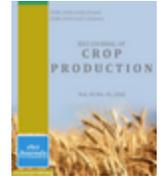




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## INCREASING MILLABLE CANE YIELD OF SWEET SORGHUM THROUGH ALTERED NITROGEN, POPULATION LEVEL AND PLANT GROWTH REGULATORS SPRAY

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### ABSTRACT

Sweet sorghum (*Sorghum bicolor*) has the potential to become a multipurpose feedstock for large-scale ethanol production from stem juice, cellulose/hemicellulose from stalks, and starch from grain. Maximizing the feedstock yield is the first step for large-scale production of ethanol. Feedstock yield in sweet sorghum can be improved by various nitrogen levels, spacing and plant growth regulators. Field experiments were conducted to evaluate the responses of sweet sorghum to nitrogen levels (60, 120 and 180 kg N ha<sup>-1</sup>), populations (21.7, 10.9 and 7.2 plants m<sup>-2</sup>), plant growth regulators (Naphthalene acetic acid; NAA 40 ppm and salicylic acid 200 ppm) and pollen sterilants spray (ethrel 200 ppm, p-coumaric acid 400 ppm and deheading) on millable stalk yield and brix value. Increased millable stalk yield was observed in 120 kg N ha<sup>-1</sup>. However, maximum brix value was recorded in 180 kg N ha<sup>-1</sup>. Increased millable stalk yield and brix value (%) was recorded in 7.2 plants m<sup>-2</sup> and it could be due to less competition between the plants for the resources. Naphthalene acetic acid (NAA) application increased brix, sucrose content and theoretical ethanol yield compared to control and it could be due to enhanced sugar mobilization from the leaf to the stem. Ethrel 200 ppm sprayed plants had higher stem girth, millable stalk yield, brix value and sucrose content and this may be due to increased pollen sterility percentage. The study revealed population of 7.2 plantsm<sup>-2</sup>, nitrogen level of 120 kg N ha<sup>-1</sup>, NAA 40 ppm spray at booting stage and ethrel 200 ppm spray at anthesis stage resulted in higher millable stalk yield and brix value.

**Keywords:** Brix value, Chemical sterilants NAA - Salicylic acid - Stem weight, Sweet sorghum.

### INTRODUCTION

Renewable resources can contribute towards meeting energy requirements with the added advantage of greater environmental protection, especially in terms of lower CO<sub>2</sub> emissions (Basavarajet *al.*, 2012). Among different crops, sweet sorghum (*Sorghum bicolor* L. Moench) is of particular interest because its biomass is used for the production of energy, fiber or paper, as well as for syrup and animal feed. Sweet sorghums are typically characterized by low grain yields, but high biomass production. The stalks contain 10-25% sugars (mainly sucrose, glucose, and fructose) at maturity (Byrtet *al.*, 2011). Sweet sorghum has advantages in ethanol production processing because it requires fewer chemical reaction steps and less energy from feedstock to the end product than grain and forage sorghums.

Furthermore, the cost to cultivate sweet sorghum can be as little as three times lower than that of sugarcane Reddyet *al.*, 2005; Audilakshmiet *al.*, 2010; Xinet *al.*, 2011 Sweet sorghum contains approximately equal quantities of soluble (glucose and sucrose) and insoluble carbohydrates (cellulose and hemicellulose) and has been considered as an important source for the production of fuel ethanol (Mamma *et al.*, 1995). However, its potential as a source of ethanol production has not been fully exploited. To make sweet sorghum a sustainable and profitable crop, there is a need for standardize the best agronomic practices, apart from breeding high-yielding cultivars, which can contribute to increased yields. The various agronomic practices include use of optimum nitrogen fertilizer rate, plant population rate, use of plant growth regulators and chemical sterilants for getting maximum millable stalk yield. Nitrogen (N) is one of the expensive nutrients to supply; simultaneously, it is an important factor limiting

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crop productivity. Approximately 85 to 90 million metric tons of nitrogenous fertilizers are added to soil worldwide each year. Worldwide, N use efficiency for the production of cereals such as wheat (*Triticumaestivum*), rice (*Oryza sativa*), corn (*Zea mays*), barley (*Hordeumvulgare*), sorghum, pearl millet (*Pennisetumglaucum*), oat (*Avena sativa*), and rye (*Secale cereal*) is approximately 33%, and the remaining 67% goes as loss (Raun and Johnson, 1999). Furthermore, producers are looking for ways to improve their ability to manage N fertilizers more effectively because of recent sharp increases in N fertilizer prices. Generally sweet sorghum responds well to N (Turgut *et al.*, 2005). Hence, we hypothesize that application of N fertilizer will enhance the vegetative biomass resulting in higher millable cane yield. The plant population provides the best chance to produce the most biomass per given area. It is common to think that in narrower row spacing, the more plants can be grown in that area would result in more biomass. However, the competition between plants will impact on biomass per given area. Compared to narrow rows, plants grown in wider rows have more spatial room to grow and more nutrients within their root zone to utilize. Studies conducted have shown that narrower row spacing does indeed increase biomass. Martin and Kellcher (1984) reported increased dry matter and water soluble carbohydrate yields with denser spacing in sweet sorghum. They also reported fewer internodes and thicker stems in wider spacings than closer spacings, whilst in closer spacings thinner stems with slightly more tillers. However, Kumar *et al.* (2008) reported that sweet sorghum stalk and grain yield is unaffected by plant population. Hence the response of sweet sorghum to plant population levels is not clear and has to be standardized.

Auxin is involved in plant growth and development (Zhao, 2010). High auxin levels in the sink could create an “attractive power,” leading to an increased accumulation of sugar in the developing plant parts and enhanced yield (Yang *et al.*, 2001; Sivakumaret *al.*, 2006). Hence, it is hypothesized that yield of millable sweet sorghum stalks can be improved by foliar spray of plant growth regulators, which are known to enhance sink capacity. Salicylic acid has been reported to induce significant effects on various biological aspects in plants (Raskin, 1992). Obvious effects on net photosynthetic rates in corn and soybean plants (Khan *et al.*, 2003) and yield of various crop species have been achieved following exogenous application of salicylic acid

(Raskin, 1992). However, there is no report available on effect of NAA or salicylic acid foliar spray on millable cane yield in sweet sorghum. The physiology of sweet sorghum is different from that of grain and fibre sorghum in reference to juice composition (Byrt *et al.*, 2011). The sucrose is accumulated in large amounts in the stem during the development of the inflorescence (McBee and Miller, 1982). During this period there may exist a competition for carbohydrates between developing inflorescence and stem. Pollen sterilants could help us to induce male sterility more efficiently causing poor seed set and causing sucrose accumulation in the stem.

A strong correlation between phenolic compounds and retardation of reproductive growth was clearly established (Kefeli and Kalevitch, 2003). Ethrel was reported to cause functional male sterility in rice and other crops (Praba and Thangaraj, 2005). Hence it is hypothesized that, if the panicle is removed or spikelet sterility is achieved by foliar spray of sterilants, assimilates that ought to have been utilized for the development of grain would be diverted to the stalk, which may be resulting in enhanced sugar accumulation in stem. Sorghum for non-food destinations can be part of agricultural systems only if its cultivation proves to be economically sustainable and if its benefit is equal or higher than that of traditional crops. Several research groups are developing new cultivars with higher stalk and grain yields potential. Apart from breeding, crop management options like nitrogen fertilizer and plant density levels, plant growth regulators and sterilants spray also have the potential to increase the stalk yields in sweet sorghum. Therefore, the present study aims to (i) optimize the planting population and nitrogen level for getting maximum millable stalk yield (ii) assess the effect of plant growth regulators and chemical sterilants spray on millable stalk yield in sweet sorghum.

#### **MATERIALS AND METHODS**

Field experiments were conducted in the experimental plots (20 m<sup>2</sup>) of Agricultural Research Station, Bhavanisagar (11°29' N, 77°8'E), Tamil Nadu, India. The meteorological data in the experimental site were given in Fig. 1. The complete details about the weather station are available in [www.tnau.ac.in](http://www.tnau.ac.in). The distance between experimental field and weather station was around 2.5 km. The soil type of experimental plot was an Alfisol with low in available N (173 kg ha<sup>-1</sup>), medium in available P (7.3 kg ha<sup>-1</sup>) and high in available K (393 kg ha<sup>-1</sup>). The pH and EC ranged from 7.3 to 7.8 and 0.21 to 0.25 dS m<sup>-1</sup>, respectively.

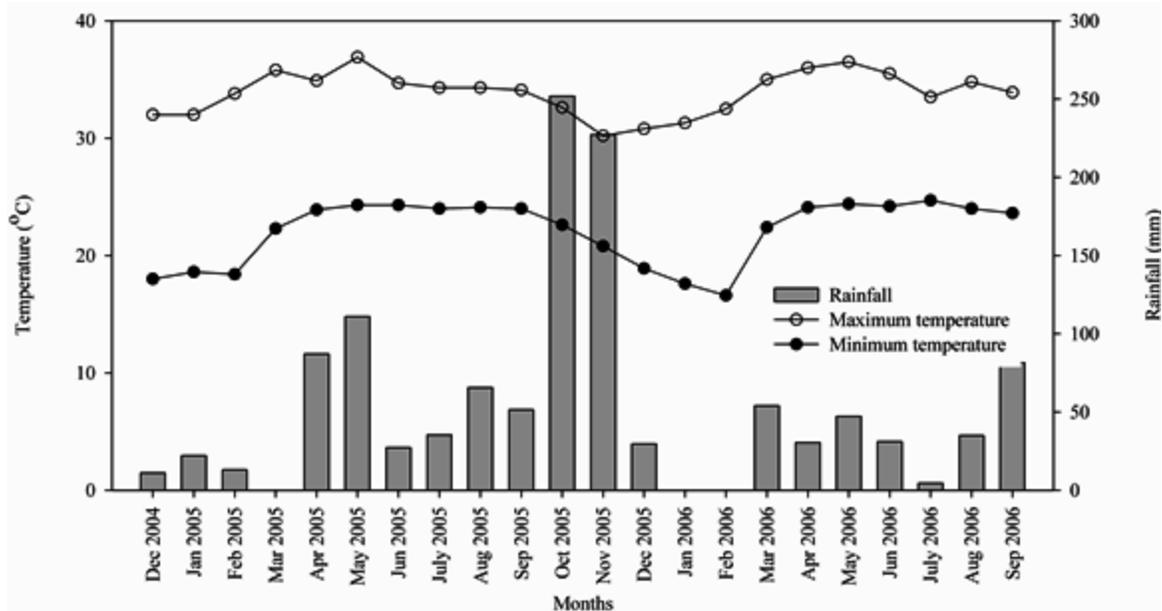


Fig. 1. Mean monthly minimum and maximum temperature ( $^{\circ}\text{C}$ ), rainfall (mm) and relative humidity (%) recorded at Agricultural Research Station, Bhavanisagar weather station (approximately 2.5 km from the study site) from December 2004 to September 2006.

**Nitrogen and plant population levels study:** The experiments were conducted in split plot design with seven replications. The date of sowing was 28.12.2004 and 14.12.2005, for experiment I and II, respectively. Nitrogen levels and plant population level was considered in main plot and sub plot, respectively. The N, P and K levels of the experimental soil were quantified and the balance fertilizers were added to each plot (each treatment). Three levels of nitrogen were compared (60, 120 and 180 kg N  $\text{ha}^{-1}$ ) in main plot. Three level of plant populations, i. Intra-row spacing of 10 cm (21.7 plants  $\text{m}^{-2}$ , higher density), ii. Intra-row spacing of 20 cm (10.9 plants  $\text{m}^{-2}$ , control) and iii. Intra-row spacing of 30 cm (7.2 plants  $\text{m}^{-2}$ , lower density) were adopted. Row spacing was 45 cm for each intra-row spacing treatment. Sweet sorghum variety SSV 84 (suitable for all season under irrigated condition) was used for the study. The variety SSV 84 was released from National Research Centre for Sorghum (NRCS), Hyderabad. Before sowing 50% N, 100% P and K in the form of urea, single super phosphate and KCl, respectively, were applied to each plot as basal per the treatment schedule to each plot. The remaining 50% N was applied as top dressing during 30 days after sowing (DAS). Atrazine @ 0.2 kg a.i.  $\text{ha}^{-1}$  was applied on third day after sowing followed by hand weeding at 30 DAS. The plant was irrigated by surface irrigation method if the top soil is dry (~ every 6

days). Shoot fly and stem borer were controlled by applying Carbofuran 3G granule @ 8 kg  $\text{ha}^{-1}$  at 20 days after emergence along the ridges. Mancozeb @ 1 kg  $\text{ha}^{-1}$  was applied during ear head emergence to avoid head mould and downy mildew. The spraying was done using backpack sprayer and the spray volume was 150 L  $\text{ha}^{-1}$ . Plant height and stem girth were recorded at physiological harvest (110 days from date of sowing) in 10 randomly selected plants from each replication. Plant height was measured in cm, the maximum distance from base of the stem (above soil surface) to the base of the panicle. Stem girth was recorded by using a Vernier caliper. The interior rows in each plot (~4  $\text{m}^2$  area) were harvested for recording millable stalk yield (kg  $\text{m}^{-2}$ ) and seed yield (g  $\text{m}^{-2}$ ) at physiological maturity stage (hard dough stage; 110 DAS). Ten plants from each plot were harvested at physiological maturity, and the stalks were stripped and topped before juice was extracted from a three-roll cane press mill. The weight of the millable portions of each sample was recorded before crushing and expressed in kg (fresh weight basis). The juice weight of each sample was used to calculate juice yield (%). A standard hand refractometer (Omega Engineering Inc., USA) was used to determine the total soluble solids (brix) of extracted juice. A theoretical yield of ethanol from the millable stalk yield of sweet sorghum was calculated using the method of Mamma *et al.* (1996) and expressed as L  $\text{ha}^{-1}$  (5.2 g ethanol per 100

g of millable stalk yield). The seeds in the panicle was threshed manually and cleaned, dried in hot air oven at 40°C for 7 days and weighed and expressed in dry weight basis.

**Plant growth regulators study:** Field experiments were conducted to evaluate the effects of foliar application of plant growth regulators on millable stalk yield and sucrose content. The date of sowing was 14.03.2005 and 24.03.2006, for experiment I and II, respectively. A randomized block design with seven replications was used. The plots were of 12 m<sup>2</sup>. The sweet sorghum variety SSV 84 was sown in a spacing of 45 x 30 cm and a fertilizer level of 120:40:40 kg NPK ha<sup>-1</sup> was applied (Blanket recommendation for grain sorghum). The form of fertilizer, timing of application and rest of plant production package was explained earlier. The treatments *viz.*, control, naphthalene acetic acid (NAA) 40 ppm, and salicylic acid 200 ppm were applied at boot leaf stage (just before panicle emergence). The spraying was done using backpack sprayer and the spray volume was 150 L ha<sup>-1</sup>. Water management, weed management, pest and disease control were described earlier. Plant height, stem girth, millable stalk yield, seed yield, brix value, juice yield and theoretical ethanol yield were recorded as described earlier. Glucose content in juice was determined as described by Miller (1959), as follows: 1 mL water (blank), standard (0.05, 0.1, 0.15, 0.2, 0.25, 0.3, and 0.4 g L<sup>-1</sup> glucose), or sample was added to test tube. Next, 0.5mL anthrone solution (freshly prepared) was added to each test tube and mixed gently but thoroughly, and incubated at 80°C in a water bath. After 3 min, the test tube was cooled at room temperature for 5 min to stop the reaction. Then the absorbance (630nm) was recorded and expressed as per cent (Scanning spectrophotometer, Beckman Coulter, Inc., USA). Sucrose content of the juice was determined by the Anthrone method (Van-Handel, 1963): 70 µl of reaction solution was added to 70 µl of 30% KOH, boiled for 10 min, and cooled to room temperatures; 1 mL of freshly prepared anthrone reagent, containing 76 mL of H<sub>2</sub>SO<sub>4</sub>, 30 ml of H<sub>2</sub>O, and 150 mg of anthrone, was added and the reaction was incubated at 37°C for 20 min. The intensity was recorded at 650 nm (Scanning spectrophotometer, Beckman Coulter, Inc., USA) immediately and expressed as per cent.

**Deheading and pollen sterilants:** Field experiments were conducted to evaluate the effect of foliar application of pollen sterilants and deheading on

millable stalk yield and sucrose content. The date of sowing was 30.03.2005 and 11.03.2006, for experiment I and II, respectively. The experiments were conducted in randomized block design with four treatments, control, deheading, ethrel 200 ppm and p-coumaric acid 400 ppm with seven replications. The plots were of 12 m<sup>2</sup>. The sweet sorghum variety SSV 84 was sown in a spacing of 45 x 30 cm and a fertilizer level of 120:40:40 kg NPK ha<sup>-1</sup> was applied. The form of fertilizer, timing of application and rest of plant production package was explained earlier. Ethrel and p-coumaric acid were applied at anthesis stage. The spraying was done using backpack sprayer and the spray volume was 150 L ha<sup>-1</sup>. Removal of panicles at anthesis constituted the deheading treatment. The rest of the methodology was described earlier. Apart from this, data on total number of reproductive sites (total spikes in panicle), and number of filled and unfilled seeds also were estimated. Individual spikelets were checked for seed by pressing the floret between the thumb and the index finger. Seed set was estimated as the ratio of spikelets with seed to the total number of reproductive sites and expressed as percentage. Per cent floral sterility was arrived as 100 – (the difference between seed set per cent in control plants and seed set per cent in treated plants divided by seed set per cent in control plants)(Chakraborty and Devakumar, 2006).

**Statistical analysis:** Statistical analysis was performed using SAS 9.2 program. In each experiment, all the factors were considered as random effect. All the experiments (nitrogen, plant population, plant growth regulators, deheading and pollen sterilant) were repeated. Fertilizer and plant population study was analyzed in split plot design separately (individual experiment) and in combination (two experiments; pooled analysis). Results of both experiments separately or in combination had the similar responses and significance for all the traits. Therefore mean responses across two experiments (years) are presented. Plant growth regulators spray and sterilants spray experiment was analyzed in completely randomized block design separately (individual experiment) and in combination (two experiments; pooled analysis). Results of both experiments separately or in combination had the similar responses and significance for all the traits. Therefore mean responses across two experiments are presented. Standard error is shown as an estimate of variability and significant means were separated by LSD test at the 0.05 level.

## RESULTS

**Nitrogen levels:** The present study indicates there were no effects of year, interaction of year and N levels on all the observed traits of sweet sorghum. The maximum and minimum temperature during the experiments was found to be optimum for sweet sorghum growth and development. However, the rainfall received during Nov and Dec, 2004 was higher than other years. The crop was continuously irrigated to avoid water stress. Hence, there were no significant effects of weather on growth and yield.

Nitrogen levels significantly altered plant height ( $P < 0.001$ ), stem girth ( $P < 0.001$ ), millable stalk yield ( $P < 0.001$ ), seed yield ( $P < 0.001$ ), brix ( $P < 0.001$ ) and theoretical ethanol yield ( $P < 0.001$ ). The plants were consistently taller in plots fertilized with  $180 \text{ kg N ha}^{-1}$ . The  $60 \text{ kg N ha}^{-1}$  plants were thin and recorded a 41.3% decrease in stem girth compared to  $120 \text{ kg N ha}^{-1}$  plants. Maximum millable stalk yield was recorded in  $120 \text{ kg N ha}^{-1}$ . Low level of nitrogen ( $60 \text{ kg N ha}^{-1}$ ) decreased the millable stalk yield by 13.4%. Maximum seed yield was recorded in  $180 \text{ kg N ha}^{-1}$ . The increase in seed yield by higher N level ( $180 \text{ kg N ha}^{-1}$ ) was 37.1% over the  $120 \text{ kg N ha}^{-1}$ . The highest brix value was recorded in  $180 \text{ kg N ha}^{-1}$  (16.0%). The low level of nitrogen ( $60 \text{ kg N ha}^{-1}$ ) decreased the brix value by 27.1% compared to  $120 \text{ kg N ha}^{-1}$ . The lower level of nitrogen ( $60 \text{ kg N ha}^{-1}$ ) decreased the theoretical ethanol yield by 13.4% compared to  $120 \text{ kg N ha}^{-1}$  (Table 1).

**Plant population:** The present study indicates there were no effects of year, interaction of year and plant population levels on all the observed traits of sweet

sorghum. Plant population levels significantly altered plant height ( $P < 0.05$ ), stem girth ( $P < 0.05$ ), millable stalk yield ( $P < 0.05$ ), seed yield ( $P < 0.05$ ), brix ( $P < 0.001$ ) and theoretical ethanol yield ( $P < 0.05$ ). The plants were taller in 10.9 and 7.2 plants  $\text{m}^{-2}$  population (intra-row spacing of 20 and 30 cm). The plant height in 21.7 plants  $\text{m}^{-2}$  population (intra-row spacing of 10 cm) had a per cent decrease of 7.2% compared to 10.9 plants  $\text{m}^{-2}$  population (intra-row spacing of 20 cm) (Table 1). The stem girth was higher at 7.2 plants  $\text{m}^{-2}$  population (intra-row spacing of 30 cm) (6.97 cm), which was 12.4 and 30% increase compared to 10.9 and 21.7 plants  $\text{m}^{-2}$  population (intra-row spacing of 20 and 10 cm), respectively (Table 1). Maximum millable stalk yield was recorded in 7.2 plants  $\text{m}^{-2}$  population followed by 10.9 plants  $\text{m}^{-2}$ . Wider spacing (7.2 plants  $\text{m}^{-2}$  population) increased the millable stalk yield by 11.4% compared to 10.9 plants  $\text{m}^{-2}$  population. Higher population (intra-row spacing of 10 cm) decreased the millable stalk yield by 15.2% compared to lower population (intra-row spacing of 20 cm) (Table 1). Maximum seed yield was recorded in 21.7 plants  $\text{m}^{-2}$  population which was 11.6% higher compared to 10.9 plants  $\text{m}^{-2}$  population. Brix value, juice yield and theoretical ethanol yield were higher in 7.2 plants  $\text{m}^{-2}$  population. The per cent increase was 1.4, 8.3 and 10.9% for the above mentioned traits, respectively. However, the high density decreased brix value, juice yield and theoretical ethanol yield by 24.8, 26.6 and 15.9%, respectively compared to 10.9 plants  $\text{m}^{-2}$  population (intra-row spacing of 20 cm: Table 1).

Table 1. Main effects of nitrogen levels [ $60, 120$  and  $180 \text{ kg ha}^{-1}$ ] and plant population levels [intra-row spacing of 20 cm ( $10.9 \text{ plants m}^{-2}$ ), intra-row spacing of 10 cm ( $21.7 \text{ plants m}^{-2}$ ) and intra-row spacing of 30 cm ( $7.2 \text{ plants m}^{-2}$ )] on plant height, stem diameter, millable stalk yield, seed yield, brix value, juice yield and theoretical ethanol yield in sweet sorghum. Data presented here is the mean of two independent experiments (Pooled analysis). Each value is the mean of five observations. Means with different letters in each row are significantly different at  $P \leq 0.05$ .

Trait	Nitrogen levels ( $\text{kg N ha}^{-1}$ )				Plant population levels			
	60	120	180	LSD	IR- spacing 10 cm	IR- spacing 20 cm	IR- spacing 30 cm	LSD
Plant height (cm)	197 <sup>b</sup>	198 <sup>b</sup>	221 <sup>a</sup>	6.1 <sup>***</sup>	190.3 <sup>b</sup>	205 <sup>a</sup>	222 <sup>a</sup>	7.2 <sup>*</sup>
Stem diameter (cm)	4.51 <sup>b</sup>	7.69 <sup>a</sup>	7.91 <sup>a</sup>	0.26 <sup>***</sup>	5.36 <sup>c</sup>	6.20 <sup>b</sup>	6.97 <sup>a</sup>	0.37 <sup>*</sup>
Millable stalk yield ( $\text{kg m}^{-2}$ )	7.1 <sup>b</sup>	8.2 <sup>a</sup>	8.1 <sup>a</sup>	0.36 <sup>***</sup>	6.7 <sup>c</sup>	7.9 <sup>b</sup>	8.8 <sup>a</sup>	0.46 <sup>*</sup>
Seed yield ( $\text{g m}^{-2}$ )	242 <sup>b</sup>	243 <sup>b</sup>	300 <sup>a</sup>	10.7 <sup>***</sup>	280 <sup>a</sup>	251 <sup>b</sup>	254 <sup>b</sup>	11.6 <sup>*</sup>
Brix value (%)	10.2 <sup>c</sup>	14.0 <sup>b</sup>	16.0 <sup>a</sup>	0.49 <sup>***</sup>	10.9 <sup>b</sup>	14.5 <sup>a</sup>	14.7 <sup>a</sup>	0.49 <sup>***</sup>
Juice yield (%)	41.2 <sup>b</sup>	56.4 <sup>a</sup>	58.3 <sup>a</sup>	3.1 <sup>*</sup>	40.8 <sup>c</sup>	55.6 <sup>b</sup>	60.2 <sup>a</sup>	4.2 <sup>*</sup>
Theoretical ethanol yield ( $\text{L ha}^{-1}$ )	3709 <sup>b</sup>	4281 <sup>a</sup>	4229 <sup>a</sup>	240 <sup>***</sup>	3484 <sup>b</sup>	4142 <sup>a</sup>	4593 <sup>a</sup>	255 <sup>*</sup>

IR- spacing - Intra-row spacing

(\*), (\*\*), Significant at  $P < 0.05$ , and  $< 0.001$ , respectively.

**Interaction of N and plant population:** The present study indicates that there were significant interaction of nitrogen levels and plant population levels for all the traits. Interaction of nitrogen and plant population revealed significant changes in plant height ( $P < 0.01$ ), stem girth ( $P < 0.05$ ), millable stalk yield ( $P < 0.01$ ), seed yield ( $P < 0.001$ ), brix value ( $P < 0.05$ ) and theoretical ethanol yield ( $P < 0.01$ ). The plant height in 10.9 and 7.2 plants  $m^{-2}$  population were same at 120 and 180 kg N  $ha^{-1}$ , however at 60 kg N  $ha^{-1}$ , 7.2 plants  $m^{-2}$  population were taller than 10.9 plants  $m^{-2}$  population and 21.7 plants  $m^{-2}$  population (Fig. 2a). High density plants recorded maximum decrease in plant height at 180 kg N  $ha^{-1}$  (9%) followed by 120 kg N  $ha^{-1}$  (7%) and 60 kg N  $ha^{-1}$  (5%) compared to 10.9 plants  $m^{-2}$  population. High

density plants recorded maximum decrease in stem girth at 180 kg N  $ha^{-1}$  (17.4%), followed by 120 kg N  $ha^{-1}$  (17.1%) and 60 kg N  $ha^{-1}$  (12.5%) compared to 10.9 plants  $m^{-2}$  population (Fig. 2b). High density decreased the millable stalk yield by 13.8 and 6.9% at 60 and 180 kg N  $ha^{-1}$ , respectively (Fig. 2d). Low density decreased the millable stalk yield by 15.3% at 60 kg N  $ha^{-1}$  compared to 120 kg N  $ha^{-1}$  (Fig. 2d). Low N level (60 kg N  $ha^{-1}$ ) decreased the brix value (Fig. 2c). Low N level (60 kg N  $ha^{-1}$ ) decreased the theoretical ethanol yield by 13.8 and 15.0% in high density and low density, respectively compared to 120 kg N  $ha^{-1}$  (Fig. 2e). Maximum seed yield ( $g\ m^{-2}$ ) was recorded in high density plants and maximum increase was recorded in 180 kg N  $ha^{-1}$  (20.9%) (Fig. 2f).

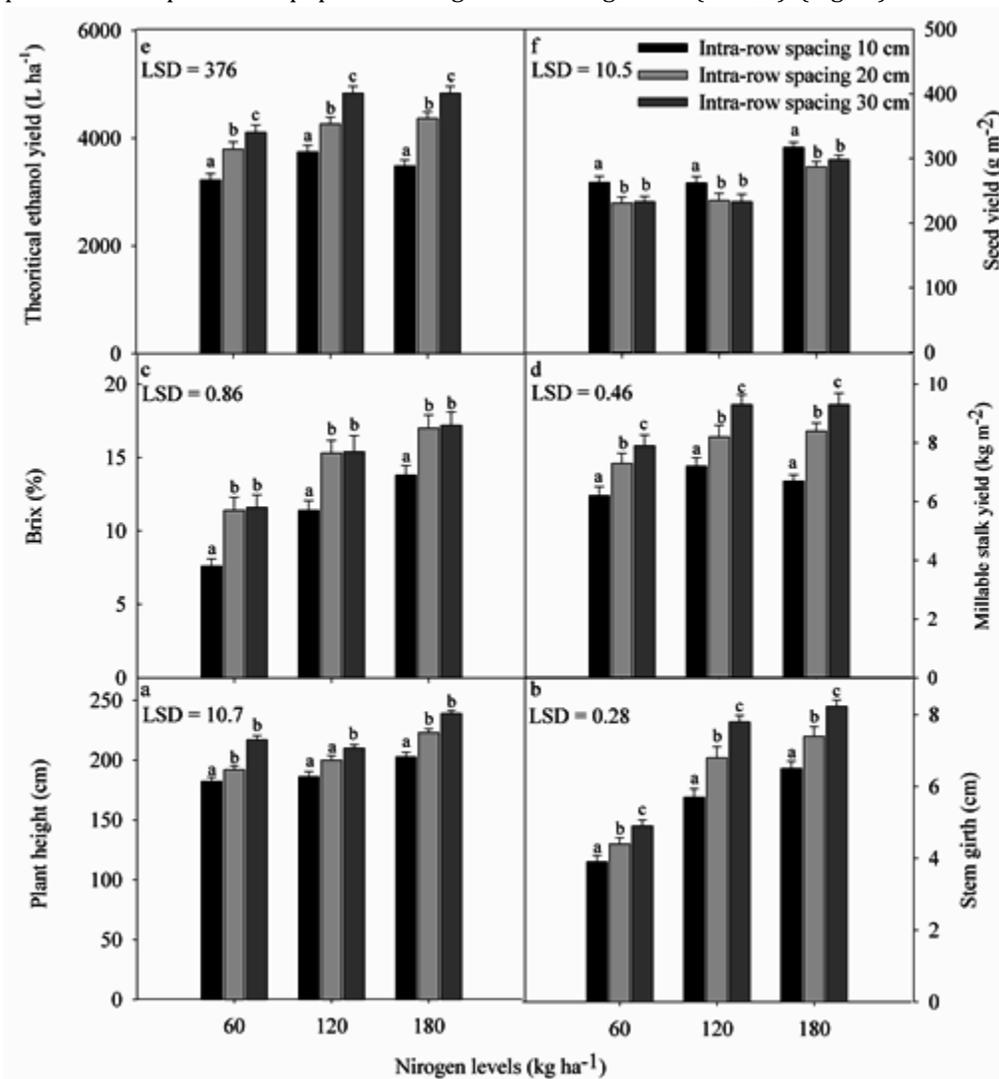


Fig. 2. Effect of nitrogen levels [60, 120 and 180 kg  $ha^{-1}$ ] and plant population levels [intra-row spacing of 10, 20 and 30 cm] on plant height (cm), stem girth (cm), brix value (%), millable stalk yield ( $kg\ m^{-2}$ ), theoretical ethanol yield ( $L\ ha^{-1}$ ) and seed yield ( $g\ m^{-2}$ ) of sweet sorghum. Each data point is the average of two independent experiments. A vertical bar denotes  $\pm$  S. E. of means.

**Plant growth regulators:** The present study indicates that there were no effects of year and interaction of year and plant growth regulators spray on all the traits recorded in the study. Foliar application of plant growth regulators significantly ( $P<0.01$ ) increased the plant height compared to the control. The tallest plant was observed in plots sprayed with salicylic acid 200 ppm (228.1 cm) and this was comparable with NAA 40 ppm (225.2 cm) (Table 2). Growth regulators significantly ( $P<0.01$ ) increased the stem girth (cm) of sweet sorghum. The crop sprayed with NAA and salicylic acid enhanced stem girth by 17.5 and 13.1% compared to unsprayed control plants (Table 2). There was significant difference among the treatments on millable stalk yield ( $P<0.01$ ), seed yield plant<sup>-1</sup> ( $P<0.01$ ) and brix value ( $P<0.01$ ).

Application of NAA 40 ppm increased millable stalk yield, seed yield plant<sup>-1</sup> and brix value by 26.5, 7.6 and

22.9%, respectively over unsprayed control. Likewise, salicylic acid also increased the above trait but the per cent increase was lower than NAA 40 ppm except for seed yield per plant (Table 2). NAA and salicylic acid significantly increased the juice yield ( $P<0.05$ ) to a tune of 27% over control. Reducing sugars content in the juice was significantly ( $P<0.01$ ) reduced by plant growth regulator application. Maximum reduction (25%) was recorded in NAA 40 ppm spray. NAA application significantly ( $P<0.01$ ) increased sucrose content (25.6%) followed by salicylic acid (11.9%) compared to unsprayed control (Table 2). Theoretical ethanol yield was significantly ( $P<0.01$ ) increased by foliar spray of plant growth regulator. NAA 40 ppm and salicylic acid increased the theoretical ethanol yield by 26.5 and 23.7%, respectively compared to unsprayed control (Table 2).

Table 2. Effect of plant growth regulators [NAA 40 ppm and salicylic acid 200 ppm] on plant height, stem diameter, millable stalk yield, seed yield, brix value, juice yield reducing sugars content, sucrose content and theoretical ethanol yield in sweet sorghum. Data presented here is the mean of two independent experiments (Pooled analysis). Each value is the mean of five observations. Means with different letters in each row are significantly different at  $P\leq 0.05$ .

Trait	Unsprayed control	NAA 40 ppm	Salicylic acid 200 ppm	LSD value
Plant height (cm)	194.0 <sup>b</sup>	225.2 <sup>a</sup>	228.1 <sup>a</sup>	13.0**
Stem diameter (cm)	4.73 <sup>c</sup>	5.56 <sup>a</sup>	5.35 <sup>b</sup>	0.18**
Millable stalk yield (kg m <sup>-2</sup> )	6.45 <sup>c</sup>	8.16 <sup>a</sup>	7.70 <sup>b</sup>	0.38**
Seed yield (g m <sup>-2</sup> )	436 <sup>c</sup>	469 <sup>b</sup>	482 <sup>a</sup>	14.3**
Brix value (%)	10.9 <sup>c</sup>	13.4 <sup>a</sup>	12.6 <sup>b</sup>	0.75**
Juice yield (%)	42.4 <sup>b</sup>	54.1 <sup>a</sup>	54.0 <sup>a</sup>	3.1**
Reducing sugars (%)	4.13 <sup>a</sup>	3.09 <sup>c</sup>	3.64 <sup>b</sup>	0.24**
Sucrose (%)	6.64 <sup>c</sup>	8.34 <sup>a</sup>	7.43 <sup>b</sup>	0.44**
Theoretical ethanol yield (L ha <sup>-1</sup> )	3354 <sup>c</sup>	4243 <sup>a</sup>	4149 <sup>b</sup>	197**

**Deheading and pollen sterilants spray:** The present study indicates that there were no effects of year and interaction of year and pollen sterilants spray on all the traits recorded in the experiment. Deheading and pollen sterilants application did not increase the plant height (Table 3). Deheading and pollen sterilants application significantly increased stem girth ( $P<0.01$ ) and millable stalk yield ( $P<0.05$ ). Sweet sorghum plants sprayed with ethrel (200 ppm) had greater stem girth (6.29 cm) and millable stalk yield (8.72 kg m<sup>-2</sup>) than p-coumaric acid 400 ppm and deheading (Table 3). Ethrel and p-coumaric acid increased stem girth and millable stalk yield by 35.2 and 33.5%, 35.8 and 26.1%, respectively compared to untreated control (Table 3).

Significant changes in sterility percentage ( $P<0.001$ ) and seed yield ( $P<0.001$ ) was achieved by foliar spray of pollen sterilants. Maximum sterility percentage was recorded in ethrel 200 ppm (69.3%) followed by p-coumaric acid (63.8%). The control plants had a sterility percent of 12.8% (Table 3). The seed yield was maximum in control (562.5 g m<sup>-2</sup>) followed by p-coumaric acid (455.8 g m<sup>-2</sup>) and ethrel (413.4 g m<sup>-2</sup>). Ethrel and p-coumaric acid decreased seed yield by 26.5 and 18.9%, respectively compared to unsprayed control (Table 3). Foliar spray of pollen sterilants significantly increased brix ( $P<0.001$ ) and juice yield ( $P<0.05$ ). Foliar application of ethrel and p-coumaric acid increased brix value by 21.9 and 11.4% respectively compared to

untreated control; however deheading decreased the brix value by 7.8% compared to untreated control. The juice yield was higher in p-coumaric acid (55.9%) which was on par with ethrel treatment (55.3%) followed by deheading treatment (22.8%) (Table 3).

Reducing sugars content was not significantly changed by deheading and pollen sterilants spray. However, sucrose contents of the juice was significantly ( $P < 0.01$ ) increased by pollen sterilants spray. Ethrel and p-

coumaric acid spray increased the sucrose content of the juice by 22.1 and 13.1%, respectively over unsprayed control; however, deheading decreased the sucrose content of the juice by 11% (Table 3). Significant ( $P < 0.01$ ) increase in theoretical ethanol yield was observed by foliar spray of pollen sterilants. The maximum theoretical ethanol yield was observed in ethrel foliar spray treatment (4534 L ha<sup>-1</sup>) followed by p-coumaric acid (4212 L ha<sup>-1</sup>) (Table 3).

Table 3. Effect of deheading and pollen sterilants [ethrel 200 ppm and p-Coumaric acid 400 ppm] on plant height, stem diameter, millable stalk yield, seed yield, brix value, juice yield reducing sugars content, sucrose content and theoretical ethanol yield in sweet sorghum. Data presented here is the mean of two independent experiments (Pooled analysis). Each value is the mean of five observations. Means with different letters in each row are significantly different at  $P \leq 0.05$ .

Trait	Control	Deheading	Ethrel 200 ppm	p-Coumaric acid 400 ppm	LSD value
Plant height (cm)	176.9 <sup>a</sup>	181.0 <sup>a</sup>	171.3 <sup>a</sup>	171.6 <sup>a</sup>	NS
Stem diameter (cm)	4.65 <sup>c</sup>	5.37 <sup>b</sup>	6.29 <sup>a</sup>	6.21 <sup>a</sup>	0.29**
Millable stalk yield (kg m <sup>-2</sup> )	6.42 <sup>d</sup>	7.53 <sup>c</sup>	8.72 <sup>a</sup>	8.10 <sup>b</sup>	0.40*
Sterility percent	12.8 <sup>c</sup>	-	69.3 <sup>a</sup>	63.8 <sup>b</sup>	3.2**
Seed yield (g m <sup>-2</sup> )	562 <sup>a</sup>	-	413 <sup>c</sup>	455 <sup>b</sup>	14.8**
Brix value (%)	11.4 <sup>c</sup>	10.5 <sup>d</sup>	13.9 <sup>a</sup>	12.7 <sup>b</sup>	0.39**
Juice yield (%)	41.2 <sup>c</sup>	50.6 <sup>b</sup>	55.3 <sup>a</sup>	55.9 <sup>a</sup>	3.1*
Reducing sugars (%)	4.46 <sup>a</sup>	4.22 <sup>a</sup>	4.23 <sup>a</sup>	4.19 <sup>a</sup>	NS
Sucrose (%)	7.22 <sup>c</sup>	6.42 <sup>d</sup>	8.82 <sup>a</sup>	8.17 <sup>b</sup>	0.35**
Theoretical ethanol yield (L ha <sup>-1</sup> )	3338 <sup>d</sup>	3915 <sup>c</sup>	4534 <sup>a</sup>	4212 <sup>b</sup>	202**

## DISCUSSION

The present study indicates that low density plants had increased plant height than high density plants (Fig. 2). It could be due to better radiation capture and improved conversion of radiation to assimilate (Whaley et al., 2000). In high density due to competition between the plants for the resources like N and light growth of the plant may be hampered. The plant height did not differ significantly between 120 and 180 kg N ha<sup>-1</sup>, indicating that 120 kg N ha<sup>-1</sup> is sufficient for optimal plant growth at low plant density compared to high plant density. The low density plants accumulated significantly increased biomass (millable stalk yield) and it was due to increased stem girth (Ponnusamy and Santhi, 2009; Djanaguiraman et al., 2005). Even though at high density there were three times more number of plants compared to low density, because of less stem girth (thin stem) the millable stalk yield was low. Low level of

nitrogen (60 kg N ha<sup>-1</sup>) reduced the biomass accumulation (Turgut et al., 2005) and there was no difference between 120 and 180 kg N ha<sup>-1</sup>, which indicates sweet sorghum can respond up to 180 kg N ha<sup>-1</sup> but optimum economic level was achieved in 120 kg N ha<sup>-1</sup> level itself (Ponnusamy and Santhi, 2009; Gutte et al., 2008).

High plant density increased seed yield and it could be due to more number of panicle per m<sup>2</sup> (Whaley et al., 2000; Turgut et al., 2005). Maximum brix value was observed in 120 and 180 kg N ha<sup>-1</sup>. Similar finding was reported by Gutte et al. (2008) that juice quality (brix value) was improved in 120 kg N ha<sup>-1</sup>. Maximum theoretical ethanol yield is the product of millable stalk yield and brix value, maximum millable stalk yield was recorded in low density and 120 kg N ha<sup>-1</sup>, and brix value was high in low density and higher N level.

However to achieve maximum biomass and brix 120 kg N ha<sup>-1</sup> with low density was found to be the best.

Foliar application of salicylic acid and NAA significantly increased the plant height compared to the control (Table 3). Increased plant height by salicylic acid was also observed in corn and beans and the increase in plant height was correlated with photosynthetic rate enhancement by salicylic acid (Khan *et al.*, 2003; Senaratna *et al.*, 2000; Bekheta and Talaat, 2009). Enhanced height with NAA application might be due to classical auxin mode of action namely cell wall extensibility and cell wall loosening (Sivakumar *et al.*, 2006). The crop sprayed with NAA had enhanced stem girth compared to unsprayed control plants. The increased stem girth by NAA may be due to enhanced cell division and cell enlargement of vascular cambium tissue (Jadhav *et al.*, 2007). The enhanced stem girth by salicylic acid spray is in accordance with the findings of Larque-Saavedra and Martin-Mex (2007) and Hussein *et al.* (2007), that foliar spray of salicylic acid (200 ppm) improved the stem diameter in maize through altered amino acids concentrations.

Salicylic acid increased seed yield to a higher per cent than NAA compared to control. Exogenous application of plant growth regulators can increase the transport of photosynthates from leaf to the developing parts (Nanjareddy *et al.*, 2003). The enhanced stalk yield by plant growth regulators application indicates that carbohydrate produced were mobilised and stored in the stalk. The enhanced stalk yield by salicylic acid may be associated with the increase in the endogenous level of auxin (Shakirova *et al.*, 2003). This can be explained by the inhibitory action of salicylic acid on IAA oxidase enzyme activity (Sivakumar *et al.*, 2006). Apart from the carbohydrate mobilization the increased millable stalk was due to increased stem girth by foliar application of plant growth regulators (Table 3). The present study clearly shows that NAA mobilized the carbohydrates from the leaf and accumulated in the stem; whereas, salicylic acid mobilized the carbohydrates to seed that has been seen as increased seed yield plant<sup>-1</sup> compared to NAA. The increase in sucrose content by NAA and salicylic acid spray may be due to repartitioning of carbon from insoluble matter and/or by continuous cleavage and synthesis of sucrose during the accumulation of sucrose in storage tissue (Rohwer and Botha, 2001). The enhanced glucose content in control plant might be due to cleavage of sucrose to glucose by either invertases or sucrose synthase activity

(Balibrea *et al.*, 2006). The increase in theoretical ethanol yield by plant growth regulator spray was due to increased millable stalk yield which was achieved by increased stem girth.

In our study, the highest percentage of floret sterility was obtained in ethrel 200 ppm. Similar results were reported in rice by Aswathanarayana and Mahadevappa (1991) and Praba and Thangaraj (2005) and in wheat by Rowell and Miller (1971) Luo *et al.* (1993) reported that the ethylene release rate of young panicles were higher when treated with ethrel. The ethylene release rate of young panicles was negatively associated with pollen fertility (Luo *et al.*, 1993). Hence, it can be concluded that ethrel was involved in the regulation of fertility change, and might have played a key role in the process of floret sterility in sweet sorghum. The male sterility inducing property of coumaric acid was reported in *Trigonella* by Kaul and Singh (Kaul and Singh, 1967). The greater accumulation of phenols in the leaves of thermo genic male sterile (TGMS) lines was found to be positively correlated with pollen sterility (Praba and Thangaraj, 2005).

The increase in millable stalk yield in ethrel, p-coumaric acid and deheading treatment over the control suggested that the stem served as a substitute for the grain as storage sink. Assimilates that ought to have been utilized for the development of grain, which usually constitutes the sink, were diverted to the stem. The enhanced stem girth by this treatments may be due to increased assimilates accumulation in the stem in lieu of panicle. The increase in millable stalk yield and stem girth by deheading is in accordance with the findings of Rajendran *et al.* (2000). The reduction in millable stalk yield in control plants over other treatments may be ascribed to the fact that the stem was a less powerful sink than the developing grains, hence decreased assimilate supply to the stem (Rajendran *et al.* (2000). The sucrose content of the juice was expected to rise as an immediate response to the deheading treatment; the subsequent growth of new tillers may trigger substantial losses in stored sucrose which can reduce the sucrose content in the juice of deheaded plants. As a consequence of the reduced sucrose content of the juice, the deheaded plants had lower brix value. The enhanced sucrose content in the juice of ethrel and p-coumaric acid sprayed plants may be due to higher mobilization of sugars from leaves to the stem because of higher floret sterility, but more likely due to an accumulation in the stem in the absence of an effective outlet into the

panicle as the primary “sink of choice”. The stem is then the primary sink, rather than a conduit to the primary sink. The use of ethrel or p-coumaric acid to achieve floret sterility is confirmed in our research. Since, the stem was a less powerful sink in sweet sorghum the use of pollen sterilants can increase the millable stalk yield coupled with higher sucrose content.

### CONCLUSIONS

From the study it was evident that maximum millable stalk yield and optimum brix value in sweet sorghum could be achieved by adopting 120 kg N ha<sup>-1</sup>, 7.2 plants m<sup>-2</sup> population (intra-row spacing of 30 cm with 45 cm inter-row spacing), foliar spray of NAA 40 ppm at boot leaf stage and ethrel 200 ppm spray at anthesis stage. However, to achieve maximum millable stalk yield with brix value, 180 kg N ha<sup>-1</sup> with a 7.2 plants m<sup>-2</sup> population (intra-row spacing of 30 cm) can be adopted. Further studies in respect to effect of various environmental conditions such as temperature, water level, light on millable cane yield are required for sustained production of sweet sorghum.

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