 1           | 2454             | 11              | 168             |
| *Miscanthus species*         | 1           | 1537             | 332             | 105             |
| *Saccharum officinarum* | 9           | 1464             | 270             | 100             |
| *Saccharum robustum*      | 11          | 1748             | 461             | 119             |
| *Saccharum sinense*       | 8           | 1929             | 530             | 131             |
| *Saccharum spontaneum*    | 5           | 2349             | 899             | 160             |

| Table 3. Starch content (ppm/°Brix) among *S. spontaneum* clones compared to *S. officinarum* control. |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Species         | Number of clones | Starch (ppm/°Brix) | Standard Deviation | % of *S. officinarum* |
| *S. officinarum* | 1           | 2144.1           | 86.2             | 100             |
| *S. spontaneum* | 52          | 3753.5           | 1304.2           | 175             |

| Table 4. Starch content (ppm/°Brix) among cultivars, F1, and BC1 clones. From Zhou et al., 2007. |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Entry           | Number of clones | Starch (ppm/°Brix) | Standard error |
| Cultivars       | 6               | 1264             | 75              |
| BC1             | 29              | 1944             | 38              |
| F1              | 41              | 2436             | 34              |
ed in South Africa (Natal Uba and NCo 310) and Australia (CP 29-116 and NCo 310) (Agarwal et al., 1998). The only active research in addressing the sugarcane starch problem has been at the factory despite the acknowledgement by several authors (Godshall et al., 1994; Cuddihy et al., 2000; Eggleston et al., 2007b; Muangmontri et al., 2007) that cultivar effects were important in determining starch content. This is because problems associated with starch in sugarcane juice have been viewed by most breeders as one that can be alleviated more economically by adding α-amylase during processing. This view has stemmed from the need to minimize the number of selection criteria in order to maximize genetic gain (Hogarth and Berding, 2006). While adding α-amylase has proved to be an economical control method in countries such as Australia, it may not be feasible or entirely efficient and economical in other countries. In Thailand, Muangmontri et al. (2007) recently surveyed starch content among sugarcane cultivars and recommended low starch cultivars should be used to alleviate that country’s starch problems. In Louisiana, only one factory has a large enough incubation tank to allow the natural amyloses to work (Eggleston et al., 2006). Also, in sub-tropical environments like Louisiana the nine month growing period imposed by freezing temperatures has meant that more immature sugarcane is processed than in tropical environments. Starch levels are generally higher in less mature cane and tend to decline as the cane matures.

Genetic and environmental effects on starch accumulation

Starch accumulation appears to be under genetic control although it is also highly affected by the environment. Depending on the precision of the experiment and type of genetic material being evaluated, broad sense heritability estimated (degree of genetic determination) in our studies have ranged from 75 to 80% (Table 5, Kimbeng, unpublished data). This range supports the feasibility of selecting clones low in starch either from among cultivars or inter-specific hybrids.

Clones selected for their relative differences in starch content are also expected to maintain the difference over time providing further support of genetic control for this trait. Studies reported by Godshall et al. (1994) showed that differences in starch among cultivars were more consistent than seasonal differences. Recent studies have corroborated this result (see Figures 6, 7 and 8; Table 5). Generally, correlation coefficients of r > 0.70 were found between replications, locations or crop years in these studies. In one study, 76 clones including 6 cultivars and 70 unselected clones of F1 and BC1 origin derived from crosses

Table 5. Variance components and broad sense heritability estimates for starch content in different sugarcane populations.

<table>
<thead>
<tr>
<th>Population parameters</th>
<th>Population 1</th>
<th>Population 2</th>
<th>Population 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population and trial description</td>
<td>One hundred twenty clones derived from a S. officinarum x S. spontaneum cross evaluated in a single environment using three replicates.</td>
<td>Seventy clones of F1 and BC1 origin derived from crosses between cultivars and S. spontaneum evaluated over 2 years in a single environment using 3 replicates.</td>
<td>19 varieties planted at three locations and each location harvested on a different date</td>
</tr>
<tr>
<td>σ²g</td>
<td>7754</td>
<td>127786</td>
<td>9422.26</td>
</tr>
<tr>
<td>σ²γv</td>
<td>-</td>
<td>31372</td>
<td>-</td>
</tr>
<tr>
<td>σ²lv</td>
<td>-</td>
<td>-</td>
<td>8441.73</td>
</tr>
<tr>
<td>σ²e</td>
<td>7629.72</td>
<td>85805</td>
<td>225.23</td>
</tr>
<tr>
<td>Heritability</td>
<td>σ²g/(σ²g+σ²e/r)</td>
<td>σ²g/(σ²g+σ²γv+y²+σ²e/γ)</td>
<td>σ²g/(σ²g+σ²lv+y²+σ²e/rl)</td>
</tr>
<tr>
<td>Heritability</td>
<td>75.3</td>
<td>80.9</td>
<td>76.8</td>
</tr>
</tbody>
</table>
between cultivars and S. spontaneum (see Table 4), were evaluated in 3 replicates over 2 crop years. The starch content for each clone was averaged over replicates and crop years and the lowest and highest 10% of clones were plotted for each replicate within a crop year (Figure 6). Starch content varied across replicates within crop years but amid all these environmental variations, clones in the lowest 10% group produced consistently less starch than those in the highest 10%.

Temperature is an environmental factor most likely to influence starch accumulation in sugarcane. In a separate study, the mean starch value from a population of 300 clones derived from selfing the cultivar LCP 85-384 (Milligan et al., 1994) was averaged across two replications and ranked from lowest to highest based on the starch content. The mean 10% of clones with the lowest and highest starch content were plotted for each replication (Figure 7). Replication 1 was sampled on November 13, 2006 (before a freeze) and replication 2 was sampled on December 27, 2006, after 5 days of freezing temperature (Table 6). The clones accumulated significantly (P<0.0001) more starch before than after the freeze (Replication 1) (Table 6). In comparison, the low starch clones accumulated comparatively higher levels of starch before and after the freeze and showed very little decrease in starch content after the freeze. The high starch clones showed comparatively higher levels of starch before the freeze and experienced a large decrease in starch levels after the freeze (Figure 7).

In another freeze study, 19 advanced clones from a commercial breeding program were grown in two replicates across three locations (Table 6). Starch content for the 19 clones was averaged across replications and locations and the mean of the lowest and highest 5 clones was plotted against locations (Figure 8). The low starch clones consistently accumulated relatively lower levels of starch across the locations (Figure 8). The locations' harvest dates with lower mean freeze temperatures were associated with lower starch content compared to locations' harvest dates with higher mean freeze temperatures (Table 6).

Although in each of the two aforementioned experiments replications or locations was confounded with sampling dates, when the results from both experiments are considered together, they suggest that low-starch clones were relatively more stable and less susceptible to environmental fluctuations than high-starch clones. High-starch clones would accumulate high levels of starch when conditions are favorable for starch accumulation and the starch levels could decrease sharply when conditions change, particularly after experiencing freezing temperatures. Thus, from a breeding standpoint, cultivars developed or selected for low levels of starch are likely to produce relatively low and stable starch content over a wide range of conditions.

The decrease in starch after a freeze was proportional to the severity of the freeze (Table 6 and Figure 8). A similar dynamic where stress causes starch levels to drop has been reported for other crops. In alfalfa, starch levels in the roots of plants of a cloned genotype were drastically reduced following defoliation compared to their non-defoliated counterparts (Gana et al., 1998). In the green algae Chlorella vulgaris, analysis of products formed in cells during photosynthesis in air containing 3,000 ppm 14CO2 at various temperatures, revealed that the level of 14C-starch was maximum around 20–24°C and decreased with further rise in temperature until 40°C, while 14C-sucrose greatly increased at temperatures above ~28°C (Nakamura and Shigetoh, 1982). Elevating the temperature from 20 to 38°C during photosynthetic 14CO2 fixation resulted in a remarkable decrease in 14C in starch and a concomitant increase in 14C in sucrose and this conversion of starch to sucrose when shifting the temperature from 20 to 38°C proceeded even in the dark. Therefore, the dynamics between starch and sucrose in sugarcane following temperature (freeze, heat) and non-temperature (application of chemical ripeners) related stresses may follow a similar pattern and warrants further investigation. The results may have implications in the way starch is managed during processing when harvesting is preceded by exposure of the crop to stress.

### Conclusions and future outlook

Starch is a sugarcane impurity that adversely affects the quantity and quality of sugar products during processing. Starch has increased in sugarcane delivered to factories in recent years because of increased production of combine and green (unburnt) harvested sugarcane. The behavior of starch granules on hydration and heating influences directly its effects on sugar processing. α-Amylase used to hydrolyze starch in the factory is expensive and not always efficient. Low starch cultivars would be a more preventative, economical, and efficient solution to starch in sugar processing.

Motivated by the need to minimize the number of selection criteria in sugarcane breeding programs, problems associated with starch in sugarcane juice have traditionally been alleviated through the application of α-amylase during processing. This may have worked in some countries but is not universally feasible or entirely efficient and economical in subtropical areas where immature sugarcane is harvested before the onset of frost tem-
temperatures. Furthermore, the widespread adoption of billeted and green (unburnt) sugarcane harvesting techniques has exacerbated the problem because starch is higher in green leaves and tops.

It may be possible to bio-engineer an intermediate-temperature α-amylase to be more active in high °Brix syrups. However, bio-engineering of enzymes is extremely expensive, and such an investment by a large enzyme company is not foreseen as the sugar market is viewed as being too small. Moreover, water is a necessary reactant in the enzymatic hydrolysis reaction and would, therefore, limit any progress of bio-engineering the enzyme to be high °Brix tolerant. Alternative methods such as selection and crossing among cultivars low in starch content most likely will alleviate the problem and provide a more economical and long term solution without adding an extra trait at the selection stages.

Starch content is known to be high among some Saccharum germplasm (e.g., Saccharum spontaneum) frequently used to improve cultivated sugarcane in Louisiana. However, little is known about how the starch content may affect selection progress during introgression because in the past no effort was made to screen for starch content in the germplasm or their progeny. Screening of germplasm for starch content and subsequent selection of low starch clones for introgression may now offer an opportunity to lower starch concentration during introgression.

Figure 6. The mean starch content of the highest 10% and lowest 10% of clones in replications 1, 2, 3 for crops sampled in 2005 and 2006. The mean starch content for each of 76 clones in the study was derived by averaging starch content over three replicates and two crop years.

Moderate to high broad sense heritability estimates for starch content indicate the potential to select for low starch genotypes among cultivars or introgression lines.

Starch analysis of the Saccharum species collection showed significant variation among the species and clones within the species. The magnitude of the variation indicated strong genetic control. These factors offer the opportunity of using “among species” and “within species” variation to develop populations and parents for developing low starch parents. Crossing low starch parents produces progenies with low starch while crossing high starch parents produces progenies with high starch. Therefore, introgression with selected low starch parents can result in progeny with low starch for variety selection. Use of germplasm with low starch should result in fewer backcrossing cycles to reduce starch and increase sucrose content in clones.

Low starch clones consistently produced lower and more stable starch across replications, years and locations compared to high starch clones. A reduction in starch content was associated with the severity of freeze temperatures before sampling. Low

Figure 7. Starch content of the high (10%) and low (10%) starch clones sampled before a freeze R1 (Replication 1) and after the freeze R2 (Replication 2) at Houma, Louisiana. The mean starch content for each of 300 clones in the study was derived by averaging starch content over the two replicates.
starch clones produced low and more stable starch levels as temperatures fluctuated.

To avoid increasing selection traits for breeding programs, future research to lower starch in cultivars should focus on selecting parents with low starch in introgression and crossing programs. Low starch clones are stable and consistently produce low starch, which warrants further investigation into the potential of scheduling of cultivars based on their starch content. It is likely to be more beneficial to harvest low starch cultivars early and high starch cultivars later, when their starch content would have declined due to decreasing temperatures. This approach may have the overall effect of lowering the amount of starch delivered to the factory and can potentially lower the costs associated with high starch in sugarcane juice.

References


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