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RESEARCH ARTICLE

Characterization of Improved Sweet Sorghum Genotypes for Biochemical Parameters, Sugar Yield and Its Attributes at Different Phenological Stages

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Abstract Sweet sorghum is a multipurpose biofuel feedstock that offers grain for human consumption, fodder for livestock and ethanol for transportation purposes. The knowledge on sugar components at different phenological stages of crop growth and identification of appropriate stage of harvesting is critical for sweet sorghum commercialization and value chain sustenance. In this regard, sweet sorghum stalk yield, juice yield, Brix%, pH, sugars (sucrose, fructose and glucose) and their content were analyzed at three different phenological stages i.e. the dough stage, physiological maturity and post-physiological maturity. Variations in sugar content at different growth stages revealed that the sugar yield was high at physiological maturity, but highest at post-physiological maturity. Sucrose accounts for major fermentable sugar (about 70%) and it sharply increased by 146% from dough stage to post-physiological maturity. The variation in the monosaccharides content (glucose and fructose) is not statistically significant. This study points to the potential scope for widening the harvesting window of sweet sorghum, by cutting the stalks from physiological maturity stage and beyond up to 15 days (post-physiological maturity), thus helping the commercial distilleries by addressing a major impediment in sweet sorghum value chain. The entries SP 4495, SP 4511-3 and SPV 422 are suitable for harvesting in a wider window of time as the sugar levels are sustained at same level from physiological maturity to post-physiological maturity.

Keywords Sweet sorghum · Genotypes · Sucrose · Glucose · Fructose · Brix%

The first three authors have contributed equally to this work.

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Introduction

Exploring the renewable energy from different sources is the focus of current research, as the present energy resources, i.e., fossil fuels, are rapidly declining. Plant biomass is one of promising renewable energy source that has been widely explored for biofuel production. It has low CO_2 emissions and its production cost is low (Antonopoulou et al. 2008). The predominant bio-ethanol feedstocks cultivated worldwide are sugarcane, maize, sweet sorghum, cassava and sugar beet (Srinivasa Rao et al. 2009). Sweet sorghum [*Sorghum bicolor* (L.) Moench] is a promising energy crop that can be cultivated worldwide under diverse agro-climatic conditions with a requirement for a relatively less nitrogen fertilizer and water when compared to sugarcane and maize and yields more ethanol per hectare per unit time (Geng et al. 1989; Reddy et al. 2005). Intensive research efforts are in progress in various countries viz., USA, China, India, Africa, Indonesia, Iran and Philippines in assessing the agro-industrial potential of sweet sorghum (Reddy et al. 2005, 2008; Ranola et al. 2007; Tsuchihashi and Goto 2008; Bennett and Anex 2009; Pillay and Da Silva 2009; Zhang et al. 2010).

Crop management suitable for climatic and soil conditions and cultivar choice depending on the location are important to attain higher stalk and juice yield in sweet sorghum. The increase in the sugar content of sorghum stalk will also enhance its palatability and the forage quality (Poehlman 1994; Blümmel et al. 2009). Therefore, sweetness along with juiciness and biomass are the important targets in sweet sorghum breeding. It's potential as a sugar source for bio-ethanol production at industrial scale has not been fully exploited owing to the availability of raw material for a limited period(s) in any given year resulting in the poor performance of commercial ethanol distilleries. It is therefore, necessary to increase the window of sweet stalk availability, which can either be achieved by breeding cultivars that mature at varying periods or by identifying cultivars that sustain high sugar levels over a longer period of time even after reaching maturity (Reddy et al. 2005; Srinivasa Rao et al. 2009). A few cultivars like Brandez, Wray and ME 84-1 have been identified to possess sustained sugar levels for a longer period (between 25 and 40 days) for industrial utilization in Brazil (Schaffert, EMBRAPA, personnel communication).

Studies aimed at determining hexoses at physiological maturity (Smith et al. 1987; Hunter and Anderson 1997; Almodares et al. 2008) established that sucrose is major component of sugars followed by glucose and fructose in sweet sorghum juice. To date there are no studies established on the flux of hexoses (glucose and fructose, the monosaccharides and sucrose, a disaccharide) at different phenological stages, i.e. at dough, physiological maturity and post-physiological maturity (15 days after physiological maturity) of the sorghum cultivars. Therefore, nineteen improved sweet sorghum hybrids and varieties along with CSH 22SS (a nationally released sweet sorghum hybrid) and SSV 84 (a popular sweet sorghum variety) as checks were chosen for this study to determine the optimum harvest stage for realizing high sugar yield at post-flowering and also to understand the dynamic flux of component stalk sugars like glucose, fructose and sucrose in the juice with respect to three different phenological stages.

Materials and Methods

Experimental Design and Crop Management

The selected improved sweet sorghum hybrids and varieties (Table 1) were evaluated during the post-rainy (*rabi*) season (October–February), 2009–2010 in vertisols of the experimental farm of the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), located in Patancheru, Andhra Pradesh, India (altitude 545 m above mean sea level, latitude. 17.53°N and longitude 78.27°E). The experimental design consisted of a randomized complete block design (RCBD) with three replications and each

Table 1 The list of improved sweet sorghum varieties and hybrids used in the study i.e. dough stage, physiological maturity and post-physiological maturity

Entry No.	Genotype/Pedigree
1	SP 4511-3
2	SPV 422
3	SP 4487-3
4	SS 2016
5	SP 4495
6	SP 4511-2
7	ICSV 93046
8	ICSA 84 × E 36-1
9	ICSA 38 × ICSV 700
10	ICSA 84 × SPV 1411
11	ICSA 675 × ICSV 700
12	ICSA 474 × SSV 74
13	ICSA 475 × NTJ 2
14	ICSA 702 × SSV 74
15	ICSA 475 × SSV 74
16	SSV 84 (Check)
17	CSH 22SS (Check)
18	Urja
19	JK Recova

cultivar was sown in a plot size of 3 m wide and 4 m long, i.e. four rows of 4 m long spaced at 75 cm × 15–20 cm. The planting was done on ridges with a plant stand of about 100,000 ha^{-1}. Sweet sorghum was initially planted dense but later (15 days after seedling emergence, DAS) thinned to one plant in each hill. Hand weeding was done following by two inter-cultivations. Surface irrigation was applied in furrows to the crop to maintain proper growth. Standard agronomic package of practices (80–40–0 NPK ha^{-1}; 2/3rd N and total P as basal dose and 1/3rd at 25 DAS) and plant protection measures were followed throughout the crop growth period in all the plots. At flowering, sorghum heads were covered with fine mesh bags for protection against bird damage on the developing grain. Ten plants randomly chosen in the central two rows, leaving the two guard rows were harvested with the panicle (ear head) at three different phenological stages i.e., dough (i.e. about 15–20 days after 50% flowering), physiological maturity (i.e. about 40–45 days after 50% flowering, when hilum turns black) and post-physiological maturity (i.e. about 55–60 days after 50% flowering) for each plot. Before juice extraction, the leaves were stripped and the panicles along with the peduncles were removed from each plant. The stripped stems were tied into loose bundles and shifted to the crushing site. The stripped stalks were squeezed once to extract the juice on a three-roller cane press mill. The juice was collected into sterile sample bottles and then transported under cold ice-jacketed conditions to the laboratory for further analysis. Grain yield was obtained from a sample of 10 randomly chosen plants from the center of each plot at physiological maturity and was estimated for each cultivar by adjusting the moisture content to about 14% (data not shown).

Chemical and Other Analyses

Sugar concentration in the stems was measured in terms of Brix (%) using a hand-held pocket refractometer (Atago, Japan) taking a sample of juice extracted from each plot. Data on juice weight (t ha^{-1}), pH and the stalk weight (t ha^{-1}) were collected following standard procedures for each plot. Approximate sugar yield (t ha^{-1}) is estimated as the product of Brix% and juice weight (t ha^{-1}). The contents of hexose sugars i.e., glucose, fructose and sucrose in the extracted juice were analyzed on a HPLC system (Shimadzu, Kyoto, Japan) equipped with a Luna 5 μm NH_2 100R column (4.6 × 250 mm, 5 μm particle size, Phenomenex, Inc., USA). The detection of the separated sugars was carried out with a refractive index detector (Model RID-10A, Shimadzu, Kyoto, Japan) using a mobile phase of acetonitrile–water (80:20, v/v) at a flow rate of 1.0 ml min^{-1} in isocratic mode and the column temperature was maintained at 40°C. All solvents for mobile phase optimization were degassed before use. Standard stock solution (1,000 μg/ml) of different sugars was prepared in distilled water as a diluent for analysis. After stabilizing the HPLC system, standard sugar solution was injected and using operating parameters a standard calibration was prepared for checking the reproducibility of the chromatograms. Different parameters like retention time, component concentration, peak area of each component was used for calculation of the content of respective sugars. The juice sample analysis was done by manual injection of 20 μl of pre-filtered sample. The chromatographic and integrated data were recorded using HP-Vectra (Hewlett Packard, Waldron, Germany) computer system interfaced with LC-20 AD data acquiring software for data management. The HPLC detection allowed the measurement of sugars in lower sample volume that is usually 20 μl of the sample. There is excellent correlation between peak area and concentration of sugars. The concentration of each sugar in the juice was determined using peak area from the chromatograms and expressed in terms of percentage of total sugars.

The SAS software (SAS Institute Inc. 1991) was employed for the analysis of variance and to calculate the significant differences among the varieties and hybrids. The statistical significance of the differences between the means was estimated by the least significant difference (LSD) and all significant results were reported at the $P \leq 0.05$ levels.

Results and Discussion

Genotypic Variability for Biochemical Traits and Candidate Sugar Traits

The analysis of variance (ANOVA) revealed that the mean sum of squares of stalk weight, juice yield, Brix%, sugar yield, sucrose, glucose and fructose contents, and pH were significantly ($P \leq 0.05$) different at all the three different phenological stages, i.e. dough, physiological maturity and post-physiological maturity (Table 2) indicating quantitative and qualitative changes in sugar yield and allied traits vis-a-vis crop phenology. The genotypes evaluated also exhibited highly significant ($P \leq 0.01$) differences for all the above traits except for fructose content. However, there is significant genotype x stage interaction for juice yield, Brix% and glucose content, at $P \leq 0.05$ level, while highly significant genotype x stage interaction was observed for sugar yield, sucrose & fructose levels besides pH ($P \leq 0.01$). The LSD for studied parameters were, 2.09 t ha^{-1} (stalk yield), 1.3 t ha^{-1} (juice yield), 0.16 t ha^{-1} (sugar yield), sucrose, 0.9 (Brix%), 0.52 (sucrose), 0.15 (glucose), 0.13 (fructose) and 0.09 (pH). This data suggests that there is high degree of variability among the genotypes for the sugar yield and its components and offers opportunity to harness high sugar yield owing to genotypic differences, stage-wise

Table 2 Analysis of variance (ANOVA) for metric traits and biochemical parameters at three phenological stages

Source	DF	MS for stalk yield (t ha^{-1})	MS for juice yield (t ha^{-1})	MS for Brix%	MS for sugar yield (t ha^{-1})	MS for sucrose (%)	MS for glucose (%)	MS for fructose (%)	MS for pH
Stage	2	1367.88**	546.45**	108.28**	13.24**	159.79**	8.99**	2.49**	0.95**
Replication	6	49.23	17.51*	1.12	0.15	0.87	0.28	0.12	0.29**
Genotype	18	106.51**	25.22**	25.14**	1.08**	5.46**	0.52**	0.19	0.55**
Genotype × stage	36	25.24	6.38*	3.97*	0.39**	4.27**	0.33*	0.28**	0.14**
LSD		8.53	3.34	2.59	0.36	0.52	0.15	0.13	0.09

DF degrees of freedom; *MS* mean squares

* Significant at $P \leq 0.05$; ** Significant at $P \leq 0.01$

differences and also from the significant interaction of genotype with phenological stage for sucrose content. These results are similar to the earlier reports (Almodares et al. 2008; Reddy et al. 2009; Srinivasa Rao et al. 2009). This is the first study reporting data at post-physiological maturity stage.

Characterization of Sweet Sorghum Genotypes for Component Sugars at Dough Stage

The stalk yield at dough stage among the 19 genotypes studied varied from 35.65 (ICSA 84 × E 36-1) to 46.32 t ha^{-1} (SPV 422) with a mean of 42.76 t ha^{-1} (Fig. 1), highest among the three phenological stages studied. In case of juice yield (Fig. 2), at dough stage, it ranged between 3.03 (SP 4511-2) and 9.03 t ha^{-1} (ICSA 38 × ICSV 700); while the Brix (%), a measure of total soluble solids in the juice varied between 8.83 (JK Recova) and 14.83 (SP 4495) (Fig. 3); sugar yield ranged between 0.37 (ICSA 84 × E 36-1) and 1.02 (ICSA 38 × ICSV 700) (Fig. 4). The sucrose content (%), a major disaccharide in sweet sorghum juice that contributes to the bulk of non-reducing sugars, ranged between 2.58 (ICSA 702 × SSV 74) and 5.48% (SP 4495) at dough stage (Fig. 5); The glucose content (%), a major monosaccharide in sweet sorghum juice which has a significant bearing on the ethanol yield, showed variation in a narrow range of 1.12

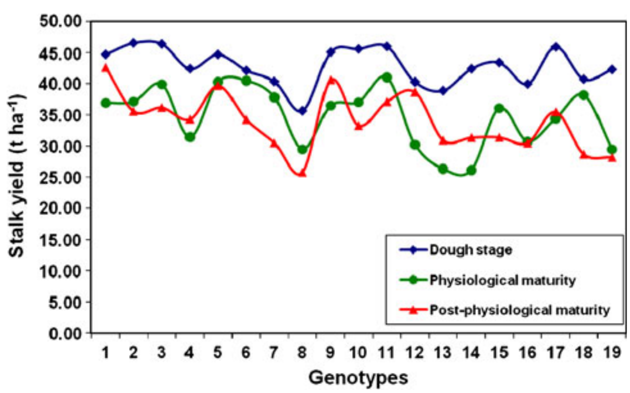

Fig. 1 Performance of sweet sorghum genotypes for stalk yield (t ha^{-1}) in three phenological stages, i.e. dough stage, physiological maturity and post-physiological maturity

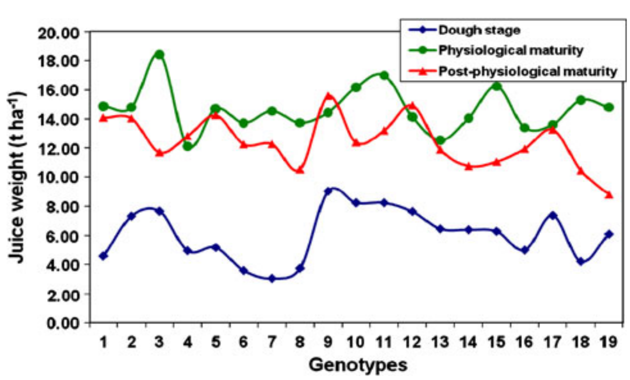

Fig. 2 Performance of sweet sorghum genotypes for juice yield (t ha^{-1}) in three phenological stages, i.e. dough stage, physiological maturity and post-physiological maturity

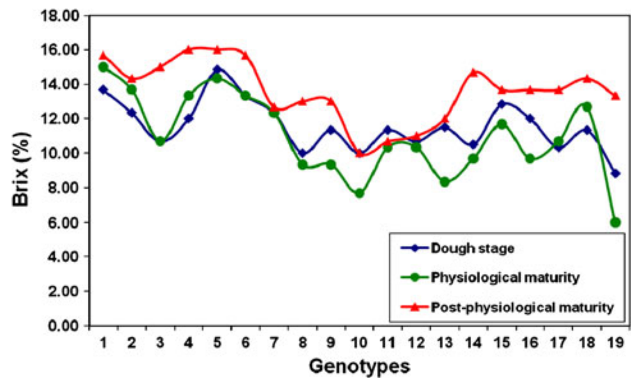

Fig. 3 Performance of sweet sorghum genotypes for Brix (%) in three phenological stages, i.e. dough stage, physiological maturity and post-physiological maturity

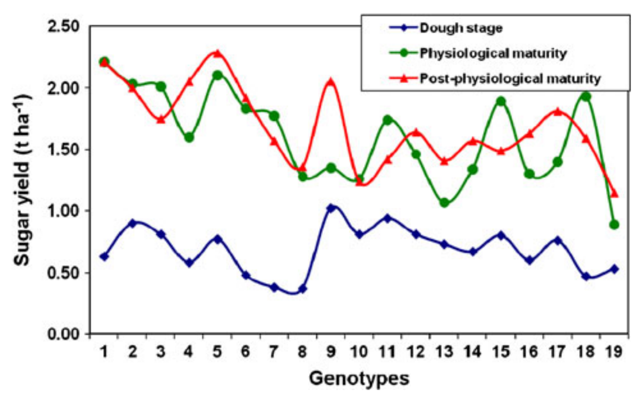

Fig. 4 Performance of sweet sorghum genotypes for sugar yield (t ha^{-1}) in three phenological stages, i.e. dough stage, physiological maturity and post-physiological maturity

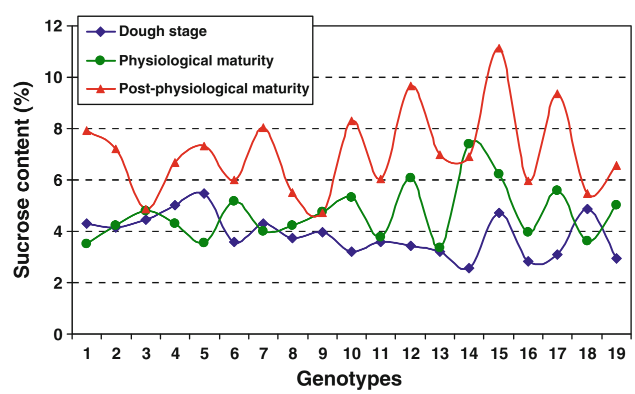

Fig. 5 Performance of sweet sorghum genotypes for sucrose content (%) in three phenological stages, i.e. dough stage, physiological maturity and post-physiological maturity

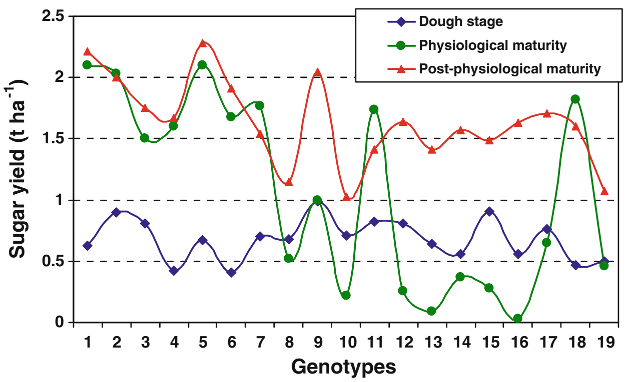

Fig. 6 Performance of sweet sorghum genotypes for glucose content (%) in three phenological stages, i.e. dough stage, physiological maturity and post-physiological maturity

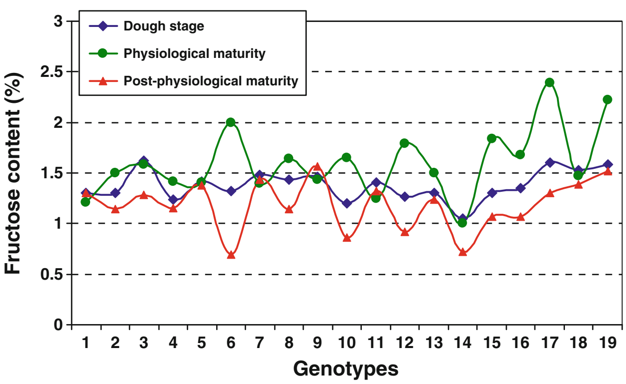

Fig. 7 Performance of sweet sorghum genotypes for fructose content (%) in three phenological stages, i.e. dough stage, physiological maturity and post-physiological maturity

(ICSA 702 × SSV 74) and 2.94 (CSH 22SS) at dough stage (Fig. 6). Another prominent monosaccharide in the juice, fructose (Fig. 7), ranged between 1.05 (ICSA 702 × SSV 74) and 2.39% (CSH 22SS) while the pH was in a range of 4.97 (ICSA 38 × ICSV 700) and 5.6 (ICSA 475 × SSV 74) (data not shown).

Characterization of Sweet Sorghum Genotypes for Component Sugars at Physiological Maturity

To harness maximum benefit, farmers and researchers believe that harvesting the crop, when hilum i.e. point of connection between individual grain and peduncle turns black as grain can be sold in grain markets and sweet stalks to the bio-ethanol distilleries. The stalk yield at physiological maturity stage among the 19 genotypes studied varied between 26.11 (ICSA 702 × SSV 74) and 40.99 (t ha^{-1}) (ICSA 675 × ICSV 700) with a mean of 34.69 t ha^{-1} (Fig. 1), lower by 19.28% with that of dough stage. In case of juice yield (Fig. 2), it ranged between 12.08 (SS 2016) to 18.41 t ha^{-1} (SP 4487-3) with a mean of 14.64 t ha^{-1}; while the Brix (%) varied between 6.0 (JK Recova) and 15.0 (SP 4495) (Fig. 3); sugar yield ranged between 0.89 (JK Recova) and 1.99 (ICSA 38 × ICSV 700) (Fig. 4). A significant variation in Brix% values among different sweet sorghum genotypes was noticed by earlier researchers (Ikegaya et al. 1993; Almodares et al. 1994; Channappagoudar et al. 2007). Further, in sweet sorghum, a high correlation was observed between Brix% and soluble sugar content which is consistent with previous findings (Yasui 1984; Guiying et al. 2000; Srinivasa Rao et al. 2009). The sucrose content (%) varied between 3.34 (ICSA 475 × NTJ 2) and 6.07 (ICSA 474 × SSV 74) at physiological maturity (Fig. 5); The glucose content (%) showed variation in a narrow range of 0.83 (SP 4511-2) and 1.73 (JK Recova) with a mean of 1.53 showing a sharp decline of over 36.1% compared to that of dough stage (Fig. 6). Fructose (Fig. 7) ranged between 1.05 (ICSA 702 × SSV 74) and 2.39% (CSH 22SS) with a mean of 1.59% showing a moderate increase of 16.1%, while the pH was in a range of 4.22 (JK Recova) and 5.73 (SP 4511-3) (data not shown).

Characterization of Sweet Sorghum Genotypes for Component Sugars at Post-Physiological Maturity

Most of the present day sweet sorghum lines are photo and thermo sensitive and therefore even under staggered planting situation too, these lines comes to flowering in about the same time period forcing the farmers to harvest the crop in a narrow window of time span, which reduces the realized sugar yield by distilleries vis-a-vis potential sugar yield (Reddy et al. 2009; Srinivasa Rao et al. 2009). Therefore, the crop was harvested at 15 days post-physiological maturity and data agronomic and biochemical parameters were collected. The stalk yield among the genotypes varied between 25.76 (ICSA 84 × E 36-1) to 42.76 t ha^{-1} (SP 4511-3) with a mean of 33.91 t ha^{-1} (Fig. 1), lowest among the three phenological stages studied as it declined by 20.7% in comparison with that of dough stage. However, the reduction was marginal (2.2%) vis-a-vis that of physiological maturity. In case of juice yield (Fig. 2), it ranged between 8.79 (JK Recova) and 15.57 t ha^{-1} (ICSA 38 × ICSV 700) with a mean of 12.42 t ha^{-1}; while the Brix (%) varied between 10.67 (ICSA 675 × ICSV 700) and 15.67 (SP 4511-3 and SP 4511-2) with a mean of 13.60% (Fig. 3); sugar yield ranged between 1.15 (JK Recova) and 2.28 t ha^{-1} (SP 4495) with a mean of 1.69 t ha^{-1} showing an increase of 146% over that of dough stage and 5.5% over that of physiological maturity (Fig. 4). The sucrose content (%) varied between 4.73 (ICSA 38 × ICSV 700) and 11.15% (ICSA 475 × SSV 74) at post-physiological maturity (Fig. 5) while the glucose content (%) showed variation in a narrow range of 1.07 (ICSA 475 × SSV 74) and 2.26 (ICSV

93046) (Fig. 6). Another monosaccharide in sweet sorghum juice, fructose (Fig. 7), ranged between 0.95 (JK Recova) and 1.67% (ICSA 675 × ICSV 700) while the pH was in a range of 4.97 (ICSA 38 × ICSV 700) and 5.6 (ICSA 475 × SSV 74) (data not shown).

Comparative Analysis of Sweet Sorghum Genotypes for Component Sugars Across the Phenological Stages

The mean stalk yield of the entries at dough stage was highest with 42.7 t ha^{-1} compared to that of physiological maturity (34.5 t ha^{-1}) and post-physiological maturity (33.9 t ha^{-1}). This observation is in tune with the earlier reports (Reddy et al. 2009; Almodares and Hadi 2009). However, such decline in stalk yield is not reflected in terms of juice realization as the mean juice yield at dough stage was low (6.04 t ha^{-1}) as against 14.64 t ha^{-1} at physiological maturity and 12.42 t ha^{-1} at post-physiological maturity. The overall mean of total soluble solids i.e. Brix% was marginally high at dough stage, 11.57% vis-a-vis 10.96% at physiological maturity, but majority of the genotypes recorded the highest Brix% at post-physiological maturity as vindicated by the highest mean Brix% value of 13.6 owing to rapid accumulation of sucrose from dough stage (3.86%) to physiological maturity (4.67%) and also to post-physiological maturity (7.08%). It is reported in the literature that sucrose begins to accumulate after heading and shows maximum accumulation after the soft dough (McBee and Miller 1982) because the developing panicle represents a less competitive sink than elongating internodes (Lingle 1987). In the present study it was observed that there was about a twofold increase of this component in all the genotypes at post-physiological maturity ranging from 4.74% (ICSA 38 × ICSV 700) to 11.15% (ICSA 475 × ICSA 74), which is in agreement with the earlier reports on sweet sorghum (Jadhav et al. 1994; Hoffmann-Thoma et al. 1996; Channappagoudar et al. 2007). A perusal of the data (Figs 6 and 7) revealed that the reducing sugars, i.e., glucose and fructose, did not increase significantly ($P \leq 0.05$) from dough stage to either physiological or post-physiological maturity in the 19 improved sweet sorghum varieties and hybrids. The mean glucose levels fluctuated between 1.35% at physiological maturity, 1.9% at post-physiological maturity, but peaking at dough stage (2.12%). However, the fructose level is highest at physiological maturity, 1.6% followed by dough stage 1.37% and post-physiological maturity, 1.18%. A birds eye view of the over all data supports the observation that the relative percentages of each sugar present in the juice were approximately 70%, 20% and 10% for sucrose, glucose and fructose, respectively. The results on sugar content in the present investigation are consistent with the earlier reports on similar studies in sweet sorghum (Smith et al. 1987; Channappagoudar et al. 2007). The incremental rise in sugar content during the physiological maturity stage has been attributed to decrease in the activity of amylases due to the aging processes and increase in temperatures during the maturation of the crop (Ikegaya et al. 1994; Channappagoudar et al. 2007). Further, it was observed that in sweet sorghum the Brix% values and the sucrose, glucose and fructose content were lower than that estimated in sugarcane (Ritter et al. 2004). These observations shed light on the extent of variability for different sugars at three phenological stages and provides new window of opportunity in hybrids like ICSA 475 × SSV 74, ICSA 38 × ICSV 700 and varieties such as SP 4495 and SP 4511-3.

Conclusions

The major outcome of this study is that all the sweet sorghum genotypes showed a significant increase in sugar yield from dough stage to physiological maturity and most of them from physiological maturity to post-physiological maturity as there is no trade-off in terms of Brix (%) and juice yield (t ha^{-1}). Although the highest stalk yield was recorded in dough stage the concomitant impact on sugar yield was not observed owing to the decreased levels of component parameters such as Brix (%) and juice content (t ha^{-1}). Stage of harvesting has a pronounced influence on the stalk and grain yield and the carbohydrate contents of the sweet sorghum. The standalone conclusion that can be drawn from the point of increasing the window of raw material supply to biofuel distilleries is that the enhanced sugar yields will be realized at post-physiological maturity and physiological maturity as compared to the dough stage, where in farmers can also get benefited by selling grains to grain markets, in addition to the sugary stalks to commercial distilleries. This has a significant impact on economic viability of sweet sorghum based distilleries. The earlier studies points to high genotype × environmental interaction for sugar yield. Therefore, it is suggested to observe the fluctuations of component sugars of juice in relation with locations and seasons vis-a-vis location of the commercial distillery to arrive at reliable period of industrial utilization of sweet stalks. Based on the results of the present study, genotypes such as SP 4495, SPV 422, SP 4487-3 and SP 4511-2 t ha^{-1} are recommended for harvesting at physiological maturity as they recorded sugar yield near or above 2 t ha^{-1} and SP 4495, SPV 422, SS 2016, SP 4511-3 and ICSA 38 × ICSV 700 for harvesting at post physiological maturity. The entries SP 4495, SP 4511-3 and SPV 422 are suitable for harvesting in a wider window of time as the sugar levels are sustained at same level from physiological maturity to post-physiological maturity. Further studies on enzymes involved in metabolism of glucose, fructose and sucrose such as amylase, invertase, sucrose phosphate synthase will help in better understanding of the dynamics of sugars vis-a-vis phenological stages under controlled conditions (temperature and photoperiod in particular), that may further aid in strategizing higher sugar productivity besides orienting breeding programs to develop photo-thermo insensitive cultivars.

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