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Executive summary

Trial 1K.Ph-11: Characterizing and identification of new sorghum sources for high biomass for second generation biofuels traits: Evaluation sixteen sweet sorghum entries for both first and second generation biofuel traits indicated that SSRG 254 and SSRG 236 were the earliest ones. Entries SSRG204 (9%), SSRG 200 (13%) & SSRG 214 (15%) gave higher fresh biomass (9.0-15%). In fresh stalk yield, entries SSRG 204 (36%), SSRG 164 (19%) and SSRG 200 (15%) were superior. Sugar and bioethanol yields ranged from 0.63 to 2.62 t/ha and 335 to 1395 L/ha, respectively. SSV 74 alone recorded 28 % more sugar and bioethanol yields than control CSV19SS.

Trial 2K-Ph-11: Assessment of sweet sorghum for post-harvest deterioration of stalks and juice quality: Significant differences were observed for stalk yield, juice yield, TSS, RS and sucrose content. The stalk yield declined with increase in storage time. Mean juice yield declined significantly by 14% at the end of 3 days of storage. Mean total soluble sugars (TSS) had marginally increased (22%) across five day storage. Sucrose content decreased from 10.28% (control) to 7.39% at the end of 5 days of storage with a 47 % decline by 24hours. These results suggest that the sugar content in the stalks and their weights can be retained up to 2-3 days after harvest in the ambient field storage conditions under the mild winter conditions of November.

Trial 3K-Ph-11: Effect of staggered planting on stalk yield, sugar content and ethanol yield of sweet sorghum for increased harvest window: There was 18 day delay for days to flower when planted in 1st week of August than 1st week of June. Fresh stalk yield decreased by 11, 25 and 52%, respectively in 1st July (D3), 16th July (D4) and 1st Aug (D5) plantings over 1st June (D1). CSH22SS produced 11% more stalk yield than best variety SSV74. The yield among the varieties was similar across planting dates. Juice yield decreased significantly as planting delayed from June to August. CSH22SS gave 13% more juice yield than best variety SSV74. Mean sugar yields decreased significantly as plantings delayed from 1st June to 1st August. CSH22SS produced 18% more sugar yields than best variety SSV74. Computed bioethanol yields ranged from 526 to 1369 L/ha with a mean of 951 L/ha across plantings and cultivars. Highest ethanol yields were obtained with 1st June plantings (1127 L/ha). At Phaltan, 1st July (D3), 16th July (D4) and 1st August (D5) plantings produced 22, 49 and 129% lower bioethanol yields, respectively over 1st June (D1). Among the cultivars, hybrid CSH22SS (1113 L/ha) produced 18% more bioethanol yields than best variety SSV74 (941 L/ha). In varieties, both SSV84 and SSV74 were statistically on par for ethanol yields. These results clearly indicate that best sowing window for increasing stalk, sugar and bioethanol yields is between 1st June and 1st July.

Detailed report

The objectives of kharif physiology sweet sorghum evaluation program are:

i) To assess the performance and stability of sweet sorghum entries across a range of environments (latitudes) and planting dates and identify superior genotypes those are better than standards (SSV 84, CSV19 SS and CSH 22 SS).
ii) To characterize plant traits that contribute higher stalk yield, juice yield and total sugar content leading to higher ethanol recovery
iii) To quantify the staling effects on stalk and juice quality
iv) To evaluate and identify high biomass (sweet & non sweet) genotypes for second generation biofuel trait.
v) To characterize test environments for soil and climatic variable that determines sweet sorghum productivity and quality.

Sweet sorghum trials have been organized during kharif, 2011 at Parbhani, Rahuri, Phaltan, Bijapur and DSR, Hyderabad.

Trial 1K.Ph-11: Characterizing and identification of new sorghum sources for high biomass for second generation biofuels traits

Sixteen promising sweet and high biomass sorghum entries along with two checks (SSV 84, CSV 19SS) have been evaluated at 5 locations during kharif 2011. The data are presented in tables 1K1.1 to 1.6.

Crop phenology: Mean days to flowering varied from 77 to 106 days. SSRG 254 and SSRG 236 were the earliest ones among the test entries (Table 1K1.1). Days to maturity was also followed the similar trend those of flowering at all locations. Plant height ranged from 272 cm to 367 cm with a mean of 335 cm.

Total fresh biomass: Total fresh and dry biomass ranged from 42.0 – 60.5. Entries SSRG204 (9%), SSRG 200 (13%) & SSRG 214 (15%) gave higher fresh biomass (9.0-15%) more than control SSV 84.

Fresh stalk yield: Fresh stalk yield differed significantly across locations. It ranged from 31.1 to 57.2 t/ha with a mean of 38 t/ha. Among the test entries, SSRG 204 (36%), SSRG 164 (19%) and SSRG 200 (15%) produced higher stalk yields than control SSVR37. Stalk yield across locations ranged between 30.3 t/ha (Hyderabad) and 42.7 t/ha (Rahuri). This variation was due to the variability in climatic and edaphic conditions across the locations. Stalk yield had shown high significant positive correlation with juice yields ($r=0.837$; $p\leq0.01$).

Juice brix: Brix at physiological maturity varied between 13.44 and 18.5% with a mean of 16.4%. Among the test entries, none was superior to control SSRV37 (18.3%). Interestingly, brix content has shown very high positive correlations with TSS, sucrose content, and sugar yields ($r=0.900$, 0.907 & 0.497 res.; $p\leq0.01$)

Juice extraction: Juice extraction ranged from 27 to 37% with a mean of 32%. Among the test entries, none was superior to control. Furthermore, juice extraction had shown a high positive relationship with juice yields and ethanol yields ($p\leq0.05$).

Juice yield: In juice yield, test entries, SSRG164 (20% more), SSRG 200 (19%) and SSRG 204 (18%) and SSRV43 (16%) produced higher yields than control SSRV37. It was ranged from 8.2 to 14.0 KL/ha with a mean of 11.1 KL/ha.

Components of total sugars: Total soluble sugars (TSS) ranged from 9.54 to 15.22% with an average of 12.4%. None was significantly superior to check SSRV37. In non-reducing sugars (sucrose) too, the range observed was 7.90-13.7% with a mean of 10.76% and none was significantly superior to check.

Total sugar and bioethanol yields: Sugar yields ranged from 0.63 to 2.62 t/ha with a mean of 1.63 t/ha. Bioethanol yields too ranged from 335 to 1395 L/ha with mean of 870 L/ha. Among the test entries, SSV 74 recorded 28 % more sugar and bioethanol yields than control CSV19SS.

Trial 2K-Ph-11: Assessment of sweet sorghum for post-harvest deterioration of stalks and juice quality

Sweet sorghum cultivar CSV19SS and CSV24SS have been evaluated with an objective of assessing post-harvest deterioration of stalks and juice quality under ambient storage conditions. The stalks have been harvested at physiological maturity and were stored in the ambient field conditions for five days. The juice was extracted at one day interval up to five days. Data on stalk weight, juice brix, components of sugar were analyzed every day up to five-days after harvest along with a control (0 hour after harvest).Significant differences were observed for stalk yield, juice yield, TSS, RS and sucrose content. The stalk yield declined with increase in storage time (Tables 2 and 2K1.1-1.3). There was a marginal decline in stalk weight until 120 h due to rapid moisture loss but differences were not statistically significant.

As the days from ambient field storage increases, there was an increase in brix content (17.6 – 21.3%) mainly because of concentration of sugars. Juice extraction was slightly declined (6%) by 48 hr. Mean juice yield declined significantly by 14% at the end of 3 days of storage. Mean total soluble sugars (TSS) had marginally increased (22%) across five day storage, by the end of 3 days followed by no change, similar trend was observed for reducing sugars (RS) 1.31 to 9.15% primarily due to inversion. In case of non-reducing sugars (sucrose content), there was a decrease from 10.28% (control) to 7.39% at the end of 5 days of storage. The magnitude of decline was 47 % by 24hours. These results suggest that the sugar content in the stalks and their weights can be retained up to 2-3 days after harvest in the ambient field storage conditions under the mild winter conditions of November. The results need confirmation in the next kharif season.

Trial 3K-Ph-11: Effect of staggered planting on stalk yield, sugar content and ethanol yield of sweet sorghum for increased harvest window

This trial was organized at Rahuri, Parbhani, and Phaltan during kharif 2011. Four cultivars (V1: SSV 84, V2: SSV 74, V3: CSV 19SS and V4 CSH 22SS) were planted across five planting dates (D1: 1^{st} June, D2: 16^{th} June, D3: 1^{st} July, D4: 16^{th} July and D5: 1^{st} August 10) in a split-plot design adopting dates of planting as the main-plots and cultivars as subplots. The crop was managed adopting recommended packages practices. Minimal crop protection measures were followed to contain the insect pests. Data on phenology, stalk yield, sugar content were recorded as per standard protocols. Significant differences were observed for main effects and simple effects and their interactions for major traits. The data are presented in 3K3.1 to 3K3.4.

Crop phenology: Days to flowering varied between 73 and 89 days across planting dates and cultivars. Mean days to flowering were significant across planting dates and varieties, while, the interaction effects were non-significant. In general, days to flowering decreased as planting delayed from 1^{st} June to 1^{st} August 10. There was 18 day delay for days to flower when planted in st week of August than 1^{st} week of June. Similar trends were observed for days to maturity too.

Plant height (cm): Mean plant height varied from 245 to 375 cm. significant differences were observed for cultivars and planting dates but not for interaction. Mid-July and early August planting decreased plant height by 7% and 12% over 1^{st} June planting. Similarly, CSH 22SS grew taller than rest (3K3.1 to 3K3.4).

Fresh biomass: Fresh biomass differed significantly across planting dates and among cultivars, but the interaction effects were found non-significant (Table 3K3.3). Mean biomass ranged from 39.0 to 74 t/ha. Biomass production decreased (11%) when planted in 1^{st} week of July over 1 week of June, although they are statistically on par. Mid-July (D4) and early August (D5) plantings produced 30% and 62% lower biomass respectively, over 1st week of June (D1) planting. Among the cultivars, hybrid CSH22SS gave 10 %more biomass than CSV 74, while, it was similar among varieties.

Fresh stalk yield: Stalk yields varied from 28.0 to 52.0 t/ha across planting dates and cultivars. Differences for stalk yield were significant for both plantings and cultivars and their interaction effects at both Rahuri and Phaltan (Tables 3, 3K3.3 & Figure 2). As planting time delayed from June to August, stalk yield decreased. Mean stalk yield of 1^{st} June (D1) and 16^{th} June (D2) and 1^{st} July (D3) were statistically on par. Interestingly, stalk yield decreased by 11, 25 and 52%, respectively in 1^{st} July (D3), 16^{th} July (D4) and 1^{st} Aug (D5) plantings over 1st June (D1). Among the cultivars, hybrid CSH22SS produced 11% more stalk yield than best variety SSV74. The yield among the varieties was similar across planting dates.

Juice brix: Brix content varied from 16.3 to 20.0 % across planting and cultivars. Differences in mean brix were significant for plantings and cultivars and not for their interaction.

Juice extraction: Juice extraction ranged from 32.0 to 43.1% across planting and genotypes. Differences for extraction were found to be significant for cultivars, plantings but not for interaction.

Juice yield: It varied between 7.9 and 18.0 KL/ha. Significant differences were observed for, cultivars only. Juice yield decreased significantly as planting delayed from June to August. Juice yield among 1st June (D1), 16th June (D2) and 1st July (D3) were statistically on par. Among the cultivars, hybrid CSH22SS gave 13% more juice yield than best variety SSV74.

Components of total sugars: Total soluble sugars (TSS) varied from 13.0 to 14.4%. Mean TSS did not differed for plantings, cultivars and their interactions. 16 th June (D3) plantings gave marginally higher TSS. Similar trends were seen in non-reducing sugars (sucrose) too and it was ranged from 3.79 to 9.33% across plantings and cultivars.

Total sugar yield (t/ha): Mean total sugar yields ranged from 0.99 to 2.52 t/ha across plantings and genotypes. Differences in mean sugar yields were significant for cultivars only. At Rahuri and Phaltan significant differences observed for plantings and cultivars. Mean sugar yields decreased significantly as plantings delayed from 1st June to 1st August. Among cultivars, hybrid CSH22SS produced 18% more sugar yields than best variety SSV74.

Bioethanol yields (L/ha): Computed bioethanol yields ranged from 526 to 1369 L/ha with a mean of 951 L/ha across plantings and cultivars. Differences in bioethanol yields were significant for plantings and cultivars. Staggered plantings from 1st June to 1st August resulted in decreased bioethanol yields. Highest ethanol yields were obtained with 1st June plantings (1127 L/ha). Mean bioethanol yields were similar across locations. At Phaltan, 1st July (D3), 16th July (D4) and 1st August (D5) plantings produced 22, 49 and 129% lower bioethanol yields, respectively over 1st June (D1). It was evident from these results that planting of sweet sorghum between 1st June and 1st July has resulted in almost similar stalk sugar yields. This period is the best sowing window for increasing stalk and bioethanol yields. Among the cultivars, hybrid CSH22SS (1113 L/ha) produced 18% more bioethanol yields than best variety SSV74 (941 L/ha). In varieties, both SSV84 and SSV74 were statistically on par for ethanol yields. SSV74 produced 15% more ethanol yield than check CSV19SS.

Annexure 1: Selection criterion for evaluation and promotion of sweet sorghum for stalk yield and sugar traits

1. *Breeding objective*: To increase the total stem sugars (fermentable sugars such as glucose, fructose, and sucrose etc including starch in the juice) in sweet sorghum combining stalk yield, wide adaptation, and insect pest tolerance. The primary use of sweet sorghum currently for ethanol fermentation (as biofuel).
2. Sugar yield from sweet sorghum is a function of total stalk yield, juice extractability (juice volume) and the concentration of sugars (brix). Therefore, selection on the basis of their superiority for all these parameters along with the grain yield rather than any single or few of these parameters should be followed.
3. Test entries proposed for biofuel (bioethanol) production should have more than 15% total soluble sugars (TSS) with minimum levels of starch in their juice.
4. *Fresh stripped stalk yield*: 35 t/ ha minimum, or 500g /plant or above
5. *Juice extractability*: 45% and above in a 2 or 3-roller extractor based on 10 stalk data
6. *Juice brix*: 15% or above; Stalk brix (field)-16% or above with hand refractometer. *Sample size*: A sample size of 10 plants drawn at random front different rows leaving border rows from each replication should be taken for the estimation of stalk yield, juice yield and juice brix
7. *Estimation of total soluble sugars (TSS):* It is the total fermentable sugars such as glucose, fructose, and sucrose etc including starch in the juice. It can be estimated by according to the phenol sulphuric acid method of Dubois *et al* (1956) especially in the entries tested in the replicated trials when the brix is minimum 15%.
8. For segregating population or unreplicated data, it is suggested to use regression equation for predicting the total sugars by using juice brix. The equation is TSS= 0.1516 + brix % x 0.8746 (R^2 0.994**)- Source: Corleto and Cazzato E (1997); Reddy et al 2005)- NB:ICRISAT also follow the same procedure in advancing the breeding materials
9. *Recent additional information:* Recent results suggest that one can identify QTLs for sugar related traits based on stalk brix alone rather than elaborate or slow process of sample analyses using HPLC or (Please see Ritter *et al* 2008) in Mol. Breeding;22:367-384 .
10. *Juice extraction (%):* Crush the ten selected stalks using two-or three roller crusher so that the last drop of juice comes out from the stems. Take the juice weight, and calculate the extractability in percent using the data of fresh weight of ten defoliated stems [Juice Extraction (%) = 10 Plants Juice weight /10 plants fresh stem weight*100].
11. *Juice yield (KL/ha):* Measure the juice volume in liters from the above ten plants and then convert to net plot based on the plant stand in net plot and compute to KL/ ha.
12. *Sugar yields (t/ha):* This is the total sugar yields calculated taking account of TSS and juice volume. It is the important parameter for calculating computed ethanol yields in initial and advanced trials
13. *Sugar yields (t/ha) = TSS%/100 x Juice yields (KL/ha) -Source*: Corleto and Cazzato E (1997); Reddy et al 2005)- NB: AICSIP and ICRISAT follow similar procedure to calculate this parameter and advancing the materials
14. *Computed ethanol yields (L/ha):* This can be computed based on the sugar yields for the entries already entered in the initial and advanced trials.
15. Additionally, sweet sorghum and high biomass sorghums (with minimal level of stalk sugar, Brix14%) genotypes are evaluated for first and second generation biofuels.