

Original Research Article

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Molecular Characterization and Evaluation of Special Sugarcane Varieties Suitable for Co-Generation of Power

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ABSTRACT

Sugarcane is a major agro-industrial crop in India. The fibre content in sugarcane is gaining importance as fuel for co-generation of electricity. Hence, the present study was proposed to genetically characterize and identify suitable clones for co-generation with high yielding, optimum sucrose per cent, erect, non-lodging and good ratooning ability characters. Genetic analysis of 10 sugarcane hybrids was carried out using STMS primers. Out of 110 markers amplified, 77.27 % were polymorphic with an average of 8.5 polymorphic products per STMS primer. Similarity coefficient value of 0.79 and 0.48 was detected with closely and distantly related hybrids respectively. The average genetic similarity among the hybrids was ~78.6%. These results indicated the existence of moderate level of genetic diversity among these high biomass hybrids. Evaluation trial in replicated randomized block design including five early and five mid late clones was conducted at Regional Agricultural Research Station (RARS), Anakapalle during 2007 to 2010. Early clones viz., CoA 03081 (123.0 t/ha and 14.93 t/ha), CoA 02081 (122.0 t/ha and 14.78 t/ha) and CoA 07321 (118.7 t/ha and 14.10 t/ha) were significantly superior for cane and CCS yields than the best standard Co 7508 (104.0 t/ha and 13.34 t/ha). Midlate clones viz., CoA 07322 (137.0 t/ha and 17.12 t/ha) and CoA 02082 (132.0 t/ha and 17.00 t/ha) were significantly superior over the standard CoV 92102 (125.0 t/ha and 16.95 t/ha) for cane and CCS yields. The fibre content of the early and midlate clones ranged from 18.67 (CoA 02081) to 22.50 (CoA 03081) and 20.17 (CoA 07322) to 23.00 (CoA 03082), respectively. These elite clones recorded resistant reaction to red rot disease. The results revealed the potential of these clones suitable for co-generation of power with high yielding, high fibre content, optimum sucrose per cent and good ratooning ability.

Keywords

Sugarcane, high biomass, high fibre content, co-generation

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Introduction

Sugarcane (*Saccharum spp*) is a major agro-industrial crop in India. The byproducts of sugar industry provide raw material for

distilleries, pulp, paper industries and co-generation of power energy (Brian, 1995). To support cogeneration, there is need for identification of hybrids (energy canes) which is capable of producing high biomass with

higher fibre and higher total sugars content. In addition, hybrids must be amenable for multiple ratooning. It is estimated that one hectare of sugarcane can produce annually about 5 tonnes of fibre which is twice the yield of one hectare of forest with the same management at a fraction of the duration to renew the supply (Almazon *et al.*, 2001).

Among the byproducts of sugarcane, fibre is gaining importance now a day as fuel for co-generation of power energy. As sugar price is fluctuating from year to year, co-generation has become one of the important additional revenue to the industry. Interspecific crosses between conventional sugarcane (*Saccharum* spp. hybrid) and *Saccharum spontaneum*, *Saccharum barberi* and *Saccharum sinense* were conducted with the goal of improving desirable traits such as high biomass, high fibre, growth, vigour and ratooning ability (Wang *et al.*, 2008). *Saccharum barberi* and *Saccharum sinense* recorded the heritability of 0.91 and 0.92 for fibre and 0.47 and 0.87 for stalk biomass with commercial *Saccharum officinarum* and *Saccharum spp* hybrid, respectively.

Energy canes developed by making crosses of commercial sugarcane (a complex hybrid of *Saccharum spp*) with *S. spontaneum* clones produced high biomass plants with high fibre content and excellent ratooning ability besides tolerant to cold and diseases (Burner *et al.*, 2009). Govindaraj and Sreenivasa (2013) revealed that some of the interspecific and intergeneric hybrids were found promising for dual purpose energy canes with high biomass, fast growing, high tillering, high yielding, non-lodging and amenable for multiple ratooning when compared to high yielding commercial sugarcane hybrids. Hence, the present study is proposed to genetically characterize and identify sugarcane hybrids with high biomass, high fibre, high yielding, optimum sucrose content, erect, non lodging

and good ratooning ability suitable for cogeneration.

Materials and Methods

A total of ten hybrids comprising five each in early and midlate categories were evaluated in replicated randomized block design along with standards at Regional Agricultural Research Station (RARS), Anakapalle during 2007-2010. Parentages of hybrids *viz.*, early (CoA 01081, Co 86062 GC; CoA 02081, MS 6847 GC; CoA 03081, Co 8212 GC; CoA 05321, Co 740 PC; CoA 07321, 87A 298 x HR 83-65) and midlate *viz.*, (CoA 01082, 85A 261 x CoA 7602; CoA 02082, Co 97027 GC; CoA 03082, MS 6847 x Co 775; CoA 07322, 79A 28 x CoA 7602; CoA 08324, 86A 146 x 88A 162). Each clone was planted in 57.60 sq. m plot area adopting a seed rate of four three budded setts per meter.

Recommended dose of fertilizers, 112Kg N + 100Kg P₂O₅ + 120 Kg K₂O ha⁻¹ were applied. Irrigations were accorded at weekly intervals during formative phase of the crop. Weeding, earthing up, propping were carried out as per the recommendation.

Data on physiological parameters, number of millable canes, length of cane, cane diameter, single cane weight and cane yield per plot were recorded at harvest. Juice sucrose was determined at harvest (10th and 12th months) following the standard procedure (Meade and Chen, 1977). Juice extraction percent and fibre per cent was calculated at 10th month for early clones and 12th month for midlate clones.

Estimated CCS yield was determined based on CCS per cent and cane yield. Statistical analysis was performed as per the procedure of Panse and Sukhatme (1978).

Reaction to red rot disease under natural and artificial conditions was recorded against the

mixed inoculum of three predominant pathotypes (Cf 419, Cf 671 and Cf 997) in Andhra Pradesh. Reaction to smut under artificially inoculated conditions was also evaluated.

Number of green leaves

Number of green leaves on randomly selected tagged canes in each genotype was counted at 120 and 240 days after planting (DAP).

Leaf Area Index

Leaf area was measured by multiplying maximum leaf length (L) and width (W) of all green leaves (N) and constant factor (K) of individual leaf was calculated using linear measurement method standardized for sugarcane leaf area.

$$\text{Leaf area} = (L \times W) \times K \times N$$

Leaf area index (LAI) was computed by dividing leaf area and ground area at 120 and 240 DAP.

Juice extraction per cent

The juice extraction per cent was calculated by using the formula as

$$\frac{\text{Juice weight}}{\text{Cane weight}} \times 100$$
 and

Estimation of fibre content

The fibre content in cane was estimated by Rapipol extraction method and calculated as per the formula (Thangavelu and Rao, 1982)

$$\text{Fibre per cent} = \frac{A-B}{C} \times 100 \quad \text{where}$$

A=Dry weight of bag + bagasse after drying (g)

B = Dry weight of bag alone (g)

C = Fresh weight of cane (g)

Estimation of fibre per cent of cane by Rapipol Extraction Method

Top, middle and bottom portion of six canes were sampled and cut into small bits. Later, 250 gms of chopped cane bits were mixed and placed in the cup of the Rapipol extractor. Two liters of water is added and disintegrated in Rapipol extractor for 5 minutes. After decantation, 2 litres of water is added and mixed well for about 2 to 3 minutes and decanted without any loss of fibre. The above procedure is repeated once again, filtered quantitatively through a fine mesh filter and finally transferred quantitatively to a bag of known weight. It is dried at 110° C to constant weight. The per cent dry weight to fresh weight of material is calculated and expressed as fibre per cent cane.

Pol per cent of cane

$$= \text{Pol per cent in juice} \times \left[\frac{100 - (10 + F)}{100} \right]$$

Genomic DNA Isolation and PCR Amplification

Total genomic DNA was isolated as described by Doyle and Doyle (1987). PCR amplification and data analysis was performed by following the procedure of Govindaraj *et al.*, (2011).

Results and Discussion

Genotypic analysis

Twenty STMS primers were screened genetic analysis, out of which 9 were selected for diversity analysis based on clear and well resolved bands obtained. The number of amplified DNA fragments by each primer

ranged from 8 to 19. Out of 110 markers amplified, 77.27 % were polymorphic with an average of 8.5 polymorphic products per STMS primer (Table 1). Jaccard's similarity coefficient value of 0.79 and 0.48 was detected with closely and distantly related hybrids, respectively. The average genetic similarity among the hybrids was ~78.6%. These hybrids formed two major cluster based on Unweighted pair group method with arithmetic averages (UPGMA) cluster analysis (Fig 1). These hybrids further divided into subclusters based on their pedigree. These results indicated the existence of moderate level of genetic diversity among these high biomass hybrids.

Physiological Parameters

Data on number of green leaves and LAI at 120 and 240 DAP for both early and midlate clones were recorded (Table 2 & 3). The early clones, CoA 03081 (17.52 and 34.00) registered higher number of green leaves at

120 and 240 DAP followed by CoA 02081 (17.00 and 32.00) and were found to be significantly superior over the best standard Co 6907 (14.16 and 27.12), respectively. For LAI, the clones CoA 03081 (2.83 and 5.63) and CoA 02081 (2.99 and 5.22) recorded significantly higher value to the best standard Co 6907 (2.11 and 5.08) respectively at 120 and 240 DAP. Among the midlate clones, CoA 02082 (17.82 and 34.40) and CoA 07322 (17.20 and 35.60) were found to be superior over the standard CoV 92102 (15.50 and 30.84) for number of green leaves. For LAI, the clones CoA 07322 (2.98 and 5.90) and CoA 02082 (2.83 and 5.92) recorded maximum index value at 120 and 240 DAP (Table 2). The maximum biomass accumulation rate of sugarcane is as high as 550 kg ha⁻¹ day⁻¹ (Muchow *et al.*, 1994) under irrigated conditions. Large number of green leaves and high leaf area index of sugarcane grown in irrigated conditions could play important role in high biomass accumulation by manipulating the photosynthetic process.

Table.1 STMS markers used and polymorphism level detected.

| S. No. | Primer No. | Total Amplicons (No.) | Polymorphic Products (No.) | Per cent polymorphism | Band range size (bp) |
|--------|------------|-----------------------|----------------------------|-----------------------|----------------------|
| 1 | NKS16 | 08 | 07 | 87.50 | 049-285 |
| 2 | NKS21 | 11 | 07 | 63.63 | 052-169 |
| 3 | NKS25 | 19 | 14 | 73.68 | 051-638 |
| 4 | NKS26 | 08 | 07 | 87.50 | 115-341 |
| 5 | NKS28 | 11 | 09 | 81.81 | 062-499 |
| 6 | NKS34 | 08 | 08 | 100.0 | 140-462 |
| 7 | NKS40 | 09 | 06 | 66.66 | 90-512 |
| 8 | NKS45 | 09 | 07 | 77.77 | 98-600 |
| 9 | NKS48 | 18 | 14 | 77.77 | 043-818 |
| 10 | NKS50 | 09 | 06 | 66.66 | 123-725 |
| | | 110 | 85 | 77.27 | |

Table.2 Mean Performance of early clones for physiological, cane, CCS yields, quality and fibre characters over two plants and one ratoon crop

| Hybrids | Number of green leaves | | Leaf Area Index | | NMC ('000/ha) | Cane Yield (t/ha) | CCS Yield (t/ha) | Juice extraction per cent | Juice Sucrose per cent | Fibre per cent | Cane length (cm) | Cane diameter (cm) | Single cane weight (kg) |
|------------------|------------------------|---------|-----------------|---------|---------------|-------------------|------------------|---------------------------|------------------------|----------------|------------------|--------------------|-------------------------|
| | 120 DAP | 240 DAP | 120 DAP | 240 DAP | | | | | | | | | |
| CoA01081 | 15.30 | 28.20 | 2.79 | 5.00 | 119.00 | 115.67 | 12.87 | 54.00 | 17.85 | 19.36 | 282.33 | 2.43 | 1.00 |
| CoA02081 | 17.00 | 32.00 | 2.99 | 5.20 | 121.00 | 122.00 | 14.78 | 59.33 | 17.51 | 18.67 | 294.67 | 2.53 | 1.03 |
| CoA03081 | 17.52 | 34.00 | 2.83 | 5.63 | 111.33 | 123.00 | 14.93 | 54.00 | 17.17 | 22.50 | 337.67 | 2.87 | 1.06 |
| CoA05321 | 14.50 | 28.00 | 2.75 | 5.40 | 108.00 | 109.33 | 13.77 | 53.00 | 18.00 | 21.77 | 259.33 | 3.03 | 1.16 |
| CoA07321 | 16.55 | 31.60 | 2.72 | 5.10 | 112.67 | 118.67 | 14.10 | 54.00 | 17.83 | 20.47 | 276.33 | 2.97 | 1.15 |
| Standards | | | | | | | | | | | | | |
| Co 6907 | 14.16 | 27.12 | 2.11 | 5.08 | 104.67 | 99.33 | 12.13 | 55.67 | 17.77 | 14.73 | 293.67 | 2.52 | 1.02 |
| Co 7508 | 14.11 | 25.62 | 1.95 | 5.15 | 97.00 | 104.00 | 13.34 | 60.00 | 19.37 | 14.23 | 279.00 | 2.75 | 1.15 |
| CD (0.05) | 2.20 | 3.25 | 0.56 | 0.43 | 10.14 | 9.27 | 2.02 | 5.67 | 1.46 | 6.08 | 20.44 | 0.66 | 0.16 |
| CV(%) | 4.80 | 4.50 | 2.86 | 3.22 | 6.87 | 8.89 | 6.44 | 5.87 | 2.01 | 5.46 | 9.17 | 1.86 | 0.86 |

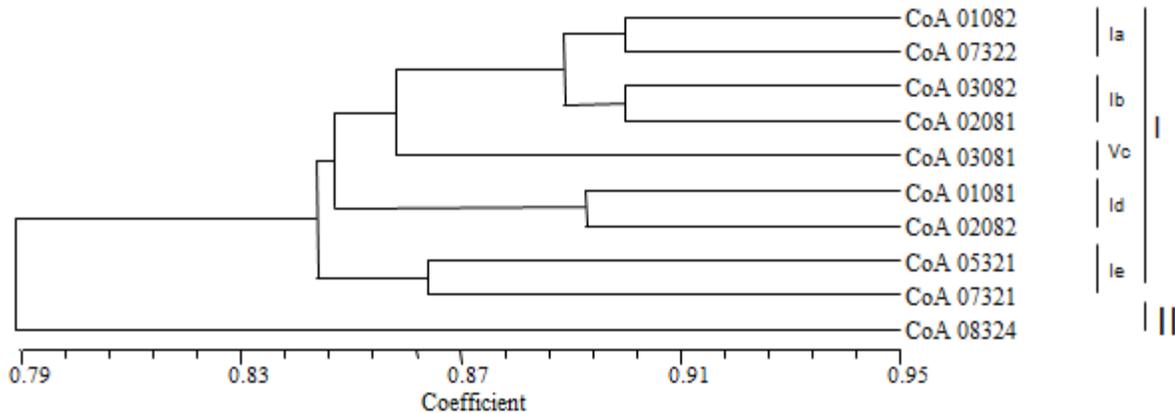
Table.3 Mean Performance of early clones for physiological, cane, CCS yields, quality and fibre characters over two plants and one ratoon crop

| Hybrids | Number of green leaves | | Leaf Area Index | | NMC ('000/ha) | Cane Yield (t/ha) | CCS Yield (t/ha) | Juice extraction per cent | Juice Sucrose per cent | Fibre per cent | Cane length (cm) | Cane diameter (cm) | Single cane weight (kg) |
|------------------|------------------------|---------|-----------------|---------|---------------|-------------------|------------------|---------------------------|------------------------|----------------|------------------|--------------------|-------------------------|
| | 120 DAP | 240 DAP | 120 DAP | 240 DAP | | | | | | | | | |
| CoA01082 | 16.80 | 32.20 | 2.78 | 5.65 | 126.00 | 124.67 | 16.15 | 45.00 | 19.50 | 20.43 | 350.0 | 2.50 | 1.04 |
| CoA02082 | 17.82 | 34.40 | 2.83 | 5.92 | 125.00 | 132.00 | 17.00 | 48.33 | 18.80 | 21.00 | 365.0 | 2.60 | 1.20 |
| CoA03082 | 16.44 | 32.25 | 2.80 | 5.64 | 138.00 | 128.00 | 15.63 | 55.33 | 18.80 | 23.00 | 320.0 | 2.60 | 1.80 |
| CoA07322 | 17.20 | 35.60 | 2.98 | 5.90 | 135.00 | 137.00 | 17.12 | 45.67 | 19.00 | 20.17 | 315.0 | 2.92 | 1.20 |
| CoA08324 | 16.22 | 33.84 | 2.52 | 5.42 | 138.00 | 130.00 | 15.56 | 47.33 | 19.50 | 21.03 | 320.0 | 2.86 | 1.15 |
| Standards | | | | | | | | | | | | | |
| Co 7219 | 14.40 | 31.83 | 2.64 | 5.50 | 131.33 | 122.00 | 15.81 | 52.33 | 20.50 | 16.00 | 350.0 | 2.84 | 1.20 |
| CoV92102 | 15.50 | 30.84 | 2.53 | 5.64 | 124.00 | 125.00 | 16.95 | 58.00 | 21.80 | 15.50 | 320.0 | 2.68 | 1.22 |
| CD (0.05) | 2.46 | 3.52 | 0.42 | 0.53 | 9.18 | 8.26 | 1.92 | 5.62 | 1.64 | 4.12 | 28.26 | 0.63 | 0.22 |
| CV(%) | 4.22 | 4.89 | 3.86 | 2.92 | 7.00 | 8.06 | 8.08 | 5.59 | 1.72 | 4.48 | 12.06 | 1.92 | 1.05 |

Table.4 Reaction to major diseases of early clones

| Early clone | Nodal method | | | Plug method | | | Smut | Mid late clone | Nodal method | | | Plug method | | | Smut |
|------------------|--------------|--------|--------|-------------|--------|--------|------|------------------|--------------|--------|--------|-------------|--------|--------|------|
| | Cf 419 | Cf 671 | Cf 997 | Cf 419 | Cf 671 | Cf 997 | | | Cf 419 | Cf 671 | Cf 997 | Cf 419 | Cf 671 | Cf 997 | |
| CoA 01081 | R | R | R | R | R | R | MS | CoA 01082 | R | R | R | R | R | R | MS |
| CoA 02081 | R | R | R | R | R | R | MS | CoA 02082 | R | R | R | S | S | S | MR |
| CoA 03081 | R | R | R | R | R | R | R | CoA 03082 | R | R | R | S | S | S | MS |
| CoA 05321 | R | R | R | R | R | R | MR | CoA 07322 | R | R | R | R | R | R | MS |
| CoA 07321 | R | R | R | R | R | R | MR | CoA 08324 | R | R | R | S | S | S | MS |
| Standards | | | | | | | | Standards | | | | | | | |
| Co 6907 | R | R | R | S | S | S | HS | Co 7219 | R | R | R | S | S | S | HS |
| Co 7508 | R | R | R | R | R | R | HS | CoV 92102 | R | R | R | R | R | R | MS |

Fig.1 Dendrogram showing genetic relationships among the 10 high biomass producing commercial varieties based on 85 polymorphic fragments generated by 10 STMS primer pairs.



Performance of early and midlate clones

Mean performance of early and midlate clones over two plants and one ratoon crop were statistically analyzed and presented in Table 2 & 3. The clone CoA 02081 (121.0 thousands/ha) and CoA 01081 (119.0 thousands/ha) recorded significantly higher NMC to that of best standard Co 6907 (104.7 thousands/ha). The clones CoA 03081 (123.0 t ha⁻¹ and 14.93 t ha⁻¹), CoA 02081 (122.0 t ha⁻¹ and 14.78 t ha⁻¹) and CoA 07321 (118.7 t ha⁻¹ and 14.10 t ha⁻¹) were found to be significantly superior over standards, Co 6907 (99.3 t ha⁻¹ and 12.13 t ha⁻¹) and Co 7508 (104.00 t ha⁻¹ and 13.34 t ha⁻¹) for cane and CCS yields. For per cent juice sucrose, the standard Co 7508 (19.37) recorded higher per cent juice sucrose than test clones. However, the clones CoA 05321 (18.00) and CoA 01081 (17.85) recorded higher percent sucrose and found to be on par with another standard Co 6907 (17.77). All the test clones recorded significantly lower juice extraction per cent to standards Co 6907 (55.67) and Co 7508 (60.00).

The test clones recorded significantly higher fibre percent compared to standards Co 6907

(14.73) and Co 7508 (14.23). Among the test clones, CoA 03081 (22.50) and CoA 05321 (21.77) recorded higher fibre per cent. The fibre percent improvement of these clones over Co 6907 was around 52% and 48%, respectively while fiber percent improvement of these clones over Co 7508 was around 58% and 53%, respectively. Overall, test clones recorded 23 % improvement of fibre content. Hence, with the improved fibre content of these test clones could be suitable for cogeneration of power. The standards Co 6907 (14.26) and Co 7508 (15.65) recorded higher pol percent cane than test clones.

For cane yield characters, the clones CoA 03082 and CoA 08324 (138.0 thousands/ha) followed by CoA 07322 (135.0 thousands/ha) recorded significantly higher NMC than Co 7219 (131.3 thousands/ha) and CoV 92102 (124.0 thousands/ha). For cane and CCS yields, the clone CoA 07322 (137.0 t/ha and 17.12 t/ha) followed by CoA 02082 (132.0 t/ha and 17.00 t/ha) and were found to be significantly superior over the best standard CoV 92102 (125.0 t/ha and 16.95 t/ha). Lower juice extraction values were recorded by all the test clones when compared to standards. It was observed from the study that the clones

with lower juice extraction values recorded higher fibre content and pol percent of cane values proved the nature of bio energetic canes (Govindaraj and Sreenivasa, 2013).

Average performance of early and midlate clones in two plants and one ratoon crop for yield components were presented in Table 2 & 3. All the test clones recorded higher cane length, medium cane diameter and single cane weight. Among the test clones, CoA 03081 (337.67 cm, 2.87 cm and 1.06 kg) and CoA 02081 (294.67 cm, 2.53cm and 1.03 kg) recorded higher yield components. Similar results of higher quality characters, high fibre, more NMC, higher cane length and medium thickness with high yielding energy canes were also reported by Govindaraj and Nair (2014) and Anna Durai (2014) for early clones in sugarcane.

Under midlate category, clone CoA 02082 recorded higher cane length (365.0 cm) and single cane weight (1.20 kg) but for cane diameter, the clone CoA 07322 (2.92 cm) was found to be significantly superior than the best standard Co 7219 (350.0 cm, 2.86 cm and 1.20 kg), respectively. Similar results of high biomass, high fibre, moderate quality parameters, maximum NMC and cane yield for biparental crosses were also reported by Burner *et al.*, (2009) and Alarmelu *et al.*, (2014) in sugarcane.

Disease resistance reaction of early and midlate clones

The early clones recorded resistant reaction for all the three pathotypes of red rot disease causing fungi under nodal and plug methods of inoculation. Highly susceptible to resistant reactions were recorded by the early clones for smut under artificially inoculated conditions (Table 4). The midlate clones, CoA 01082 and CoA 07322 recorded resistant reaction both under nodal and plug methods of inoculation for red rot disease whereas the clones, CoA

02082, CoA 03082 and CoA 08324 noted resistant reaction under nodal method of inoculation but susceptible reaction under plug method for red rot disease. A highly susceptible to moderately resistant reaction for smut was noticed. This indicates that these clones can also suitable for cultivation in disease affected areas.

This study revealed that the early clones (CoA 02081 and CoA 03081) and midlate clones (CoA 02082 and CoA 07322) were significantly superior for physiological traits, NMC, cane yield, fibre content, optimum sucrose content with multiple ratooning ability besides possess resistance to major diseases and were identified as high biomass varieties. Development of varieties with high tillering, high fibre and high biomass production, optimum sucrose, non lodging varieties not only help in increasing average cane yields and revenue of sugarcane growers but also a feed stock for cogeneration since many sugar factories rely on the fibre as fuel for cogeneration which is additional revenue to the industry. Overall value added varieties would extremely advantageous to the millers as well as farmers.

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