

Full Length Research Paper

Agro-industrial potential of sweet sorghum accessions grown under semi-arid conditions

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The current world-wide interest in alternative and sustainable forms of energy is providing an opportunity for carving new pathways of development in many countries. Plants are particularly attractive vehicles in this regard because of their innate capacity to self-renew and to produce chemical forms of energy every time. Carbohydrates and oils as primary products from plants are attractive raw materials for the production of alternative forms of energy such as bioethanol and biodiesel. Against this background, this study was undertaken to assess the potential of sweet sorghum as a feedstock for bioethanol production. To this end, a comparative analysis of non-structural carbohydrate amounts in mature stems of several local accessions of sweet sorghum was carried out. For comparison, the same products were also determined in mature stems of sugarcane. Sucrose was the predominant sugar in the mature stems of sweet sorghum as was the case with sugarcane. However, the overall carbohydrate profiles of sweet sorghum were markedly different from those of sugarcane. Mature stems of sweet sorghum contain more readily fermentable sugars than mature sugarcane stems. In addition, sweet sorghum also accumulates starch in the stems as well as in the seeds. Through hydrolysis, this serves as an additional source of glucose for fermentation into ethanol. These data clearly demonstrate the renewable energy resource potential of sweet sorghum. Nonetheless, further studies on the basic biology of carbohydrate partitioning in sweet sorghum are warranted. Such studies could provide rational basis for improvement of wet-fermentation substrate yield of this crop.

Key words: Metabolism, non-structural carbohydrates, renewable energy, substrates, sucrose, trade-offs, wet fermentation.

INTRODUCTION

The current world-wide interest in sustainable production of energy is providing an opportunity for agro-industrial development in many countries. Plants present an attractive option in this regard because of their innate ability to harness sunlight and convert it into renewable chemical energy forms. Such energy forms, especially carbohydrates and oils are primary substrates for the production of biofuels like bioethanol and biodiesel, respectively. Agricultural crops such as maize and sugarcane and or its products are the traditional sources of fermentable substrates for bioethanol production (Leiper et al., 2006; Calvino et al., 2009). However, the high water demands of these crops and their food use preclude their widespread adoption as biofuel feedstocks, particularly in developing countries where food security is

a significant concern. This calls for careful evaluation of options for biofuel production in order to avoid the food-for-fuel conflict that is already envisaged across many parts of the world. Recent developments in the supply of electricity and petroleum-based fuels provide the much needed impetus for evaluation alternative energy forms to support development programmes. For semi-arid climates as is the case for Botswana, there is need to explore the potential of crops with high water-use efficiency and short growth cycle as possible feedstocks for biofuel production. Sweet sorghum has these characteristics (Almodares and Hadi, 2009; Wu et al., 2010) and this makes it an attractive candidate feedstock crop for bioethanol production.

Sorghum [*Sorghum bicolor* (L.) Moench] belongs to the

Saccharinae clade which includes sugarcane. Not surprisingly sorghum types described as sweet sorghums accumulate soluble sugars in the stem at maturity (Vieter and Miller, 1990; Calvino et al., 2008). But in contrast to sugarcane which matures in 12 to 16 months, sweet sorghum matures in 5 months and it is predominantly grown under rain-fed conditions (Schaffert, 1992). In view of these factors, sweet sorghum may have the potential to be developed into a feedstock crop for bioethanol production under semi-arid conditions. Currently, sweet sorghum is grown successfully (and with minimum input) under conditions where few or no other crop performs better (Reddy et al., 2005).

This research has thus been initiated to investigate whether sweet sorghum has potential to be developed into a feedstock for bio-energy production under semi-arid conditions. Demonstration of such potential could have a significant positive impact on the socio-economic livelihoods of many inhabitants of Botswana, especially those who live in rural areas. As in many other developing countries, most people in the rural areas of Botswana rely on agriculture for their livelihoods. In view of this, research into crops with agro-industrial potential is particularly relevant. Therefore, it was deemed prudent to investigate whether sweet sorghum grown under rain-fed conditions has sufficient capacity to accumulate fermentable carbohydrates in the stem at maturity. Existence of such capacity could pave the way for development of agro-industrial projects based on this marginalized crop. This manuscript presents evidence to this effect.

MATERIALS AND METHODS

Chemicals

Enzymes and chemicals were obtained as follows: amylogluco-sidase (EC 3.2.1.3), ATP, glucose-6-phosphate dehydrogenase (EC 1.1.1.49), hexokinase (EC 2.7.1.1), NADP⁺, phosphoglucose isomerase (EC 5.3.1.9) from Roche Products, South Africa. Sodium acetate from SAARCHEM, South Africa. D-fructose, D-glucose, ethanol, glacial acetic acid, invertase (EC 3.2.1.26), magnesium chloride and sucrose were from Sigma Chemical Company, South Africa. All the chemicals used were of analytical grade. Water used was de-ionized unless otherwise stated.

Plants

Different local accessions of sweet sorghum were grown in a field at the department of Agricultural Research at Sebele in Gaborone. Mature (14 months old) stems of sugarcane grown under normal sugarcane agronomy practices were obtained from a local farm. The rainfall during the growth period was around 350 mm.

Sampling

Samples of three mature (5-months old) stems from hard-seed stage plants of each accession were chosen at random from crop stands in the field. Mature sugarcane stems were included as a positive control.

These were transported to the laboratory within an hour of harvesting. Internode tissue samples were taken from each stem as follows: second internode from the pedicel, middle internode as well as from the second internode from the base. The fresh weight of the samples varied from 0.6 to 1.5 g. Each sample was quickly weighed and immediately killed in boiling 80% (v/v) aqueous ethanol. Samples killed in boiling 80% (v/v) aqueous ethanol were stored at 4°C.

Separation of sugars from insoluble material

The ethanol used to quench the sample was decanted and retained. Each sample was homogenized to a fine suspension with a pestle and mortar. The homogenate was extracted with 80% (v/v) aqueous ethanol (Stitt et al., 1978). All the ethanolic extracts were combined and concentrated by vacuum evaporation at 28°C. The residue was recovered in 5 ml of distilled water and stored at 20°C. The ethanol-insoluble material (containing starch) was homogenized with an all-glass homogenizer and suspended in 20 ml of distilled water. All the samples were frozen.

Assays

The contents of sucrose, glucose and fructose were determined by following the reduction of NADP⁺ at 340 nm (Jones et al., 1977) using a Milton Roy model 301 spectrophotometer. The reliability of the assays was always checked by adding 10- μ L aliquots of authentic glucose or fructose (1 mM) to the cuvette on completion of the sample assays. Starch in the insoluble fraction was determined after a 2-h digestion with amyloglucosidase (EC 3.2.1.3) from *Aspergillus niger* according to the method of Stitt et al. (1978). For assay of starch in the seeds, separate seed samples were pulverized using mortar and pestle prior to digestion with amyloglucosidase as described above.

Statistical analysis

Data were subjected to one way analysis of variance. Significant differences between data points were designated according to the least significant difference approach.

RESULTS

Sucrose profile in mature stem tissue from different accessions of sweet sorghum

Since the production of bioethanol from cane crops is largely dependent upon their sucrose yield (Moreira, 2000; Chohan et al., 2011), the first experiment under this study investigated amounts of sucrose in mature stems of sweet sorghum. Tissue samples for this analysis were obtained from internode positions as follows; second internode from the top, middle internode and second internode from the base. Similar tissue samples from mature sugarcane stem were analysed for comparison (Table 1). Marked differences in amounts of sucrose were observed between the sweet sorghum accessions. Four of the accessions (971 to 974) showed different but high amounts of sucrose in all the sampled stem positions. Of these, there were significant differences in the top internode sucrose amounts between accessions 972 and 973 as a pair and between

Table 1. Comparative amounts of sucrose (mg/g FW) in mature stems of different accessions of sweet sorghum and sugarcane.

Accession code	Internode position		
	Top	Middle	Base
971	78 ± 6 ^b	74 ± 8 ^b	45 ± 5 ^b
972	108 ± 12 ^a	85 ± 14 ^b	39 ± 5 ^b
973	114 ± 10 ^a	114 ± 5 ^a	67 ± 10 ^a
974	99 ± 14 ^b	81 ± 4 ^b	31 ± 2 ^c
975	22 ± 7 ^d	12 ± 2 ^c	5 ± 1 ^d
976	39 ± 2 ^c	16 ± 4 ^c	6 ± 2 ^d
977	19 ± 4 ^d	11 ± 4 ^c	5 ± 1 ^d
978	17 ± 2 ^d	7 ± 2 ^d	3 ± 1 ^d
Sugarcane	170 ± 8	168 ± 12	177 ± 4

Each table value represents mean ± S.E.M., n=3. Values followed by different letter(s) within columns are statistically different (p < 0.05).

Table 2. Comparative amounts (mg/g FW) of hexose sugars in mature stems of different accessions of sweet sorghum and sugarcane (samples were obtained from the top, middle and bottom internodes).

Accession code	Internode position					
	Top		Middle		Base	
	Glucose	Fructose	Glucose	Fructose	Glucose	Fructose
971	54 ± 6 ^a	34 ± 4 ^a	42 ± 6 ^a	28 ± 2 ^a	51 ± 6 ^a	29 ± 2 ^a
972	32 ± 3 ^b	19 ± 3 ^b	27 ± 6 ^{bc}	27 ± 5 ^a	37 ± 6 ^b	23 ± 7 ^a
973	16 ± 5 ^c	15 ± 2 ^b	21 ± 6 ^c	20 ± 3 ^a	33 ± 6 ^b	15 ± 2 ^b
974	26 ± 5 ^{bc}	15 ± 2 ^b	36 ± 6 ^{ab}	25 ± 3 ^a	48 ± 6 ^a	32 ± 3 ^a
975	26 ± 8 ^{bc}	28 ± 9 ^a	22 ± 5 ^c	24 ± 7 ^a	18 ± 4 ^c	21 ± 4 ^a
976	30 ± 4 ^b	34 ± 4 ^a	31 ± 4 ^{ab}	32 ± 6 ^a	40 ± 2 ^a	27 ± 7 ^a
977	23 ^{bc} ± 4	22 ± 2 ^a	26 ± 5 ^c	25 ± 2 ^a	32 ± 5 ^b	27 ± 4 ^a
978	22 ^{bc} ± 4	23 ± 2 ^a	26 ± 4 ^c	26 ± 3 ^a	28 ± 4 ^b	26 ± 4 ^a
Sugarcane	9 ^d ± 3	9 ± 3 ^c	10 ± 3 ^d	11 ± 3 ^b	10 ± 5 ^d	9 ± 5 ^c

Each row of values in the table represents mean ± S.E.M., for glucose and fructose, n = 3 for sweet sorghum and, n=4 for sugarcane. Values followed by the same letter within the same column are not significantly different (P = <0.05).

accessions 971 and 974 as another pair (Table 1). The highest amounts of sucrose (8 to 11% of the fresh weight of tissue) were recorded for uppermost internodes of these four accessions.

In terms of sucrose amounts at different stem positions, all the accessions showed consistently decreasing amounts of sucrose from top internode to basal internode (Table 1). These were significantly different for each accession. In contrast to sweet sorghum, no significant differences in amounts of sucrose were observed in the sampled tissue sections of mature sugarcane stem.

Quality of juice derived from the stems of sweet sorghum

Since the method used to extract sucrose from plant

tissue also extracts invert sugars (glucose and fructose), it was deemed necessary to explore the quality of juice derived from sweet sorghum in terms of invert sugar levels. This was important because data on invert sugar profiles may provide a reasonable basis for explaining the differences in the patterns of sucrose accumulation between the sweet sorghum accessions and sugarcane (Table 1). With the exception of accessions 971 and 975, most accessions showed high amounts of glucose and fructose in internodes from the base of the stem than in internodes nearest to the panicle (top internode) of sweet sorghum (Table 2). Accession 971 showed comparable hexose sugar content between the same internode positions, whereas accession 975 had the lowest hexose sugar content in the internodes at the base of the stem. Based on Fisher's LSD method of analysis, a greater degree of significant difference in invert sugar amounts was observed for glucose than fructose between the

Table 3. Comparative amounts of starch (mg/g FW) in mature stems of different accessions of sweet sorghum and sugarcane.

Accession code	Internode position		
	Top	Middle	Base
971	8 ± 2 ^c	2 ± 1 ^b	2 ± 1 ^b
972	9 ± 2 ^c	5 ± 2 ^b	1 ± 0 ^b
973	13 ± 1 ^b	4 ± 1 ^b	3 ± 0 ^b
974	7 ± 2 ^c	3 ± 0 ^b	1 ± 0 ^b
975	32 ± 13 ^a	13 ± 0 ^a	6 ± 0 ^a
976	35 ± 6 ^a	10 ± 0 ^a	6 ± 0 ^a
977	15 ± 3 ^b	9 ± 4 ^a	9 ± 3 ^a
978	19 ± 4 ^b	3 ± 0 ^b	3 ± 0 ^b
Sugarcane	nd	nd	nd

Each table value represents mean ± S.E.M., n=3. nd = Not determined; values followed by different letters within the same column are statistically different (p<0.05).

Table 4. Comparative amounts of starch (mg/g DW) in seeds of different accessions of sweet sorghum.

Accession code	Amount of starch	S.E.M.
971	230 ^c	2
972	261 ^{bc}	12
973	196 ^d	19
974	220 ^c	13
975	336 ^a	3
976	268 ^b	4
977	241 ^c	11
978	264 ^b	24

Each table value represents mean ± S.E.M. n=3. Values followed by the same letter(s) are not significantly different (p = < 0.05).

accessions of sweet sorghum (Table 2). In general, higher content of glucose than fructose was recorded for most of the sampled tissue sections from the stem of sweet sorghum. Sugarcane had contrastingly low and comparable quantities of these sugars along the stem (Table 2).

An explanation for the differences in invert sugar amounts in the sweet sorghum stem was sought by assaying starch in the same tissues of sweet sorghum sampled for soluble sugar analysis. The results (Table 3) revealed the presence of starch in the stem of sweet sorghum. The pattern of the starch accumulated in the sampled tissue sections of sweet sorghum was generally similar to that of sucrose in the same tissue samples (Table 1). Also, sweet sorghum accessions that accumulated less sucrose in the stem tended to accumulate more starch than those that accumulated more sucrose in the same tissue.

Starch accumulation in seeds of different accessions of sweet sorghum

To assess whether starch accumulation in the seeds of

sweet sorghum has any bearing on the sucrose and hexose sugar accumulation pattern in the stem of sweet sorghum (Tables 1 and 2), starch was assayed in the seeds. These were obtained from sweet sorghum plants used for soluble sugar analysis. The results (Table 4) show that different accessions of sweet sorghum accumulate different amounts of starch in the seeds as indeed sucrose in their stems. Accessions that accumulate low amounts of sucrose tended to accumulate more starch in the seeds.

DISCUSSION

The primary objective of this study was to assess the potential of sweet sorghum as a feedstock for fermentable substrate production under semi-arid conditions. With this in mind, the first experiment of this study determined the sucrose accumulation potential of sweet sorghum. Sugarcane was included as positive control. Tissue samples for this analysis were obtained from internode positions from; the top, middle and at base of the stems of these respective crops. This was

important because sucrose is the predominant long-distance transport sugar in many grasses including sugarcane (Murray et al., 2008) which is a C4 grass species like sweet sorghum. Also, production of bioethanol from cane crops is largely dependent upon the sucrose obtained from their stems.

Sucrose was the predominant sugar in all the accessions of sweet sorghum as well as in sugarcane (Table 1). The results (Table 1) also showed contrasting patterns of sucrose accumulation between sweet sorghum and sugarcane. In sweet sorghum, sucrose was more concentrated in the upper parts of the stem. Amounts of sucrose in the top and lower parts of the stem of sweet sorghum were significantly different ($P \leq 0.001$), while no significant difference existed between amounts of this sugar along the mature stem of sugarcane. The most promising sucrose-accumulating accession of sweet sorghum (no 973) had amounts of sucrose accounting for 11% of the fresh weight of tissue in the top and middle internodes and 8% of the fresh weight of tissue in the basal internodes. These amounts contrast with 17 and 14% of the fresh weight of tissue from the respective top and middle internodes of sugarcane. Since sweet sorghum matures in 4 to 5 months while sugarcane reaches maturity in 14 to 16 months, the results shown here underlie a strong agro-industrial potential of sweet sorghum even under semi arid conditions. This is so because with supplementary irrigation, two crops can be produced within a twelve months cycle. In addition, the data (Table 1) lend support to the proposition that physiological mechanisms of sugar accumulation may differ between sorghum and sugarcane (Tarpley and Vietor, 2007).

The variation in the pattern of sucrose accumulation observed for sweet sorghum (Table 1) is not an isolated case. Similar observations have been reported from other studies with sweet sorghum (Lingle, 1987; Hoffman-Thoma et al., 1996) and sugarcane (Lingle, 1997; Zhu et al., 1997). Although, no hypothesis was proposed to explain observations made in these separate studies, interplay of activities of key enzymes of sucrose metabolism (Zhu et al., 1997) is likely to be the cause of such variation. The key enzymes of sucrose metabolism include sucrose phosphate synthase as well as sucrose synthase and invertase(s) (Sturm and Tang, 1999). The individual or combined activities of these enzymes can influence the sucrose status of sampled tissue. Indeed, the presence of invertase-mediated post-harvest catabolism of sucrose in sugarcane stems has been reported by Mao et al. (2006). This may be indicative of a wound response expression and activity pattern for invertase in sugarcane. However, this is not supported by the sugarcane data of the present study.

Currently, alcoholic fermentations based on sucrose are carried out by yeasts and the efficiency of such fermentations is reportedly sensitive to the sugar composition of the fermentation substrate (Phowchinda and Strehaiano, 1999). In view of this, the quality of juice derived from both sweet sorghum and sugarcane was

evaluated for invert sugar content (Table 2). This was also important because invert sugar levels are additional means of assessing the potential of sweet sorghum as a feedstock for biofuel production. This study has revealed the presence of high amounts of invert sugars in the stem of sweet sorghum at maturity. They ranged from 2 to 5 orders of magnitude above those present in sugarcane stem. This confirms the existence of metabolic differences between sweet sorghum and sugarcane as reported by Tarpley and Vietor (2007). In this study, amounts of glucose were generally higher than those of fructose at similar stem positions. An investigation of a potential source of additional glucose in the sweet sorghum samples revealed the presence of starch (Table 3) with a quantitative pattern similar to that of sucrose from the same tissue samples.

Taken together, the results in Tables 1, 2 and 3 suggest the existence of age-related mobilization of non-structural carbohydrates in mature stem tissues of sweet sorghum. Such phenomena may involve expression and or activation of sucrolytic enzyme(s) and α -glucan hydrolase(s). In view of the data presented herein (Tables 1 and 2), invertase(s) are likely to have the strongest influence on the observed pattern of sucrose and invert sugar accumulation in the stem of sweet sorghum. It is yet to be determined whether the proposed sucrolytic activity is mediated by a cell wall-bound or soluble acid invertase. Maturity-linked increase in activity of cell wall-bound invertase has been reported in sugarcane (Lontom et al., 2008), and this has a direct bearing on sucrose levels in the internodes. Since sweet sorghum and sugarcane belong the same clade (Calvino et al., 2008), and they both accumulate sucrose in their stems at maturity, it can be expected that they use similar gene products in sucrose metabolism. In view of this, studies of the expression patterns of key enzymes of sucrose catabolism in the stem of sweet sorghum are necessary.

Given that sweet sorghum is a species that bears fertile seeds, this study has also investigated the possibility of trade-offs (Murray et al., 2008) between sugar accumulation in the stem and starch deposition in the seeds. This was particularly important in view of the report (Yang and Zhang, 2006) that the grain filling process in cereals is supported by photosynthesis and remobilization of stored carbohydrates in the stem. The results (Table 4) indicate that starch accumulation in the seeds may not have a strong influence on fermentable carbohydrate accumulation in the stem. Murray et al. (2008) reached similar conclusions from their studies with Rio (sweet sorghum) and BTX623, a grain sorghum type. Generally, these findings suggest that starch in the seeds and sucrose in the stem may be semi-independent photoassimilate sinks in sweet sorghum. Nonetheless, it is conceivable that some of the non-structural carbohydrates accumulated in the stem of sweet sorghum are used to support starch synthesis in seeds (Tables 1, 2 and 3). Consistent with this, Lingle (1987) and Willenbrink et al. (1998) have independently

proposed from their respective studies with sweet sorghum and wheat that the water-soluble carbohydrates obtained from the stem at harvest may represent some of the carbohydrate that could not be used for storage reserve biosynthesis (in the seeds). However, wheat is a C3 plant and therefore innate differences in sucrose metabolism between wheat and sweet sorghum may exist.

Conclusion

Sweet sorghum has good agro-industrial potential, even for semi-arid climates. The differences in the overall nonstructural carbohydrate profiles in the sample of local accessions of sweet sorghum may be indicative of genetic differences between the accessions. Sucrose and starch appear to accumulate transiently and to different extents in these accessions. Comparatively, both sweet sorghum and sugarcane accumulate sucrose in the stem at maturity even though their partitioning of fixed carbon between non-structural carbohydrates is markedly different. Regardless of this, the results of this study demonstrate that sweet sorghum has a significant capacity to serve as a feedstock for bioethanol production. It is clear therefore that improvement of the sucrose yield of sweet sorghum is prerequisite for full agro-industrial development of this crop. However, there is need to elucidate the implications of starch accumulation (in the stem) on the sucrose accumulating potential in the same tissue. Also, strategic manipulation of one or more of the key enzymes of sucrose metabolism is needed for improved understanding of sucrose metabolism in this crop.

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