Assessment of ethanol yield associated characters in sweet sorghum

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Abstract

Sweet sorghum is a crop close to sugarcane in respect to its sucrose accumulation, and the juicy composition of the stem offers an excellent alternative feedstock apart from others such as sugar beets. In the present investigation nine sweet sorghum cultivars were grown in the field, IARI, New Delhi; all the ethanol yield associated morphological characters were recorded, sorghum cultivars samples were analyzed at harvesting. The association analysis had clearly brought out that among the inherent genotypic characteristics stem girth, number of internodes per plant, juice content of sorghum were very important for increase in juice yield. Among the other associated characters, green cane yield and consequently percent juice recovery with higher total soluble sugar content were important parameters for ethanol yield in sorghum. It is therefore, suggested that these inherent genotypic characteristics could be exploited in identifying suitable cultivars for the purpose of ethanol production.

Keywords: sorghum (*Sorghum bicolor* L); genotypic characteristics; juice recovery; total soluble sugar; ethanol

Introduction

At present, fossil fuels are the principal resource of energy for transportation and economic developments in the world. Due to the depletion in its reserves and high pricing, fossil fuels are not equally available between the developing and developed nations (Asif and Muneer, 2007; Medina et al, 2009). The total dependence for energy and development on fossil fuel is also not sustainable (Sahoo and Das, 2009). The mandatory blending of ethanol in automotive fuels and dwindling supply of sugarcane molasses forced India to look for supplementary and alternative feedstock for producing ethanol, so to meet the requirements economically (Prasad et al, 2007; Xiaorong et al, 2010).

To meet the increased demand of ethanol in energy and transport sectors, it has become essential to explore the ethanol production potential of crops other than sugarcane. Present resources of ethanol are obtained through fermentation of sugarcane molasses (Ratnavathi et al, 2005). However, in the context of highly burdened water resources and long duration, the crop may not meet the projected increase in energy needs of future in India (Reddy et al, 2005). Hence, it is imperative to explore the potential of short duration crops that can be grown with low water and input requirements, and can easily integrate along with sugarcane in existing ethanol industries (Reddy et al, 2005; Prasad et al, 2007).

Sorghum [*Sorghum bicolor* (L) Moench] is the fifth most important cereal crop in the world for human, and to a lesser extent for cattle, feeding. However, it has a wide range of other applications that are being explored with worldwide interest in renewable resources. According to Dahlberg et al (2011), sorghum forages could produce high biomass yields and the theoretical estimates for ethanol production of these forages could average 6,146 l ha^{-1} of renewable fuels with a maximum production of 8,422 l ha^{-1} from the top ranged forage hybrids. The juice yield/cutting for several sweet sorghum genotypes ranged widely from 3,940 to 16,440 l ha^{-1} with ethanol yield ranging from 298 to 1,312 l ha^{-1} (Dalvi et al, 2011)

Sweet sorghum is a crop close to sugarcane in respect to its sucrose accumulation, and the juicy nature of the stem offers an excellent alternative feedstock apart from others such as sugar beets. It has many characteristics such as wide adaptability; tolerance to abiotic stresses like drought, water logging, salinity and alkalinity; and the capacity to grow quickly and also to accumulate sugars in stalks (Hill et al, 1990; Ratnavathi et al, 2005). Sugar yield from the crop is the desirable characteristic for ethanol production. However, the major hindrance in production of ethanol from sweet sorghum is the lack of knowledge among the inherent genotypic characteristics. The present study was undertaken to analyze the association among inherent genotypic characteristics of sweet sorghum and make it an attractive feedstock for ethanol production.

Materials and Methods

Experimental Site and Design

Field experiments were conducted in research farm at Indian Agriculture Research Institute, New Delhi (28°40'N, 77°12'E and 228 m above mean sea

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level). The climate is subtropical semiarid, with average annual rainfall of 750 mm, about 80% of which occurs from June to September. The mean maximum and minimum temperatures during kharif season (Indian crop growing period from June to October) were 35 and 18°C, respectively.

Nine sorghum cultivars PC1, PC6, PC9, PC23, PC121, PC129, PC601, PCH109, and SSG610, generally grown for forage purpose, were sown on the 20th of June, 2010 on a sandy loam soil in a randomized complete block design with three replications in a plot of size 5 x 5 m. In general sorghum cultivars having high sugar accumulation in their stalk ranging from 16–23° Brix are considered as sweet sorghum (Dayakar et al, 2004). Sugar accumulation in stalk varied among these cultivars and the Brix value ranged from 16-18.5°. All the selected cultivars were thus included in sweet sorghum category. The inter row and plant spacing were 45 and 15 cm, respectively. The plot was ploughed thoroughly and farmyard manure was applied uniformly at 10 t ha^{-1} before sowing. Fertilizers N:P:K was applied at normal recommended dose 60:40:30 kg ha^{-1}, respectively. Irrigation was given at seedling, primordial and flowering stages with ground water (600 mm). The crop was harvested on the 20th of October 2010 about 120 days after sowing.

Morphological characters

Seven randomly selected plants from each cultivar in all replicates were used for recording morphological characters viz., plant height, number of internodes, and girth of the stem, leaf lengths, leaf fresh weight, and stem fresh weight. For assessing the green cane yield at harvesting, plants from 2 m^2 area of each replicate were harvested.

Juice extractions and processing

The leaves were stripped off manually from the harvested stalks and seed head were removed with the help of knife. Peduncles (between top node and base of seed head) were also removed as they contain less sugar than the rest of the stalk (Morris and Joe, 2000). Juice in the stalks was extracted in a horizontal 3-roller power mill. Juice was strained through a wire screen into juice box. This straining help in removing larger pieces of suspended matter such as stalk fragments. Volume of the juice was recorded and juice recovery percentage was calculated. Collected juice was stored at -80°C until further analysis (Morris et al, 2000).

Analytical methods

The stalk juice was analyzed for total soluble sugar (TSS) content by Anthrone's method. To 50 µl of the sample and 950 µl of distilled water, 4 ml of 2% Anthrone reagent (in sulphuric acid) was added. The mixture was incubated in a boiling water bath for 10 min. Concentration of total soluble sugars was estimated using glucose as standard in spectrophotometer (UV-160 Shimadzu, Japan) at 620 nm (Thimmaiah, 2004). Juice yield was calculated by multiplying green stalk yield (tonnes ha^{-1}) with juice recovery (%). Fermentation of stalk juice was carried out in the small-scale Aplikan fermenter using yeast strain, Saccharomyces cerevisiae NCIM 3186. Fermented juice was centrifuged at 13,000 rpm (revolutions per minute) for 10 minutes at -20°C. The supernatant was analyzed for total residual sugar by a phenolsulfuric acid method (Mecozzi, 2005). Ethanol concentration was analyzed by gas chromatography (Shimadzu GC-14B, Japan, solid phase: polyethylene glycol PEG-20M, carrier gas: nitrogen, 90ºC isothermal packed column, injection temperature 160ºC, flame ionization detector temperature 230ºC; and isopropanol as an internal standard). The data were tested for statistical significance using Statistical Package for Social Sciences version 10 (SPSS Inc).The significance of correlation coefficient was tested against 'r' values given by Fisher and Yates (1963) at (n-2) degrees of freedom at 5 and 1% level of significance.

Results

Morphological characters and green cane yield

Morphological traits differed significantly among selected cultivars of sweet sorghum. Results indicate plant height was recorded highest in PC601 followed by PC9, PC121, PCH109, and PC129 while it was lowest in PC1. Cultivars SSG610, PC23, and PC6 were intermediate in terms of plant height. The variation was also apparent in stem girth which recorded highest in PC601 followed by PC9, PCH109, PC129, and PC121 while it was lowest in PC23. Cultivars

Table 1 - Morphological characters and cane yield among nine cultivars of sweet sorghum.

Cultivars	Plant height (cm)	Internodes/ Plant (No.)	Stem girth (cm)	Leaf Length (cm)	Total biomass yield (t ha^{-1})	Green cane yield (t ha^{-1})
PC1	191.3	14.7	4.5	63.8	57.6	46.8
PC6	194.6	16.3	5.0	70.5	74.6	55.9
PC9	262.7	16.7	5.5	83.2	71.4	54.2
PC23	197.2	10.3	3.9	68.7	63.8	50.3
PC121	241.3	15.2	4.7	81.5	66.2	47.4
PC129	224.8	15.5	5.2	68.7	68.5	52.7
PC601	268.2	15.8	5.6	83.2	77.5	57.7
PCH109	237.5	15.1	5.4	93.0	83.3	64.3
SSG610	208.5	13.5	4.2	74.8	63.4	48.0
LSD (P=0.05)	35.7	2.5	1.3	11.5	8.9	9.3

Table 2 - Differences in juice recovery, total soluble sugar (TSS) and ethanol yield of nine cultivars of sweet sorghum.

Cultivars	Juice recovery (%)	TSS (%)	Total juice (l ha^{-1})	TSS (kg ha^{-1})	Ethanol yield (l ha^{-1})
PC1	32.0	14.6	14,926	2,174	982.2
PC6	36.5	13.4	20,399	2,732	1,253.7
PC9	29.9	13.8	16,736	2,302	1,006.7
PC23	27.3	13.1	13,724	1,937	814.4
PC121	31.4	13.3	14,597	1,947	886.9
PC129	30.8	13.9	16,268	2,261	1,032.6
PC601	37.2	13.2	21,431	2,829	1,321.0
PCH109	40.1	13.7	25,699	3,530	1,661.4
SSG 610	30.4	13.4	14,677	1966	899.6
LSD (P=0.05)	4.2	0.9	2,047	,270	123.9

PC6, PC121, PC1, and SSG610 were intermediate in terms of stem girth (Table 1). Similarly number of internodes per plant varied among these cultivars. The PC9, which was second in plant height, had more number of internodes followed by PC6, PC601, PC129, and PCH109. The numbers of internodes were least in PC23 cultivar. While number of internodes were intermediate in the other cultivars. Apart from these traits the length of leaves showed significant cultivar differences. Leaves were the longest in PCH109 followed by PC6, PC601, and PC121. The minimum leaf length was recorded in PC23, while it was intermediate in SSG610, PC6, PC9, and PC129 (Table 1).

Significant cultivar differences were observed in green biomass yield. Cultivar PCH109 recorded the highest biomass yield followed by PC601 and PC6 while it was least in PC1. Cultivars PC9, PC129, PC121, PC23, and SSG610 yield intermediate quantity of biomass (Table 1). Green cane yields were calculated based on percent green cane weight of the total biomass yield at harvesting. This trait varied significantly among the cultivars with highest cane yield in PCH109 followed by PC601, PC6, and PC9. On the other hand green cane yield was least in PC1. The cultivars PC129, PC23, PC121, and SSG610 had intermediate green cane yields (Table 1).

Sugar and ethanol yield from different sorghum cultivars

Total juice recovery or percent juice extraction rate (Table 2) at harvest was significantly higher in PCH109 followed by PC601, PC6 and minimum in PC23. Juice yield per hectare was considered at harvest stage (Cane yield x Juice extraction %). Juice yield (per hectare) was highest in PCH109 and lowest in SSG610. The cultivars PC601, PC6 had similar juice yield per hectare. On the other hand, differences among PC1, PC9, PC23, PC121, and PC129 were non-significant (Table 2).

Total soluble sugar (TSS) content (%) at harvesting was significantly higher in PC1 followed by PC129, PC9 and PCH109 whereas it was observed minimum in PC23 (Table 2). On the other hand TSS quantified per hectare (kg ha^{-1}) was highest in PCH109, while it was recorded minimum in SSG610. Cultivars PC601 and PC6 were on par in terms of total soluble sugar yield. A significant cultivar differences were observed for ethanol yield among different sorghum cultivars which was ranged from 814.4 to 1661.4 l ha^{-1}. Ethanol yield was highest in PCH109 and lowest in PC23 among the 9 cultivars in this study. The rest of the cultivars PC601 and PC6, PC129 and PC9 had intermediate ethanol yield (Table 2).

The association analysis among the inherent genotypic characteristics

The interrelationship among the yield-associated characters viz., internodes per plant, stem girth and plant height had positive correlation with total fodder yield. These associated characters at genotypic level also had a positive correlation with green cane yield and juice recovery (Table 3). The correlation coefficients among the associated characters viz., green cane yield and juice recovery with total soluble sugar was (as expected) positive. These traits can thus be very much decisive in economic yield in terms of cane and sugar yield in sorghum cultivars. The results also indicate that green cane, juice recovery and total soluble sugar traits were positively correlated with ethanol production as well. All these correlations were highly significant with ‘r’ value more than 0.9 (Table 3).

Discussion

In India, interest in cultivation of sweet sorghum increased among farming community which is mainly due to its utilization in ethanol production. Wu et al (2008) described sweet sorghum a potential feedstock for ethanol production with high fermentable sugars, low fertilizer requirement, high water use efficiency, short growing period, and the ability to adapt well to diverse climate and soil conditions. Other studies also suggested sweet sorghum juice as a potential feedstock for ethanol production (Gibbons et al, 1986; Venturi and Venturi 2003; Huligol et al, 2004; ICRISAT 2007; Prasad et al, 2007; Rooney et al, 2007). The single-cut yields in sweet sorghum may be low but an increased growing season increases cumulative yields due to the ratoon potential of the crop (Rooney et al, 2007). Some studies considered sweet sorghum as an alternative to sugarcane as its growing period (about 4 months), water require-

Table 3 - Correlation coefficients among Ethanol yield associated characters in sorghum.

Characters	Plant height	No. of internodes	Stem girth	Green biomass yield	Green cane yield	Juice recovery	TSS (kg ha^{-1})	Ethanol yield (l ha^{-1})
Plant height	1.00	0.51	0.75*	0.56	0.43	0.27	0.32	0.32
No. of internodes		1.00	0.84**	0.46	0.36	0.51	0.42	0.45
Stem girth			1.00	0.76*	0.72*	0.63	0.68*	0.67*
Green biomass yield				1.00	0.95***	0.80**	0.89**	0.88**
Green cane yield					1.00	0.79*	0.94***	0.93***
Juice recovery						1.00	0.92***	0..95***
TSS (kg ha^{-1})							1.00	0.96***
Ethanol yield (l ha^{-1})								1.00

probability levels are indicated by ***, **, and * for 0.001, 0.01, and 0.05, respectively

ment (800 mm ha^{-1} over two crops) (Soltani and Almodares, 1994) and overall cost of cultivation (three times) were lower than that of sugarcane (Dayakar et al, 2004). The crop duration in sugarcane is about 12-16 months and water requirement is reported to be very high which is estimated at 3600 mm ha^{-1} (Soltani and Almodares, 1994). Assessment of genotypes for the potential ethanol yield is very important since sweet sorghum genotypes exhibit wide variability in juice quality and extractability (Balaravi et al, 1997). Sorghum is a drought-tolerant crop with high water-use efficiency which was estimated at 310 kg water per kg dry matter (Lima, 1998). Reports indicate that during very dry periods, sweet sorghum can go into dormancy, with growth resuming when sufficient moisture levels return (Gnansounou et al, 2005).

In the present investigation, morphological traits like plant height, leaf length, and number of internodes, stalk girth, total fodder and green cane yield were differed significantly among the sweet sorghum cultivars (Table 1). Genotypic differences for morphological characters and alcohol production have also been reported (Somani and Pandrangi 1993; Ratnavathi et al, 2003). The correlation between yield-associated characters and total fodder yield indicated that the internodes per plant, stem girth and plant height had positive correlation with green cane yield and juice recovery. The associated characters at genotypic level also had a significant and positive correlation with ethanol yield (Table 3). These results were in accordance with Ganesh et al (1995). The study also indicated that high green cane yield with total sugar content is a pre-requisite for high ethanol recovery. Hence, these traits could be utilized in the sweet sorghum breeding program for ethanol production.

Conclusion

As per the findings of the present investigation on association analysis it can be concluded that among the inherent genotypic characteristics stem girth, number of internodes per plant, percent juice recovery of sorghum cultivars were very important for increase juice yield ha^{-1}. Other plant traits such as green cane yield and consequently percent juice recovery with higher total sugar were important for ethanol yield in sorghum. Therefore these traits could be helpful in screening the sorghum cultivars for higher economic returns in terms of green cane yield and consequently higher ethanol production.

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