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RESEARCH ARTICLE

Effect of Micronutrient Treatments in Main and Ratoon Crops of Sweet Sorghum Cultivar ICSV 93046 Under Tropical Conditions

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Abstract The sweet sorghum variety, ICSV 93046 is commercially cultivated in large areas in India and the Philippines. The response of ICSV 93046 to six fertilizer treatments viz., T1 (control: 80 kg N ha^{-1} and 40 kg P_2O_5 ha^{-1}); T2 (designed fertilizer from a commercial source); T3 (N + P with Zn and B soil application); T4 (N + P with Zn and B soil application); T5 (N + P with foliar application of 0.1 % sodium borate and T6 (N + P with foliar application of 0.5 % $ZnSO_4$ and 0.1 % sodium borate) was evaluated during the post-rainy season (December–March, 2009–2010) as main (plant) crop and during summer season (April–July, 2010) as ratoon crop. The combined ANOVA showed that there were no significant differences observed between main and ratoon crops and the treatment interactions for the qualitative and quantitative component traits of sugar yield measured and also no significant differences observed for main and ratoon crop except for non-significant numerical differences giving a trend. The stalk yield was highest for treatments T5 and T6 in main crop and in the ratoon crop however, the treatment T4 recorded the highest stalk yield.

Keywords Sweet sorghum · Micronutrients · Tropics · Sugar yield · Stalk yield · Main crop · Ratoon crop

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Abbreviations

RCBD	Randomized complete block design
N	Nitrogen
P	Phosphorus
Zn	Zinc
B	Boron
$ZnSO_4$	Zinc sulphate
RE	Renewable energy
HPLC	High performance liquid chromatography
cv	Cultivar

Introduction

Renewable energy (RE) from different sources has received a renewed interest in the recent past, as global fossil fuels are rapidly declining due to increased consumption demands and concerns over climate change. The demand for RE has led to increase research on conversion of alternative (non-conventional) biomass to fuels, as RE contribution is predicted to increase from the current levels of 12.9 % of global energy use to 27 % by 2050 (Edenhofer et al. 2011). Sweet sorghum (*Sorghum bicolor* (L.) Moench) is a C4 plant with high photosynthetic efficiency and dry matter production and is considered an important energy crop for production of fuel bioethanol, due to high-yields, drought tolerance, relatively low input requirements in terms of water and fertilizer and its ability to grow under a wide range of agro-climatic conditions. It can yield significant amounts of readily soluble fermentable sugars (Reddy et al. 2005). Sugar stalk crops, such as sugarcane and sweet sorghum, offer more advantages than other crops since they produce a solid residue (bagasse) which can be used as a source of fuel to generate energy (Srinivasa Rao et al. 2009; Kumar et al. 2010), as animal feed

(Blümmel et al. 2009) or as soil fertilizer after composting with other agro-wastes (Srinivasa Rao et al. 2011). The utilization of bagasse has a most promising future for its bioconversion to cellulose-based ethanol, while the residual solids (mainly lignin) can be incinerated to co-generate heat and power (Srinivasa Rao et al. 2009). Besides, sweet sorghum has a panicle with grains that may be used either as food or feed (Blümmel et al. 2009). Some recent research reports suggest that soluble sugars produced in sweet sorghum has a potential to yield up to 8,000 l of ethanol per hectare or about twice the ethanol yield potential of maize grain and 30 % greater than the average Brazilian sugarcane productivity of 6,000 l ha^{-1}. 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; Wang and Liu 2009; Zhang et al. 2010; Srinivasa Rao et al. 2011). There are many sweet sorghum cultivars (cv) distributed throughout the world, providing a diverse genetic resource from which regionally specific, highly productive cv can be developed through diverse breeding approaches.

The biofuel distilleries need continuous supply of raw material, i.e., sweet stalks for a major period of the year to be commercially viable. Since the sweet sorghum crop takes 3–4 months to reach maturity, it is advantageous to explore the possibility of ratooning not only to extend the raw material supply to the distillery but also to reduce the cost of feedstock production as well as to facilitate relay cropping to maximize the returns on land and labour (Srinivasa Rao et al. 2009; Tsuchihashi and Goto 2008). Ratoon cropping does not involve sowing since it utilizes the regeneration stems and is an additional double-cropping scheme that can be adopted which involves the harvesting of the crop twice or more number of times from a single planting during the growing season (Duncan and Gardner 1984). Further, to increase the yield, timely application of fertilizers in adequate quantities is required. It has been reported that sugarcane responds favorably for micronutrients like zinc, copper, iron and boron (Shinde et al. 1986; Nayyer et al. 1984). Improved biomass of sorghum by Zn application was reported in sorghum (Rego et al. 2003) and micronutrient response in different semi-arid crops like groundnut and chickpea is well documented in the literature (Rego et al. 2007; Srinivasa Rao et al. 2008). In forage sorghum, maximum green fodder yield (52.9 t ha^{-1}) was obtained from 100 % recommended dose of fertilizer (RDF) + 25 kg $ZnSO_4$ ha^{-1} and a significant positive response to Zn was established (Anonymous 2009). The ICRISAT bred sweet sorghum cv ICSV 93046 is widely grown for bioethanol production as well as for fodder use in India and the Philippines. It is tolerant to shoot fly and terminal moisture stress and recommended for release owing to its performance in AICSIP multilocation trials during 2007–2009 (Reddy et al. 2011). Hence, the present study was conducted with the two objectives of the possibility of ratooning sweet sorghum cv ICSV 93046 under tropical conditions and also to assess its response to micronutrients like zinc and boron, particularly on sugar yield, stalk yield, juice yield, Brix %, sucrose %, glucose % and related traits for both biofuel and fodder production.

Materials and Methods

Experimental Design and Crop Management

The response of sweet sorghum cv, ICSV 93046, to six fertilizer treatments viz., T1 (control—80 kg N ha^{-1} and 40 kg P_2O_5 ha^{-1}); T2 (designed fertilizer from a commercial source); T3 (N + P with Zn and B soil application); T4 (N + P with Zn and B soil application); T5 (N + P with foliar application of 0.1 % sodium borate and T6 (N + P with foliar application of 0.5 % $ZnSO_4$ and 0.1 % sodium borate) was evaluated during post-rainy season (December–March, 2009–2010) as main (plant) crop and during summer season (April–July, 2010) as ratoon crop in vertisols of the Experimental farm of the International Crops Research Institute for the Semi-Arid

Table 1 Data temperature and photoperiod in main and ratoon crops of sweet sorghum cv. ICSV 93046

Crop	Vegetative/reproductive phase	Growing degree days (GDD)	Average maximum temperature (°C)	Average minimum temperature (°C)	Number of bright sunshine hours (h)	Average brix % at maturity
Main crop	Vegetative—88 days	783	28.84	14.61	8.27	–
	Anthesis and post-anthesis phase—42 days	648	36.5	20.35	8.7	16
Ratoon crop	Vegetative—81 days	1,532	37.86	25.05	8.9	–
	Anthesis and post-anthesis phase—39 days	523	29.44	22.6	3.4	18.2

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 four replications and a treatment plot size of 3 m wide and 4 m long, i.e., six rows of nine meters long spaced at 75 × 15 cm^2.

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 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. Accumulated growing degree days (GDD) from sowing to flowering and flowering to maturity were calculated considering 13 °C as the base temperature, as suggested by Ferraris and Charles-Edwards (1986). Four central rows, leaving the two guard rows were harvested at physiological maturity (when hilum turns black). The 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. Data on juice yield (t ha^{-1}), pH and the stalk yield (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 yield (t ha^{-1}).

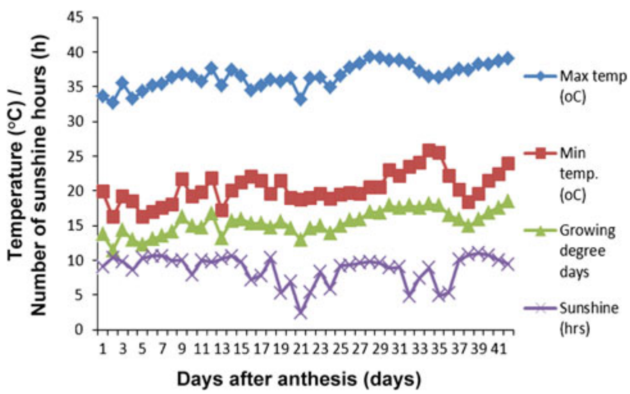

Fig. 1 Weather parameters during post-anthesis phase of the main crop of sweet sorghum cv ICSV 93046

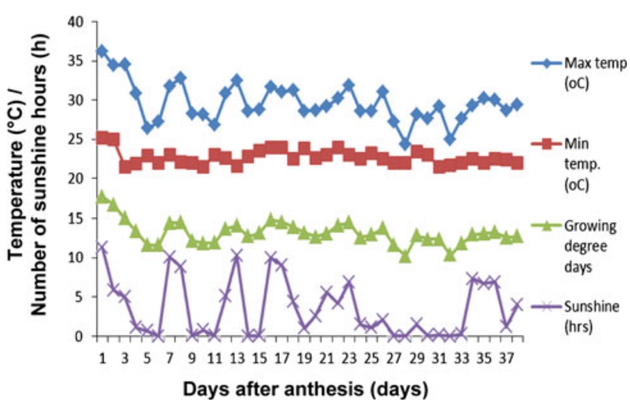

Fig. 2 Weather parameters during post-anthesis phase of the ratoon crop of sweet sorghum cv ICSV 93046

Chemical Analysis

Sugar concentration in the stem was estimated in terms of Brix % using a hand-held pocket refractometer (Model PAL, Atago Co. Ltd., Tokyo, Japan) based on the extracted juice samples taken from each plot. The pH was recorded using a microprocessor-based pH meter (Model DPH506, Global Electronics, Hyderabad, India). Between two different sample readings, the refractometer and the pH meter were cleaned with distilled water and dried with a paper towel. The contents of hexose sugars i.e., glucose, fructose and sucrose in the extracted juice were analyzed on a high performance liquid chromatography (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

Table 2 Combined ANOVA table for response of fertilizer treatments on agronomic and biochemical traits of main and ratoon crops of sweet sorghum cv. ICSV 93046

Source of variation	DF	Stalk weight (t ha^{-1})	Juice weight (t ha^{-1})	Bagasse weight (t ha^{-1})	Brix %	Sugar yield (t ha^{-1})	Fructose %	Glucose %	Sucrose %	pH
Replication	3	85.73	24.20	20.41	12.54	0.73	1.00	0.68	5.53	0.01
Treatments	5	31.20	6.23	9.89	4.14	0.11	0.17	0.22	0.36	0.00
Main vs. ratoon crop	1	194.67**	171.04**	16.33	63.03**	0.97*	4.8324**	6.64**	0.20	0.39**
Treatments × cropping	5	23.49	4.79	5.82	8.88	0.19	0.62	0.45	3.02	0.01
Pooled Residual	15	17.66	4.22	7.34	3.38	0.13	0.31	0.25	1.61	0.01

DF degrees of freedom

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^{-1}) of different sugars prepared in Milli-Q distilled water as diluent was used for calibrating the HPLC system. The juice sample analysis was carried out by manual injection of 20 µl of pre-filtered sample. The data acquisition and analysis was carried out using LC solutions software (version 1.24 SP2) (Shimadzu, Kyoto, Japan). 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 (Kumar et al. 2010).

Statistical Analysis

General linear model (GLM) was used for analysis of variance and to calculate significant differences among improved varieties using SAS software (Anonymous 1991). GraphPad Prism (GraphPad Software Inc., San Diego, CA, USA) software version 2.0 (Motulsky 1999) was used for simple linear regression analysis between traits. The statistical significance of the differences between the means was estimated by the least significant difference and all significant results were reported at the $P \leq 0.05$ levels.

Results and Discussion

Influence of Seasonal Variation

As sorghum is a short day plant, it tends to flower in the days that have a photoperiod less than 12 h. The tropical sweet sorghums are also influenced by temperature. Hence, an attempt to compare the weather parameters was made between main and ratoon crops. The main crop reached 50 % flowering in 88 days (783.4 GDD) as compared to that of 81 days (1,532 GDD). This is due to coincidence of main crop with winter season in the months of December, January and part of February. The average maximum temperature during vegetative phase in main crop was at 28.8 °C, while that for ratoon crop stood at 37.9 °C (Table 1). The average minimum temperatures are 14.6 and 25 °C in main and ratoon crop, respectively. There was no significant difference observed in number of bright sunshine hours in the vegetative phase (8.3 vs. 8.9 h) in both main and ratoon crops. However, a significant difference (8.7 vs. 3.4 h) was observed during anthesis and post-anthesis stages. The weather parameters like maximum temperature (°C), minimum temperature (°C), GDD and number of bright sunshine hours (h) recorded during post-anthesis period for main crop (Fig. 1) and ratoon crop (Fig. 2) showed significant differences particularly in post-anthesis stage where the higher maximum temperature of 36.5 °C lead to poor accumulation of sugars (16 % Brix). Further investigations are required to fully understand the relevance of weather parameters vis-a-vis sugar accumulation.

ANOVA for Agronomic and Biochemical Traits

The combined ANOVA (Table 1) reveals that there was no significant differences observed among the treatments and the interaction of treatments with cropping (main and ratoon). However, significant differences were observed for all the traits except for bagasse yield and sucrose levels in the main and ratoon crop interaction. This explains the reason for reduced sugar yield in ratoon crop and the component traits influenced in the ratoon. The mean performance of fertilizer treatments on agronomic and biochemical traits of main and ratoon crops of sweet sorghum cv ICSV 93046, for different parameters like stalk yield, juice yield, bagasse yield, Brix %, sugar yield, fructose, glucose, sucrose and pH are presented in Table 2. The average stalk yield recorded for the main and ratoon crops are 29.4 and 25.2 t ha^{-1}, respectively. The stalk yield in the ratoon was lower by 16.6 % than that of plant crop, while juice yield reduced by 42.3 % and sugar yield by 22.4 %. However the Brix % levels in ratoon increased by 12.1 %. The highest stalk yield was recorded for fertilizer treatments, T5 and T6, in the main crop (31.4 t ha^{-1}), and in the ratoon crop, the fertilizer treatment T4 recorded the highest stalk yield (28.9 t ha^{-1}). The lowest stalk yield was realized in T2 treatment both in the main/plant and ratoon crops. The juice yield was significantly lower in the ratoon crop as it was cultivated in summer season, coinciding with higher temperatures. These findings are in tune with the earlier reports (Tsuchihashi and Goto 2008; Srinivasa Rao et al. 2009). The highest Brix % was recorded for fertilizer treatments T5 (16.9 %) and T6 (16.8 %) in the main crop, while in the ratoon crop, the fertilizer treatment T1 recorded the highest Brix % (20.8 %). The variation is probably due to the low temperature differential during post-flowering stage in the post-rainy season, while high temperature differences in summer ratoon crop (Srinivasa Rao et al. 2009, 2011; Kumar et al. 2010). The average sugar yield in the main and ratoon crops are 1.5 and 1.2 t ha^{-1}, respectively. This reduced sugar yield in ratoon crop, i.e., by 25 % as compared to the main crop conforms to the earlier report of Tsuchihashi and Goto (2008). The lower mean sugar yield in summer ratoon crop is attributed to the reduced stalk yield and juice recovery, in spite of the higher Brix % in ratoon summer crop. The highest sugar yield was recorded for fertilizer treatments T5 (1.74 t ha^{-1}) and T6 (1.67 t ha^{-1}) in the main crop. This implies that foliar application on B (T5) and foliar

Table 3 Mean performance table for response of fertilizer treatments on agronomic and biochemical traits of main and ratoon crops of sweet sorghum cv ICSV 93046

Treatments	Stalk yield (t ha^{-1})		Juice yield (t ha^{-1})		Bagasse yield (t ha^{-1})		Brix %		Sugar yield (t ha^{-1})		Fructose %		Glucose %		Sucrose %		pH	
	Crop																	
	Main	Ratoon	Main	Ratoon	Main	Ratoon	Main	Ratoon	Main	Ratoon	Main	Ratoon	Main	Ratoon	Main	Ratoon	Main	Ratoon
T1	30.56	26.45	13.22	8.89	17.08	16.48	15.9	20.8	1.59	1.40	2.42	1.81	2.17	1.53	6.46	7.11	5.76	5.58
T2	23.91	22.36	10.61	8.02	13.25	14.19	15.9	18.1	1.27	1.09	2.73	1.83	2.57	1.53	7.52	7.08	5.79	5.59
T3	29.56	26.73	12.08	9.83	17.16	15.60	15.7	19.1	1.44	1.43	2.09	2.00	1.97	1.65	6.34	7.47	5.73	5.63
T4	29.51	28.95	13.28	10.82	16.22	16.57	14.5	18.1	1.48	1.49	1.87	2.00	1.70	1.62	5.88	7.61	5.70	5.61
T5	31.44	23.98	13.77	7.97	17.54	14.75	16.9	17.2	1.74	1.08	2.80	1.76	2.60	1.50	7.54	6.68	5.82	5.57
T6	31.43	22.77	13.20	7.98	17.94	14.61	16.8	16.2	1.67	0.99	2.92	1.63	2.69	1.44	7.78	6.36	5.82	5.55
Minimum	23.91	22.36	10.61	7.97	13.25	14.19	14.50	16.19	1.27	0.99	1.87	1.63	1.70	1.44	5.88	6.36	5.70	5.55
Maximum	31.44	28.95	13.77	10.82	17.94	16.57	16.88	20.76	1.74	1.49	2.92	2.00	2.69	1.65	7.78	7.61	5.82	5.63
Mean	29.40	25.21	12.69	8.92	16.53	15.37	16.0	18.2	1.53	1.25	2.47	1.84	2.28	1.54	6.92	7.05	5.77	5.59
LSD ($P < 0.005$)	6.33		3.09		4.08		2.76		0.542		0.834		0.753		1.91		0.113	
CV %	7.2		8.2		7		4.2		8.6		6.8		8.7		3.1		0.3	

LSD least significant difference, *CV %* coefficient of variation, *T1* control (N + P), *T2* designed fertilizer from TCL as per their recommendation, *T3* N + P with Zn and B soil application, *T4* N + P with foliar application of 0.5 % $ZnSO_4$, *T5* N + P with foliar application of 0.1 % sodium borate, *T6* N + P with foliar application of 0.5 % $ZnSO_4$ and 0.1 % sodium borate

application of Zn and B has increased sugar yield in the main crop. On the other hand, in the ratoon crop the fertilizer treatment T4 recorded the highest sugar yield (1.49 t ha^{-1}) that includes soil application of B and Zn along with N and P_2O_5. However, the foliar application of micronutrients did not influence sugar yield in ratoon crop. In case of sucrose levels, the ratoon crop recorded a higher sucrose % of 7.05 % as compared to the main crop's level of 6.95 %. The highest sucrose % was recorded for fertilizer treatments T5 (7.5 %) and T6 (7.7 %) in the main crop, while in the ratoon crop the fertilizer treatment T4 recorded the highest sucrose % of 7.6 %. However, the glucose % and fructose % levels in ratoon crop were considerably lower as compared to those observed in the main crop. Surprisingly, the pH content was significantly lower in the ratoon crop as compared to that of the main crop (Table 3).

Conclusion

Main and ratoon cropping pattern of sweet sorghum provides a double-cropping option for farmers to achieve maximum benefits of their resources and also helps in providing extended period of functioning of the biofuel distillers. The performance of cv. ICSV 93046 is significantly influenced by seasonal variation (temperature, GDD and sunshine hours) in late post-rainy (main crop) and early rainy season (ratoon crop) as revealed by ANOVA. The application of micronutrients (Zn and B) at Patancheru location did not yield significant gains in productivity. The decline in the ratoon crop productivity vis-a-vis main crop is due to a reduction in stalk yield (16.6 %), juice yield (42.3 %), glucose (48 %) and fructose (34.2 %) levels as reflected in the final sugar yield. In future, the breeding programs should address these traits for sustained ratoon crop yield. The present study validates the possibility of taking ratoon crop of cv. ICSV 93046 without significant compromise on biomass yield and effect of micronutrients (Zn and B) nutrition on biomass and sugar production is not significant.

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