A multiple harvest cultivation strategy for ethanol production from sweet sorghum throughout the year in tropical ecosystems

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Abstract

Sweet sorghum is a sugar rich crop that has been considered an alternative feedstock to sugarcane for ethanol production. A full year production cycle is possible as sweet sorghum is capable of multiple harvests. In this work we present experimental results for stalk biomass and sugar productivities, individual sugar distribution at harvest, ethanol yield and productivity of several sweet sorghum varieties and three harvests, the first cut during the rainy period and two ratoon crops during the dry season. The fresh stalk biomass productivity was different among varieties and harvests. All varieties had a lower than expected first harvest productivity, short waterlogging periods during the rainy period might have been the cause, and Top 76-6 showed the best value, 118 Mg per ha. The stalk sugar content dropped drastically for the second ratoon crop which also had an atypical sugar distribution, sucrose content decreased and reducing sugars increased, a premature harvest might have been the possible cause. Umbrella and Top 76-6 had the highest sugar productivity around 10 Mg per ha year. Fructose was the only remaining sugar after fermentation. Top 76-6 showed the highest ethanol productivity of around 4,800 liters per ha year and the best overall process efficiency.

Key Words

Sweet sorghum; multiple harvests; stalk productivity; sugar productivity; ethanol productivity

1. Introduction

Sweet sorghum is a sugar rich crop that has been considered an alternative feedstock to sugarcane for first generation ethanol production in tropical countries, due to its efficient C4-photosynthesis, short production cycle, nitrogen and water use efficiency, high tolerance to environmental stress and adaptability to marginal lands [1-5]. Two alternative scenarios have been considered previously for sweet sorghum planting in the tropics.

The first alternative contemplates a sweet sorghum harvest management in order to fill the idle time in the year that ethanol plants processing sugarcane byproducts have [6,7]. For example, in Central America sugarcane is harvested from middle November to late April, the period in which sugar factories supply molasses to ethanol distilleries. However, ethanol distilleries could operate all year if sweet sorghum harvests could be programmed from May to October supplying sweet sorghum juice for fermentation.

The second alternative centers on a full year production cycle as sweet sorghum in the tropics is capable of two ratoon crops as we showed recently [8]. Ratooning is a harvesting technique that leaves uncut the lower parts of the plant, from which the ratoon crop develops. Employing such method the costs associated with soil preparation and seed planting decrease. For many tropical countries this alternative could foster the much needed economic development of marginal land away from sugarcane plantations. It must be noted that sweet sorghum presents an additional advantage over sugarcane as a biofuel feedstock due to its multiproduct crop characteristic, that is, a high sugar productivity and grain production with adequate nutritional characteristics [9].

However in order to achieve an optimum full year production cycle questions regarding the proper time to sow remain. In our previous publication [8] the sweet sorghum varieties, Della, M81-E, Sugar Drip, Top 76-6, and Umbrella, planted in our South Campus located in the Guatemalan Pacific lowlands in 2011 and 2012, sowed in the middle of January and harvested in April during the dry season, with ratoon crops harvested in July and November during the rainy period, showed a significant decrease in stalk biomass productivity for the ratoon crops. Instead of such production scheme, sowing could be done during the rainy season period which runs

from May to November in order to have ratoon harvests during the dry season expecting with this change to increase the ratoon crops stalk biomass productivity.

In the present work we present experimental results for stalk biomass productivity, stalk sugar content, sugar productivity, and individual sugar distribution at harvest, ethanol yield and ethanol productivity for first cut, first and second ratoon crops of the sweet sorghum varieties, Della, M81-E, Sugar Drip, Top 76-6, and Umbrella, sowed in July during the rainy season with harvests in October, and ratoon crops in February and April during the dry season.

2. Materials and Methods

2.1 Experimental site and sweet sorghum planting

The experimental plot was located in our South Campus located in the Guatemalan Pacific lowlands at Santa Lucia Cotzumalguapa, Escuintla at 300 meters above sea level. The soil was an Andisol rich in volcanic material, with a pH of 5.6 and 227, 160 and < 9 mg/kg of total nitrogen, potassium and total phosphorus, respectively. The sweet sorghum varieties tested were Della, M81-E, Sugar Drip, Top 76-6, and Umbrella. The last two varieties had shown before higher stalk biomass and sugar productivities and grain with adequate nutritional characteristics, M81-E had consistently shown high stalk biomass productivity, and the other two varieties were in between (8,9). Sowing was done in early July 2013 and the first harvest took place in late October after 115 days, the plant development period took place entirely during the rainy season as shown in Figure 1. On the other hand, the ratoon crops took place during the dry season; the first ratoon was harvested in early February 2014 after 109 days, and the second ratoon was harvested in late May after 107 days at the start of the rainy period. In all cases harvest time was established by testing manually the grain hardness until the soft dough stage was reached. The monthly average, maximum and minimum temperatures and precipitation are shown in Figure 1. A randomized complete block with three replications was used as the experimental design with plant density the same as the one employed previously at the same site [8,9]. The fertilization employed for the first cut and the two ratoon crops was 100 kgN per ha, applied as follows, one third two weeks after sowing, one third after 1 month, and one third after 2 months. Also, 50 kg P₂O₅ and 50 kg K₂O per ha were incorporated at the first application. No irrigation was done during the first harvest as it took place when the rainy season started. Furrow irrigation was

implemented three times weekly late in the first ratoon crop and early in the second ratoon crop, when there was lower precipitation as shown in Figure 1.

2.2 Stalk sampling and handling

The stalk biomass average productivity was obtained by harvesting the two central rows of four sorghum rows cut manually at ground level for each experimental unit, cleaning and weighing the stalks, removing the top and repeating the procedure for each replication. Each time for every variety a sub-sample was taken consisting of 12 to 15 stalks chosen randomly from the three field replications and were sent on the same day to the laboratory in our central campus. They were stored at -10 °C until processed. The twelve to fifteen thawed stalks for each variety and each harvest were processed as follows: the second to four internodes, counting from the bottom, of two stalks chosen randomly were cut employing a small hand saw. The stalk segments were reduced into small pieces with a knife. These were further pulverized employing a laboratory high-speed cutting mill (*IKA Works A11*). A known amount of solid particles as described below was used for fermentation purposes. Another amount, about 5 g, of the pulverized material was placed in an oven at 65 °C (*Fisher Scientific Isotemp Incubator*) until constant weight for moisture determination. The rest of the stems, 10-13 stalks, were pressed employing a stainless steel pilot three roll crushing mill (*Vencedora Magtron Model 721*) with a 2-HP motor. Juice Brix was measured with a digital refractometer (*Model 300034 Sper Scientific Ltd*) and sugar analysis was carried out as described below.

2.3 Yeast growth, culture media and ethanol production.

The yeast employed was a commercial *Saccharomyces cerevisiae* strain used for years by Guatemalan distilleries and adapted to sugar cane molasses. Yeast was grown in a 30-g/L Sabouraud broth (Merck, 2 % glucose, 0.5 % animal peptone, and 0.5 % casein peptone) plus 1 % additional sucrose. One hundred and twenty-five milliliters of broth was added into a 250-mL flask, sterilized at 121 °C for 20 min, cooled, inoculated, and agitated at 250 rpm at 30 °C for 48 h (Incubator Shaker Lab Companion Model SI-600). The suspension was centrifuged at 1,600g, 10°C for 5 min (Eppendorf Table-top Refrigerated Centrifuge Model 5804R). The solid pellet was suspended in deionized water and the optical density adjusted to 1.2 which corresponded to approximately 1.0 g dry yeast/L. A volume of 100 mL of the suspension was used to inoculate

approximately 30 g of pulverized sweet sorghum placed in a flask. The average amount of yeast employed was 20 g of dry yeast/kg of dried sorghum particles. The flasks were kept at 30 °C for 36 h. Each sorghum variety was run in duplicate. The solid water proportions inside the flask assured that all particles were submerged in order to facilitate the simultaneous sugar extraction and fermentation. Employing this technique we avoided the cumbersome and incomplete sugar extraction achieved by stalk pressing.

2.4 Analytical determinations

Sugars in the pressed sweet sorghum juice and in the filtrate from the flasks at the end of the fermentation were determined with an Agilent 1100 high-pressure liquid chromatograph, an Agilent 1200 refractive index detector, and a Zorbax NH2, 25 cm long and 4.6 mm internal diameter column, employing acetonitrile in water (70-30) as the solvent phase. Ethanol was quantified employing an Agilent 6890N gas chromatograph, with an HPPlot/ Q, 30 m long and 32 mm internal diameter column.

2.5 Mathematical analysis

Two-way analysis of variance was used with the fresh stalk biomass productivity data using it as dependent variable and harvest and variety as independent factors. On the other hand the stalk sugar content and ethanol production data were analyzed employing one-way analysis of variance and Bonferroni corrections for multiple comparisons. The analysis was done for each variety separately having the data as dependent variable and the harvest as independent factor. This methodology was employed as the experimental data was obtained by analyzing two sub-samples of a composite sample of pulverized material from each variety. Both analyses of variance were done employing Stata version 9.2. The sugar distribution data was used to identify similar groups of samples employing principal components using MVSP Plus version 3.1.

3 Results

3.1 Fresh stalk biomass productivity

The fresh stalk biomass productivity data for the five sweet sorghum varieties and the three harvests for each experimental unit are shown in Table 1. The two way analysis of variance summary is given in Table 2. It is clearly seen that a) the two independent factors harvest and

variety and their interaction were significant, and b) in all varieties lower first harvest productivity was observed. Top 76-6 led the group in total biomass productivity with about 118 Mg per ha for the three harvests; Sugar Drip showed the lowest figure close to 79 Mg per ha.

3.2 Stalk sugar content and productivity and individual sugar distribution

The average stalk sugar content at harvest on wet basis of two sub-samples of pulverized sweet sorghum stalk is shown in Table 3 for each sweet sorghum variety and for the three harvests. The average stalk moisture among varieties at harvest did not change drastically, it was $75.87 \% \pm 3.91$, 74.88 ± 3.91 and 77.49 ± 5.93 for the first harvest and first and second ratoon crops respectively, so that expressing the sugar content on wet basis, which is commonly done, presents no problem. Table 4 lists the Bonferroni adjusted values of the difference among the means. It can be seen that the difference was significant, that is below the 5.0 % level, between the first and the second ratoon crops, with the exception of Umbrella in which the difference was above this level. The difference between the first cut and the first ratoon was not significant, with the exception of M81-E in which the difference was significant at the 0.1 % level. Looking at the average values for all varieties in the last column of Table 3 it can be concluded that the sugar content tended to drop drastically for the second ratoon crop.

Total sugar productivity was calculated from the fresh stalk yield and the fresh stalk sugar content data. Sugar productivity increased for the first ratoon crop and decreased drastically for the second ratoon crop. The reason being that for the second ratoon crop the stalk sugar content was much lower than the first cut and ratoon crops. Umbrella had the highest sugar productivity, 10.16 Mg per ha per year, followed by Top 76-6, 9.57 Mg per ha per year.

The individual sugar distribution data are shown in Table 5. With the exception of Umbrella the rest of sweet sorghum varieties had an atypical sugar distribution for the second ratoon crop. The sucrose content decreased and the reducing sugars increased.

We analyzed our individual sugar distribution data by principal components in order to identify groups according to the following indicators: a) sucrose, glucose and fructose proportions, b) the ratio between glucose and fructose, c) the ratio between reducing sugars and sucrose, and d) total sugar content. The results are shown in Figure 2. It is easily seen that the first principal component PC1, or horizontal axis, explaining 96 % of the total variation, is able to separate and

form a different group for second ratoon samples of four of the five sweet sorghum varieties, with exception of Umbrella, which make them different from the rest.

Figure 3 shows the relationship between stalk sugar content and the ratio between reducing sugars and sucrose. It is observed that for first cut samples there was a linear inverse relationship between variables, meaning that a lower ratio induced higher sugar content. The linear trend was weaker for ratoon samples.

3.3 Ethanol fermentation

The average ethanol production data employing simultaneous sugar extraction and fermentation of two sub-samples of pulverized sweet sorghum stalk are presented in Table 6. The second column lists for all sweet sorghum varieties and different harvests the experimental values expressed as g of ethanol per kg of initial dry solid particles. Umbrella and Top 76-6 produced more ethanol than the other three varieties. Table 7 lists the Bonferroni adjusted values of the difference among the means. It can be seen that the difference was not significant with the exception of: a) the first and second ratoon crop for Sugar Drip which was significant at the 3.8 % level, and b) the first cut with the second ratoon at the 2.9 % level and the first ratoon with the second ratoon at the 3.6 % level for Top 76-6.

We have also calculated the ethanol yield in terms of g of ethanol produced per g of sugar utilized. Such estimation needs to include in the calculations the extent of sugar extraction from the solid particles. This was done as follows, we estimated for both duplicates of each fermented sample the maximum ethanol yield that could be obtained for each sweet sorghum variety, taking into account a) its initial sugar content, b) a total sugar extraction from the solid particles, and c) 0.51 as the theoretical amount of ethanol produced from hexoses by yeast. The results of this calculation are listed in the third column in Table 6 which give for each variety and harvest the maximum amount of ethanol in g of ethanol per kg of initial dry solid particles. The fourth column in Table 6 lists, as %, the experimental ethanol value divided by the calculated maximum ethanol amount. We can see that higher values of simultaneous sugar extraction and fermentation were obtained with Top 76-6. The overall process efficiency for the first cut was close to 90 % of the maximum value and decreased to about 76 % for the ratoon crops. The extracted sugar was not fermented completely, consumption was $95.29\% \pm 1.32$, $93.55\% \pm 5.92$ and $86.84\% \pm 4.98$

for the first cut, first and second ratoon crops respectively, and significantly the remaining sugar after fermentation was essentially fructose.

Ethanol productivity data are presented in Figure 4. The low productivity obtained in the first cut was due to the poor stalk biomass produced per ha, and in the second ratoon was due to the low stalk sugar content. Top 76-6 gave the best annual productivity with 4,837 liters per ha, of which 64 % was obtained in the first ratoon crop.

4. Discussion

4.1 Fresh stalk biomass productivity

The experiments were done in order to find out the proper time to sow in order to achieve an optimum full year production cycle by having multiple harvests. In our previous publication [8] the same varieties at the same site planted in 2011 and 2012 were sowed in the middle of January with harvest in April all in the dry season, and ratoon crops in July and November during the rainy season. In the present article we planned ratoon crops during the dry season, hence the planting schedule was modified and sowing was done in July during the rainy season with harvests in October, and ratoon crops in February and May during the dry season. A summary of the planting schemes is shown in Table 8 including a qualitative appraisal of the stalk biomass productivity results.

It can be observed for both experiments that a lower than expected fresh stalk biomass productivity was obtained when plant development and harvest took place during the rainy season, irrespective if it was the first harvest or the ratoon crops. Other factors might have been involved associated with a precipitation increase during this period: a) an increase in daily cloud cover affecting solar radiation intensity and b) a more prevalent plant damage by soil microbial pathogens and pests.

Nevertheless, precipitation was a significant possible cause for this effect. Heavy precipitation in short tropical rain storms accompanied by heavy wind gusts were common during the rainy season on the experimental site. However extended waterlogging for several days or weeks did not occur as the soil exhibited adequate drainage, but short periods of soil waterlogging did indeed take place frequently. In general, the negative effect observed caused a thinner and shorter stalk although no data was taken on stalk measurements.

It is known that sweet sorghum development is affected by different soil moisture regimes [10] although most research done has focused on scarce water availability or drought conditions [11-17]. However, two review articles comment in general on the negative effects of waterlogging effects on the agricultural productivity of sorghum [18-19]. On the other hand, a study [20] investigated the effect of waterlogging in pots for 20 days on Wray, Keller and Bailey sweet sorghum cultivars and forage sorghum hybrid. The extensive flooding reduced plant height, leaf area, photosynthetic and transpiration rates. In a second study [21] flooding was induced in Wray, a sweet sorghum variety, and a multipurpose sorghum cultivar at three plant development stages, early vegetative, early reproductive and mid reproductive stages. Plant height, stem diameter, leaf area and dry weight, shoot dry weight, primary root length and root dry weight of both cultivars were significantly reduced by flooding at the early vegetative and reproductive stages, but not at the mid reproductive stage. At harvest, flooding at the early stages reduced the stalk yield of both cultivars. A third study [22] found that leaf dry weight and area, number of node per stalk, shoot dry weight, and stalk yield were reduced for Wray, Keller and Bailey sweet sorghum cultivars subjected to flooding after 30 days of plant emergence, but not later on. Such findings showed that the waterlogging negative effect on sweet sorghum plant productivity depends on the growth stage that it takes place, although no detailed discussion was provided related to the flooding time period. It has been known for some time that sweet sorghum recovers from short waterlogging conditions [23] fact that was experienced also for the sweet sorghum Wray variety in Indonesia [24]. The lower than expected first harvest fresh stalk biomass productivity that we observed in this experiment might have been caused then by short waterlogging periods.

4.2 Stalk sugar content, productivity and individual sugar distribution

There was no significant difference between the stalk sugar content in the first cut and first ratoon crop with the exception of M81-E, see Table 4. Similar results have been published by Erickson et al [25] for three sweet sorghum cultivars planted in Florida and found that sugar content, measured as juice Brix values, for all cultivars was similar for the first cut and ratoon crop. However other authors have reported opposite results, Han et al [26] for three sweet sorghum varieties planted in Louisiana found for the ratoon crop a drop of about 2-6 Brix units in the pressed juice compared to the first harvest. Wang et al [27] reported variable results for

sweet sorghum hybrid cultivars planted in Lubbock, Texas, in most of them the relationship between total sugar and dry mass increased, however there were a few where the inverse happened. It seems then that an increase or a decrease will depend on the variety and the plantation site.

The stalk sugar content decreased significantly for the second ratoon crop. We found the same result in our previous work [8] as indicated in Table 8. It seems then that the sugar drop happens irrespective of the time in the year that the second ratoon crop takes place, either under dry weather or during the rainy season, so there is really no evidence of any effect from an environmental variable like excess precipitation causing short time flooding. We do not have a clear explanation for this drastic sugar decrease and unfortunately, as known to us, there is no similar data available in the literature to compare it. The second ratoon crop developed normally with an acceptable stalk biomass yield. As discussed below a physiological effect seemed more possible.

Sugar accumulation in the stems is regulated by a system of enzymes as discussed recently [28,29]. It seems that a low soluble acid invertase (SAI) activity coupled with a higher sucrose phosphate synthase (SPS) can promote higher sugar contents in the stem. A significant linear correlation was found between glucose plus fructose contents and SAI activity for various cultivars, also between sucrose content and the difference in activities between SPS and SAI. We indeed found a linear inverse relationship between stalk sugar content and the amount of reducing sugars at harvest as shown in Figure 3.

High or low sucrose content at harvest might imply high or low total sugar content. The second ratoon crop had the shortest development cycle being harvested after only 107 days, time on which the grain reached the soft dough stage. It is possible then that the second ratoon crop required more time to reach full maturation. Figure 2 shows that this group of samples was different from the other two groups, the first cut and the first ratoon samples, in terms of their individual sugar contents.

The sweet sorghum production scheme consisting of various harvests per year, either one or two ratoon crops, can be compared in terms of sugar productivity with the experiments by Han et al [26] with sweet sorghum varieties planted in Louisiana and including one ratoon crop, an average value of approximately 9.6 Mg per ha per year can be calculated from their data. This figure is similar to our present results, for example, Umbrella and Top 76-6 with two ratoon

crops had sugar productivities of approximately 10.2 and 9.6 Mg per ha per year respectively. In our previous publication [8] the same varieties at the same site planted during the dry season in 2011 and 2012 with two ratoon crops showed the highest productivity also, however, the total sugar productivity then was below 9 Mg per ha per year. On the other hand, the year-round scheme for sweet sorghum can also be compared with commercial sugarcane production in Brazil. Recent typical figures for this country are a fresh stalk productivity of 85 Mg per ha per year with 14.5 % sugar content, which gives 12.32 Mg of sugar per ha per year [30]. We believe that this productivity can be reached with sweet sorghum, specifically with Top76-6 and Umbrella, by further experimentation.

All of these results suggest that ratoon crops have to be managed differently from first cuts. Ratoon crops might need more time in the field in order to reach maximum sucrose content and higher total sugars. Harvest time should not be determined by grain maturity characteristics as it is usually done for first cuts, but instead, a juice sample from the stem should be taken for each variety and sugar analysis should be performed. It is possible then that harvesting the second ratoon crop prematurely determined only by grain maturity characteristics might have caused the low sugar content data and the atypical sugar distribution found.

4.3 Ethanol fermentation

The use of g of ethanol per kg of initial dry solid particles to express ethanol production has been commonly used in solid substrate fermentation of sweet sorghum particles [31-36] and is also useful in solid suspended fermentations where sugar is extracted from the solid particles and fermented by yeast in simultaneous fashion [8,9]. Results on these units can be compared to the corresponding figure obtained from a reference sweet sorghum cultivar containing 500 g of fermentable hexoses and 500 g of structural carbohydrates per kg of dry stalk. Employing the theoretical ethanol yield of 0.51 g of ethanol per g of hexose, the maximum yield from the hexoses gives 255 g of ethanol per kg of dry matter. Our calculations for the maximum yield, showed in the third column of Table 6 were most below this figure; however the first cut and first ratoon for Sugar Drip and Umbrella and the first ratoon for Top 76-6 were in the range of 204 to 260 g of ethanol per kg of dry matter. On the other hand the overall sugar extraction and conversion to ethanol data, showed in the fourth column of Table 6, indicated that only for M81-E and Top 76-6 first cuts the efficiency was above 85 % of the maximum possible.

The final ethanol content in the flask was low enough to avoid yeast inhibition, hence, the differences in ethanol production found among sweet sorghum varieties might have originated either from inhibitors in certain sweet sorghum varieties or by differences in the lignocellulosic fraction structure and chemical composition for each variety which might influenced sugar extraction rates and extent [37]. The possible fermentation inhibitors that have been mentioned are organic acids: aconitic acid which is present in sweet sorghum juice and lactic and acetic acids which are microbial products of homo- and hetero-bacteria active during handling and storage of sweet sorghum stalks or billets [38]. Aconitic acid is metabolically produced during plant development and decreases at harvest [39]. There is limited information, according to our knowledge, on the aconitic acid content of different varieties and hybrids, a recent article [40] reports a value of 18 g/L in sweet sorghum juice. Klasson [41] has published some details on the inhibition in experiments employing 80 g/L sweet sorghum juice in fermentations with the addition of acetic, lactic and aconitic acids. He found inhibition in fermentations containing above 2 g/L of acetic acid. Lower levels and all levels of lactic and aconitic acids (1-5 g/L) were not detrimental to ethanol production. However, higher levels of aconitic acid are possible in raw sweet sorghum juice [40] and it could become critical in concentrated juice and VHG, very high gravity, fermentations. The undissociated acids seem to be the main culprits [41].

The individual sugar variation among varieties might not be a factor influencing ethanol production, as it is well known that glucose is a preferred uptake substrate for yeasts over fructose and sucrose [42]. Sucrose is hydrolyzed by invertase in the periplasmic space of the yeast cell, which is the space between the cell wall and the cytoplasmic membrane, into glucose and fructose, which then enter the cell by facilitated diffusion [43, 44]. Ethanol producing yeasts also produce extracellular invertases which usually are subjected to glucose repression and the liberated glucose and fructose in the fermentation broth are eventually transported into the cell by the same transporters. In a mixture of sucrose, glucose and fructose, for many types of yeast, glucose will be consumed first, then sucrose and finally fructose [44]. In our fermentations fructose was the only sugar remaining suggesting a relatively slower transport into the cell. This fact has also been reported before for sweet sorghum fermentations [45].

The best annual productivity was shown by Top 76-6 with 4,837 liters per ha, of those 718 were for the first cut, and 3,116 and 1,003 for the first and second ratoon crops respectively.

With sugarcane in Brazil current figures estimate around 7,580 liters of ethanol per ha per year [30].

5. Conclusion

The planting scheme of sowing and harvesting in the rainy season and ratoon crops during the dry season induced in five sweet sorghum varieties the following results. The fresh stalk biomass productivity was different among varieties and harvests. All varieties had a lower than expected first harvest productivity and Top 76-6 showed the best value, 118 Mg per ha for the three harvests. Short waterlogging periods during the rainy period might have been the cause. The stalk sugar content dropped drastically for the second ratoon crop in all varieties and with the exception of Umbrella, they also had an atypical sugar distribution, as sucrose content decreased and reducing sugars increased. A premature harvest of the second ratoon crop might have been the possible cause. More ethanol was extracted and fermented simultaneously in pulverized stalk samples of Umbrella and Top 76-6. The latter variety showed the best overall process efficiency, close to 90 % for the first cut and about 76 % for the ratoon crops, and the best annual productivity with 4,837 liters per ha. Fructose was the only remaining sugar after fermentation. Fresh stalk biomass, sugar and ethanol productivities in the present planting scheme were improved over those previously obtained [8] with the same varieties at the same site in which sowing and harvesting was done in the dry season and the ratoon crops developed during the rainy period. However, the unexpected low sugar content of the second ratoon crop in our present experiment suggests that, at least in the tropics, only one ratoon crop might be advisable.

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Table 1. Fresh stalk biomass productivity in Mg per ha

	Della	M81-E	Sugar Drip	Top 76-6	Umbrella	
First cut	28.98 ± 3.01	30.97 ± 7.27	16.48 ± 6.46	11.93 ± 1.39	17.05 ± 4.25	21.08
First ratoon	37.50 ± 3.83	36.65 ± 6.39	28.41 ± 4.25	57.39 ± 4.59	46.31 ± 3.52	41.25
Second ratoon	44.89 ± 5.29	35.51 ± 2.52	34.09 ± 3.59	48.58 ± 4.58	50.00 ± 3.35	42.61
Total per year	111.36	103.13	78.98	117.90	113.35	

Table 2. Anova results for the stalk biomass productivity data

	Partial SS	df	MS	F	Prob > F
Model	9759	14	697	33.72	0.0000
Harvest	5816	2	2908	140.67	0.0000
Variety	1276	4	319	15.43	0.0000
Harvest* Variety	2667	8	333	16.13	0.0000
Residual	930	45	21		
Total	10689	59	181		

Table 3. Fresh stalk total sugar content % on wet basis (g of sugars/100g of wet stalk)

	Della	M81-E	Sugar Drip	Top76-6	Umbrella	Average
First cut	7.54 ± 0.89	7.38 ± 0.22	7.62 ± 1.73	10.36 ± 0.28	9.76 ± 2.22	8.53
First ratoon	10.03 ± 0.05	10.59 ± 0.05	11.10 ± 0.37	10.88 ± 0.07	10.88 ± 0.07	10.70
Second ratoon	3.81 ± 0.27	2.61 ± 0.07	3.58 ± 0.79	4.15 ± 0.10	5.54 ± 0.30	3.94
Average	7.13	6.86	7.43	8.46	8.73	

Table 4. Bonferroni adjusted significance of the difference among means for the stalk sugar content data

	Della	
	First cut	First ratoon
First ratoon	0.056	
Second ratoon	0.018	0.004
M81-E		
First ratoon	0.001	
Second ratoon	0.000	0.020
Sugar Drip		
First ratoon	0.158	
Second ratoon	0.109	0.020
Top 76-6		
First ratoon	0.321	
Second ratoon	0.001	0.000
Umbrella		
First ratoon	1.000	
Second ratoon	0.142	0.078

Table 5. Individual sugar distribution as % of total sugar

	Sucrose	Glucose	Fructose
Della			
First cut	69.33 $\pm$ 3.14	18.56 $\pm$ 1.53	12.11 $\pm$ 1.61
First ratoon	61.94 $\pm$ 1.05	22.11 $\pm$ 0.40	15.95 $\pm$ 1.45
Second ratoon	46.58 $\pm$ 0.07	28.58 $\pm$ 0.04	24.83 $\pm$ 0.11
M81-E			
First cut	65.63 $\pm$ 0.30	22.47 $\pm$ 0.05	11.90 $\pm$ 0.05
First ratoon	80.06 $\pm$ 1.15	12.99 $\pm$ 0.49	6.95 $\pm$ 1.64
Second ratoon	22.30 $\pm$ 8.05	48.97 $\pm$ 5.25	28.74 $\pm$ 2.80
Sugar Drip			
First cut	64.16 $\pm$ 0.75	26.04 $\pm$ 1.98	9.80 $\pm$ 1.23
First ratoon	62.96 $\pm$ 3.25	26.52 $\pm$ 2.27	10.52 $\pm$ 0.98
Second ratoon	49.32 $\pm$ 0.08	33.48 $\pm$ 0.60	17.20 $\pm$ 0.69
Top 76-6			
First cut	79.17 $\pm$ 3.95	12.03 $\pm$ 2.15	8.80 $\pm$ 1.80
First ratoon	80.32 $\pm$ 0.84	11.73 $\pm$ 0.26	7.94 $\pm$ 0.58
Second ratoon	49.41 $\pm$ 1.20	32.51 $\pm$ 1.20	18.09 $\pm$ 0.01
Umbrella			
First cut	76.81 $\pm$ 4.17	17.38 $\pm$ 2.88	5.81 $\pm$ 1.28
First ratoon	59.29 $\pm$ 6.22	30.71 $\pm$ 3.15	10.00 $\pm$ 3.07
Second ratoon	70.65 $\pm$ 4.44	17.96 $\pm$ 3.30	11.39 $\pm$ 1.14

Table 6. Experimental ethanol production in g ethanol per kg of initial dry stalk particles and comparison with respect of the maximum value

Della			
	Experimental ethanol	Maximum ethanol	% of Maximum
First cut	71.94 ± 11.47	150.39 ± 7.77	48.10 ± 10.11
First ratoon	60.51 ± 3.25	189.20 ± 1.07	31.99 ± 1.90
Second ratoon	40.05 ± 6.12	74.18 ± 12.69	54.08 ± 1.00
M81-E			
First cut	141.08 ± 40.63	164.29 ± 6.88	86.47 ± 28.35
First ratoon	99.18 ± 0.89	194.87 ± 0.60	50.90 ± 0.61
Second ratoon	29.92 ± 6.76	47.60 ± 3.74	62.49 ± 9.28
Sugar Drip			
First cut	89.79 ± 23.07	208.49 ± 41.11	45.03 ± 19.44
First ratoon	148.56 ± 8.84	259.45 ± 0.47	57.26 ± 3.51
Second ratoon	59.20 ± 14.63	78.25 ± 22.54	76.13 ± 3.23
Top 76-6			
First cut	160.54 ± 11.36	179.62 ± 3.56	89.45 ± 8.09
First ratoon	155.06 ± 17.40	203.96 ± 9.06	75.91 ± 5.16
Second ratoon	89.08 ± 2.31	117.02 ± 12.15	76.43 ± 5.94
Umbrella			
First cut	138.17 ± 58.73	209.47 ± 69.84	64.89 ± 6.40
First ratoon	149.28 ± 47.34	257.80 ± 33.37	57.19 ± 10.96
Second ratoon	114.17 ± 1.17	195.22 ± 45.59	60.05 ± 13.42

Table 7. Bonferroni adjusted significance of the difference among means for the ethanol production data

	Della	
	First cut	First ratoon
First ratoon	0.708	
Second ratoon	0.078	0.232
M81-E		
First ratoon	0.529	
Second ratoon	0.056	0.186
Sugar Drip		
First ratoon	0.115	
Second ratoon	0.487	0.038
Top 76-6		
First ratoon	1.000	
Second ratoon	0.029	0.036
Umbrella		
First ratoon	1.000	
Second ratoon	1.000	1.000

Table 8. Summary of stalk biomass productivity, sugar content and distribution for the three harvests and comparison with previous work (8)

Month	January	February	April	July	October	November
Precipitation	Dry	Dry	Dry	Rain	Rain	Rain
Previous work	Sowing		Harvest	Ratoon harvest		Ratoon harvest
Stalk biomass productivity			Normal	Lower than expected		Lower than expected
Stalk sugar content			Normal	Normal		Lower than expected
Sugar distribution			Normal	Normal		Not determined
Present work		Ratoon harvest	Ratoon harvest	Sowing	Harvest	
Stalk biomass productivity		Normal	Normal		Lower than expected	
Stalk sugar content		Normal	Lower than expected		Normal	
Sugar distribution		Lower sucrose	Lower sucrose		Normal	

Figure 1. Average (line with circles), maximum (upper dashed line) and minimum (lower dashed line) temperatures during the yearly production cycle. Average monthly precipitation on the right vertical axis (dash dot line). The horizontal bars below indicate the plant cycle length for each harvest, the first bar, the first crop growth, the second bar, the first ratoon crop and the last bar, the second ratoon crop.

PCA case scores

PC2

PC1

■ First group ▼ First ratoon ● Second ratoon

Group	Case	PC1 (approx)	PC2 (approx)
First group (Blue Squares)	M	-13.4	-1.0
	D	-4.5	2.5
	U	5.5	-1.0
	T	6.5	1.5
First ratoon (Green Inverted Triangles)	M	-13.4	-1.0
	D	-4.5	2.5
	U	5.5	-1.0
	T	6.5	1.5
Second ratoon (Red Circles)	M	-13.4	-1.0
	D	-4.5	2.5
	U	5.5	-1.0
	T	6.5	1.5

Figure 3. Scatter diagram between the stalk sugar content in % on wet basis versus the ratio between reducing sugars over sucrose.

Figure 4. Ethanol productivity in liters per ha obtained from samples from the five sweet sorghum varieties and the three harvests done in the year

- **Three harvests were obtained per year for five sweet sorghum varieties:**

- ☐ First cut
- ☐ First ratoon
- ☐ Second ratoon

Simultaneous
sugar
extraction
& fermentation
to ethanol of
sweet sorghum
stalk particles

- ☐ The **Top 76-6** variety gave the highest fresh stalk biomass, sugar and ethanol productivities