

**International Symposium on
Sugarcane Research Since Co 205 : 100 Years and Beyond
(SucroSym 2017)**

PROCEEDINGS

**September 18-21, 2017
Coimbatore, India**



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Organized by

**ICAR - Sugarcane Breeding Institute, Coimbatore
Tamil Nadu Agricultural university, Coimbatore
South Indian Sugar Mills Association (SISMA), Tamil Nadu
Society for Sugarcane Research and Development, Coimbatore**



CONTENTS

PLENARY LECTURE

Status of sugarcane agriculture and sugar industry	i
--	---

Bakshi Ram

SESSION I

SUGARCANE GENETIC RESOURCES AND PRE-BREEDING FOR ENRICHING GENE POOL

Alloplasmcy for improvement of agronomic traits in sugarcane	1
--	---

M. N. Premachandran, Maya Lekshmi, V. Raffee Viola, A. K. Remadevi and Adhini S. Pazhany

Characterization of interspecific progenies from poly crosses on two <i>Saccharum robustum</i> clones belongs to the forma 'sanguineum'.....	3
--	---

K. Chandran, M. Nisha and P. P. Gireesan

Cytogeographical survey of <i>Erianthus arundinaceus</i> (Reitz.) Jeswiet (a wild relative of sugarcane).....	5
---	---

A. Suganya, Nandan P. Suresh and R. Gayathri

Pre-breeding utilizing the improved intraspecific clones of <i>Saccharum</i> spp.....	7
---	---

S. Alarmelu, Adhini. S. Pazhany, C. Jayabose and T. Manjunatha

Developing Multiparent Advanced Generation Inter-Cross (MAGIC) population in sugarcane; Evaluation of founder parents for drought tolerance.....	11
--	----

K. Mohanraj, G. Hemaprabha, S. Vasantha and R. Raja

Improved <i>Saccharum officinarum</i> clones as a potential parental source for sugarcane improvement.....	13
--	----

R. Karupaiyan, N. Vijayan Nair, Bakshi Ram, K. Mohanraj, A. Annadurai and P. Amudha

Exploration and collection of <i>Saccharum</i> diversity in the North Western India.....	16
--	----

S. Karthigeyan, P. Govindaraj and Adhini S. Pazhany

Study on phenotypic characterization of <i>Saccharum spontaneum</i> L. in Germplasm Collection – An Exploration and Cataloguing Perspective.....	18
--	----

C. Jayabose, Adhini S. Pazhany, S. Karthigeyan, A.K. Rema Devi and V. Aravindhan

Defuzzed true seed for maintenance of Sugarcane germplasm	19
---	----

Rajendra Prasad Narra

Performance of exotic hybrid germplasm of sugarcane for yield and quality traits.....	21
---	----

Nisha. M, Chandran K, ArunKumar. R and Gireesan P P

Assessing the inheritance of red flesh color and antioxidant activity from the polycross progenies of <i>Saccharum robustum</i> genotypes.....	24
--	----

G.S. Suresha, K. Chandran, M. Nisha, Arun Kumar and K. Hari

Phenotypic divergence among <i>Saccharum spontaneum</i> clones.....	25
---	----

V. Vinu, T. Lakshmiathy and H. K. Mahadevaswamy

Flowering behaviour of Panicoideae grasses of <i>Saccharum</i> complex.....	28
<i>C. Jayabose, Adhini S. Pazhany, A.K. Rema Devi and V. Aravindhan</i>	
An innovative prebreeding approach to develop true breeding inbreds in sugarcane by harnessing the diploidization mechanism.....	30
<i>A.J. Prabakaran</i>	
Evaluation of intra-specific hybrids of <i>Saccharum robustum</i> for yield and quality traits.....	32
<i>R. Karuppaiyan, N. Vijayan Nair, Bakshi Ram, K. Mohanraj, A. Annadurai and P. Amudha</i>	
Character association and principal component analysis in interspecific hybrids of <i>Saccharum robustum</i>	36
<i>E. Karpagam and S. Alarmelu</i>	
Genome modifications in wide hybrids involving <i>Saccharum</i> and <i>Erianthus</i>	40
<i>Maya Lekshmi, Adhini S. Pazhany, V. P. Sobhakumari, A. K. Rema Devi, M. N. Premachandran</i>	
Evaluation of <i>Saccharum</i> and <i>Erianthus</i> introgressed hybrids for Juice quality, cane fibre and biomass production.....	43
<i>V. Sreenivasa, P. Govindaraj and H K Mahadeva swamy</i>	
Identification of drought tolerant ISH and IGH clones in sugarcane (<i>Saccharum</i> spp.).....	47
<i>T. Lakshmi Pathy, P. Govindaraj and H.K. Mahadeva swamy</i>	
Genetic enhancement of sugarcane through wild related species.....	50
<i>Adhini S. Pazhany, M. N. Premachandran, Mayalekshmi and A.K. Remadevi</i>	

SESSION II

BREEDING STRATEGIES-PAST, PRESENT AND FUTURE – LEAD PRESENTATIONS

Glimpses of century old sugarcane improvement programme in India (1918-2017).....	53
<i>B.D. Singh and Bakshi Ram</i>	
The prospects of sugar beet in India.....	90
<i>A. D. Pathak, Varucha Misra and A. K. Mall</i>	
Relative performance of Coimbatore canes (Co canes) for major component traits of yield and quality and an analysis of their genealogies to measure genetic gain over a century of sugarcane breeding at ICAR Sugarcane Breeding Institute.....	98
<i>G. Hemaprabha, K. Mohanraj, S. Alarmelu and Bakshi Ram</i>	
Varietal distribution in sugar factory areas of Uttar Pradesh.....	103
<i>Ram Murti Singh,</i>	
Plant phenomics: a new era of exciting opportunities for sugarcane breeding and crop improvement.....	105
<i>Prakash Lakshmanan</i>	
Yield stability analysis on sugarcane cultivars trials in high rainfall area of Maharashtra....	110
<i>R.S. Hapase, J.M. Repale and D. S. Pawar</i>	
Sugarcane cultivation and breeding program development in Khuzestan province of Iran...	113
<i>K. Taherkhani, M. Parvizi Almani, H. Hamdi and A. Derakhshanzadeh</i>	

Appraisal of different crossing methods adopted by fluff receiving centers of AICRP(S) for sugarcane improvement programme in India.....	114
<i>A. Anna Durai, Adhini S Pachary, G. Hemaprabha and Bakshi Ram</i>	
Intrinsic root characteristics of sugarcane varieties under hydroponic conditions.....	116
<i>K. Hari, S. Vasantha, A. Anna Durai, G.S. Suresha, P. Sruthi, C. Brindha, Rajesh Kumar and P. Sivaraj</i>	
Genetic enhancement of sugarcane for organic jaggery production.....	117
<i>B. Patil Sanjay, C. Devaraj and Meti Vijaykumar</i>	
Identification of traits and development of sugarcane genotypes for improving nitrogen economy.....	121
<i>Kuldeep Singh, Vikrant Singh, RS Singh and Kulvir Singh</i>	
Family selection in sugarcane (<i>Saccharum</i> L.) for cane yield traits, reaction to red rot (<i>Colletotrichum falcatum</i> Went) and sugar content.....	122
<i>D.K. Pandey, Deeksha Joshi, P.K. Singh, J. Singh, Sanjeev Kumar and D R Malaviya</i>	
Biological hardening of micro propagated sugarcane plants with plant growth promoting bacteria to enhance the quality of seedling and yield improvement.....	124
<i>D. Neelamathi, S. Vasantha, K. Hari, BMS Baghel, L. Vidhyanandhini Nici Ashok</i>	
Response of different sugarcane varieties against salinity in in-vitro condition through callus culture.....	126
<i>S. C. Mali, V. L. Parmar, U. Jagdish and A. R. Pathak</i>	
Strategies towards development of superior sugarcane varieties for adverse climate of sub tropical India : history, challenges and opportunities	128
<i>Ravinder Kumar</i>	
Molecular characterization of sucrose specific genic microsatellites among Indian Co canes evolved through varietal improvement programmes.....	130
<i>R.M. Shanthi, G. Hemaprabha, R. Vigneshwari and T.S. Sarath Padmanabhan</i>	
Genetic diversity among 133 elite genotypes of sugarcane for their use as parents in sugarcane varietal improvement.....	135
<i>T.S. Padmanabhan Sarath and G. Hemaprabha</i>	
Population structure and genetic diversity characterization of <i>Saccharum</i> interspecific hybrids using SSR markers.....	138
<i>E. Karpagam and S. Alarmelu</i>	
Co 06022: A promising high yielding and high quality early maturing sugarcane variety for Tamil Nadu	142
<i>C. Appunu, A. Anna Durai, K. Mohanraj, G. Hemaprabha, Bakshi Ram, M. Jeyachandran, S. Ganapathy, M. Shanmuganathan, A. Thirumurugan and N.A. Saravanan</i>	
Estimation of genetic parameters in early generation clones of sugarcane.....	144
<i>Arvind Singh Negi, S P Singh, A S Jeena and K A Khan</i>	
Genetic diversity among the early generation clones of sugarcane (<i>Saccharum</i> species complex) based on morphological characterization.....	145
<i>Neetu, A. S. Jeena, Usha Pant, S. P. Singh, K. A. Khan and Deepak Koujalagi.</i>	
Cross - combination of proven parent for sugarcane yield & H.R. Brix % in Indo-Gangetic plains of Uttarakhand.....	146
<i>Sidharth Kashyap, V.K. Tyagi, Sanjay Kumar, Pramod Kumar and Shailbala.</i>	

Evaluation and identification of promising new sugarcane clones suitable for Rajshree Sugars command area.....	181
<i>S. Jayaram, B. Vijayakumar, C.G. Balaji and V. Velmurugan</i>	
Co V 16-356 - An early maturing sugarcane variety with improved yield and quality suitable to Coastal Andhra.....	182
<i>V. Satya Priya Lalitha, K. Krishnumma and K. Jhansi</i>	
Studies on flowering behaviour of commercial and hybrid sugarcane clones under Bangalore conditions in India.....	184
<i>S. Rajeswari, P. Bharathi and Raghu Sankaran</i>	
Evaluation of superior cross combinations and identification of better parental stocks from early clonal trials.....	187
<i>V. Sreenivasa, C. Mahadevaiah and G. Hemaprabha</i>	
Evaluation and selection of early sugarcane clones (<i>Saccharum</i> sp. Hybrid) for fodder yield related traits.....	189
<i>M. Shanmuganathan, V. Baskaran and R. Nageswari</i>	
Identification of early maturing sugarcane genotypes suitable for delayed harvesting conditions.....	190
<i>T. Chitkala Devi, K.V. Ramana Murthy, V. Gouri and M. Bharathalakshmi</i>	
Characterization of CCS yield component traits through correlation and principal component analysis at first clonal stage in sugarcane.....	193
<i>R. Vigneshwari, R.M. Shanthi and K. Mohanraj</i>	
Evaluation of progenies derived from genetically diverse crosses based on Coefficient of Co ancestry revealed their association and nature of gene action for number of millable cane, cane thickness and Brix in sugarcane.....	196
<i>C. Mahadevaiah, G. Hemaprabha, A. Anna Durai, Adhini S. Pazhany and Mahadevaswamy</i>	
Evaluating the potential of sugarcane families by DTOPSIS.....	199
<i>S. Alarmelu, Adhini. S. Pazhany, E. Karpagam, K. Elayaraja and G. Hemaprabha</i>	
Mixed model analysis in sugarcane varietal evaluation programme in India.....	201
<i>Rajesh Kumar, A.D. Pathak and Bakshi Ram</i>	
Tissue culture technique : a potential tool for sugarcane varietal improvement in Sub-tropical India.....	207
<i>D.N. Kamat, Balwant Kmar and S.S.Pandey</i>	
Impact of sugarcane breeder seed production through three tier seed programme in Bihar..	209
<i>A. K. Mall, Dinesh Singh, S. N. Sushil, B. D. Singh and A. D. Pathak1</i>	
Three tier clean seed nursery programme through tissue culture methods at E.I.D. Parry Sankili- Andhra Pradesh, India.....	210
<i>P. Prabakaran, V. Ganapathi, M. Bharathi, K. Chinnabbaiah and S. Raghu</i>	
Effect of media and growth hormone on invitro rooting of sugarcane varieties (<i>Saccharum officinarum</i> .L).....	212
<i>S. Sheelamary, L. Vidhya Nandhini and Braj Mohan Singh Baghel</i>	
Discriminant function analysis in mid-late maturing clones of sugarcane	215
<i>T.E. Nagaraja, V.N. Patel, P. Thimmegowda, B.T. Ravindrababu and B.P. Sunitha</i>	
Performance of sugarcane varieties for jaggery production under very heavy rainfall areas of central Travancore region of Kerala.....	216
<i>V.R. Shajan, G. Jayakumar, C.R. Rini and Jessy M Kuriakose</i>	

2n=60 were observed by Tagane *et al.* (2011). The Indian collections were more diverse with presence of three cytotypes. Presence of 2n=40 in the mainland and island indicates their geographical proximity (Nair and Praneetha, 2006). However, the lower cytotype 2n=30 was observed only in older collections of 1915-1951 in the areas of Orissa, Uttar Pradesh, Punjab and Pakistan with a frequency of 6.25%. Expedition strategies are to be prepared for collection of this cytotype based on monograph/flora of different states.

As the lowest cytotype 2n=20 was as observed to be a rare occurrence, the evolution of different ploidies might have occurred through functioning of restitution in gametes (male or female or both). Androgenic lines have been observed in intergeneric crosses involving *E. arundinaceus* (Anon, 1991). Further studies on meiotic behavior, pollen mitosis of different cytotypes and segregation in the selfs are required for confirmation.

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I-O4

PRE-BREEDING UTILIZING THE IMPROVED INTRASPECIFIC CLONES OF *SACCHARUM SPP*

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repeated use of few parents in hybridization have resulted in narrow genetic diversity in modern sugarcane cultivars. Currently sugarcane cultivars under cultivation have a narrow genetic base tracing back less than 20 *S.officinarum*, two *S.spontaneum* and a couple of *S. barberi* and *S.sinense* clones which offers a major threat to the sugarcane productivity and demands concerted efforts to utilize new germplasm. Pre-breeding aims at creating new base population by gene introgression that enhances genetic variability in the germplasm for direct use in breeding programmes. It can also assist in identifying heterotic patterns for hybrid programs. Backcrossing is an efficient way to transfer characteristics controlled by one or two genes, although it can be used also for many number of genes, including quantitative characters. Eberhart (1971) suggested that the first backcross to adapted germplasm is the best base population to start selection. In our study, elite improved clones of *S.officinarum* and *S.robustum* produced through intra population improvement programme at ICAR-Sugarcane Breeding Institute (Nair *et al.*, SBI Annual Report 1991-1996) and commercials were utilized in introgression through backcross breeding. The back cross generations and nobilized hybrids (F_1 , BC_1 , BC_2) were explored for their heterotic pattern and breeding potential.

Selection during nobilisation: The prebred clones developed (utilizing improved *S. officinarum*, improved *S. robustum* and commercial Co canes), from three nobilized generations and different mating groups exhibited extensive variability for both qualitative and quantitative traits. They expressed wide variability for various traits like cane population, cane height, cane diameter, canopy colour, internode shape, internode color, spines and flowering. Significant variation was recorded for cane growth and yield characters. The means for the F_1 , BC_1 and BC_2 generations showed progressive increase of yield traits and sucrose % with successive stages of nobilisation. Heritability estimates varied over the different nobilising generations. Heritability estimates from this study, indicate that selection of parents for sucrose % during the nobilisation process will be effective. The results indicate that gain from selection for yield traits like number of millable canes and single cane weight at the BC_1 and BC_2 stages was moderate to high. In general, sucrose % have shown moderate to moderately high heritability and clump yield of low to moderate heritability.

Genetic improvement in the backcross generations: In the improved *S.robustum* x improved *S.officinarum* group, the first generation nobilized hybrids showed an improvement of 15.12% for sucrose % at 360 days over the improved *S.officinarum* parents. BC_1 hybrids showed an improvement of 16.71% and 34.09 % for sucrose % at 360 days in comparison with improved *S.officinarum* and *S.robustum* parents respectively. BC_2 hybrids showed an improvement of 22.36% and 40.58 % for sucrose % at 360 days in comparison with improved *S.officinarum* and *S.robustum* parents respectively. Number of millable canes, cane height and single cane weight exhibited a substantial improvement compared to their improved *S.robustum* *S. officinarum* parents. BC_1 and BC_2 exhibited improvement for both yield and quality parameters suggesting further exploitation. The hybrids from the improved *S.officinarum* x improved *S.robustum* group also exhibited improvement for both yield and quality traits. There was an improvement of 23.57 % for sucrose in F_1 , 30.37 % in BC_1 and 33.35 % in BC_2 in comparison with improved *S.officinarum* parents. Similarly an improvement of 41.97 %, 49.78 % and 53.21 % was observed in F_1 , BC_1 and BC_2 respectively in comparison with improved *S.robustum* parents. The BC_3 hybrids from these two mating groups also exhibited improvement for yield and quality traits suggesting further studies on cytogenetical behaviour and breeding potential in further backcross generations.



Genetic Improvement in backcross population of Improved *S.robustum* x Improved *S.officinarum*

Improved <i>S.robustum</i> x <i>S.officinarum</i>	Performance at 360days			
	NMC /row	C.Ht (cm)	SCW (kg)	Sucrose % (360 d)
F ₁	91.00	270.00	0.99	18.12
% imp over <i>S.o</i> parents	82.00	17.39	-7.48	15.12
% imp over <i>S.r</i> parents	37.88	8.00	16.47	32.26
BC ₁	85.00	280.00	1.13	18.37
% imp over <i>S.o</i> parents	70.00	21.74	5.61	16.71
% imp over <i>S.r</i> parents	28.79	12.00	32.94	34.09
BC ₂	74.00	300.00	1.34	19.26
% imp over <i>S.o</i> parents	48.00	30.43	25.23	22.36
% imp over <i>S.r</i> parents	12.12	20.00	57.65	40.58
<i>S.officinarum</i> parents	50.00	230.00	1.07	15.74
<i>S.robustum</i> parents	66.00	250.00	0.85	13.70

Genetic improvement in backcross population of Improved *S.officinarum* x Improved *S.robustum*

	Performance at 360 days			
	NMC /row	C.Ht (cm)	SCW (kg)	Sucrose % (360 d)
F ₁	70.00	252.00	1.09	19.45
% imp over <i>S.o</i> parents	40.00	9.57	1.87	23.57
% imp over <i>S.r</i> parents	6.06	0.80	28.24	41.97
BC ₁	78.00	255.00	1.14	20.52
% imp over <i>S.o</i> parents	56.00	10.87	6.54	30.37
% imp over <i>S.r</i> parents	18.18	2.00	34.12	49.78
BC ₂	81.00	258.00	1.38	20.99
% imp over <i>S.o</i> parents	62.00	12.17	28.97	33.35
% imp over <i>S.r</i> parents	22.73	3.20	62.35	53.21
<i>S.officinarum</i> parents	50.00	230.00	1.07	15.74
<i>S.robustum</i> parents	66.00	250.00	0.85	13.70

Breeding potential of pre-bred clones

From the study, the crosses PIR 001157 x PIO 00845, PIO 001057 x PIR 00 1062, PIR 03-107 x PIO 96-475, PIO 94-345 x PIR 96-258, PIR03-107 x PIR 96-475 ,PIO 001100 x PIR 001174 gave more selections combining yield and quality traits. Crosses with PIR 96-285, PIR 001188, 99 -269 as one of the parents were identified as potential with high quality recombinants.

Significant variation was recorded for growth and yield characters in the prebreeding population. Among the prebred materials, 26 clones recorded single cane weight > 1.25 kg, 45 clones with brix % above 20 % and 41 clones with juice sucrose above 18.5 % and most of them were derived from the crosses involving improved *S.officinarum* x Improved *S.robustum*. The clones viz., 13-36,13-



38,13-39,13-44,13-78,13-80,13-125,13-150,13-151,13-198,13-272,13-266 and 13-250 with good field stand, yield and quality parameters comparable with Co 86032 were identified as genetic stocks for further utilization. The clones 13-69, 13-103 and 13-251 showed substantial improvement for sucrose % at 360 days. The clone 13-147 recorded the maximum single cane weight of 1.45 kg.

The pre-bred clones were tested for tolerance to water deficit conditions, red rot and for natural incidence of smut. The clones involving PIR 001057, PIR 001058, PIR 96-285, PIR 00 1022 as parents in the back crosses were moderately resistant and moderately susceptible to red rot. The promising clones did not show natural incidence of smut. Under water deficit conditions, 25 clones exhibited good growth and response, coupled with high yield (95-98.0t/ha) and sucrose (19.85 -20.94%) indicating their ability of adaptation under changing climate. IOR 360 showed significant improvement for cane yield (21.05%) and sucrose (10.14 %) followed by IOR 396, 258, 1100, 1136 and 1128. These clones also combined resistance to red rot. Earlier studies have indicated that clones with *S. robustum* cytoplasm performed better at high temperature and water deficit conditions and hence the pre-bred clones with genetic base of *S. robustum* might prove as a potential donor for tolerance to high temperature. Forty prebreeding clones were selected based on genetic diversity and were screened for molecular diversity using sugarcane specific markers. Variation in allelic size was detected within the range of 122-1136 bp and maximum of 33 alleles. Among the SSR primers used, NKS 34_{142 & 185} were specific to PIR 00 1057, marker NKS 8_{181, 112 & 336} were specific to PIR 00 1057, Maximum molecular diversity was detected in the crosses involving PIR 00-1174, PIR 00-1163, PIR 001057 as female parents indicating the use of these clones as diverse parents in hybridization.

Though introgression of wild germplasm is time consuming and requires considerable efforts and resources, it is very important to improve the commercial breeding populations. Pre-breeding strategy in our study through back crossing has helped in identifying clones combining yield, quality and resistance to red rot and smut. Utilization of these clones by the breeders will help in the development of new breeding populations to work upon and on identifying clones to improve resistance to red rot, smut and tolerance to drought and salinity stresses.

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I-P10

CHARACTER ASSOCIATION AND PRINCIPAL COMPONENT ANALYSIS IN INTERSPECIFIC HYBRIDS OF *SACCHARUM ROBUSTUM*

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The multiple usage of sugarcane crop necessitates a breeding program that generates gene pool which enables the identification of genotypes for multiple purposes. Once the magnitude and pattern of existing genetic base is determined for traits of interest, the breeding program has to follow an effective selection procedure that utilizes both direct and indirect methods to improve the quantitative traits. Several statistical procedures measures the magnitude and direction of interrelationship between any two casually related variables and models like principal component analysis (PCA) and cluster analysis can be further used for diversity study and for selection of genotypes. Earlier reports have explained the relationship of fibre yield with cane yield and cane quality traits, millable canes and single cane weight with highest direct effect on fibre yield. The present study reports the association of cane yield and quality characters with fibre % in improved *S. robustum* interspecific hybrids, partitioning of the total variance of original traits into a small number of uncorrelated new variables for visual differentiation among genotypes and cluster analysis to identify diverse combinations for further improvement in clonal population. The study also resulted in identification of high fibre types with *S. robustum* base that combined yield and quality.

The experimental material comprises 36 elite interspecific hybrids: 27 F₁ hybrids (improved *S. robustum* x improved *S. officinarum*: 12, 9 from improved *S. officinarum* x improved *S. robustum*, 6 from improved *S. robustum* x commercial) and nine back cross hybrids with NMC > 31 and cane yield per clump > 14 kg, were evaluated in RBD with two replications in a plot size of 6m x 0.9m during the crop season 2014 at ICAR-Sugarcane Breeding institute, Coimbatore. Observations on NMC, cane height (cm), cane thickness (cm), SCW (kg), cane yield per clump (kg), brix %, sucrose %, purity %, CCS % and fibre content were recorded at 12 months. Fibre content was calculated as per Thangavelu and Rao, 1982) 21 genotypes were identified as very high fibre types (>25 %), 9 were high fibre types (20-25%) and 6 were low fibre types (<20%). Correlation coefficient and path analysis was done using standard statistical procedures (Singh and Chaudhary, 1985). PCA and cluster analysis (using euclidean distance) was performed with PAST Statistical software, version 2.17 (Hammer et al., 2001).

Fibre % showed high significant positive genotypic association with cane yield/clump (0.716), NMC (0.537), cane height (0.515) and SCW (0.439). The association of fibre % with cane thickness was non-significant and negative (-0.011) at phenotypic level while it was positive and significant (0.172) at the genotypic level (Table 1). Correlation of fibre % with brix % was positive and significant; sucrose % showed negative but non-significant association with fibre %. This indicates the possibility to combine both high fibre and high sucrose content in same genotype since there was no significant negative correlation between fibre % and brix % and sucrose %. Hence selection could be made based on both the traits to raise genotypes with increased sugar and fibre %, thus satisfying the future demands through development of multipurpose cultivars.

Genotypic path analysis was done to estimate the direct and indirect effects of different traits on fibre %. Cane yield/clump showed a relatively high positive direct effect on fibre % (0.442). It also



exhibited high indirect positive effect through NMC (0.195) and SCW (0.103). These positive indirect effects resulted in higher correlation coefficient of cane yield/clump with fibre content (0.716). NMC recorded the highest positive direct effect on fibre % (0.624). But its negative indirect effects through brix %, sucrose %, cane thickness and juice weight results in reduced total correlation of NMC with fibre % (0.537). Cane height recorded a low positive direct effect (0.284) on fibre yield but the total correlation was significant and of higher magnitude (0.515) due to the positive indirect effects through cane yield/clump (0.177), NMC (0.082) and SCW (0.062). SCW exerted lowest direct effect on fibre % (0.198); but its positive indirect effect through cane height (0.246) resulted in high positive correlation with fibre yield (0.439). Residual effect was 0.59, suggesting the characters studied showed significant influence on fibre %.

PCA was performed to explain the combination of variable which accounts for maximum genetic variation in *S. robustum* interspecific hybrids. The first three principal components (PC) with eigen value >1 contained the majority of variance information with the cumulative variation of 76.40 %. remaining components with eigen value < 1 contributed to 23.60 % of variability. Cane yield, NMC, SCW and cane height were characterized with large factor loadings in PC₁ (Table 2). Quality parameters such as Brix %, Sucrose % and purity % were characterized with large scores in PC₂. The genotypes that appeared in quadrant (1) and (2) were characterized with high fibre %, cane yield and moderate brix %, while those in quadrant (3) and (4) were characterized with high brix % and fibre % < 20. The Eigen value of cane yield, NMC, SCW, cane height, fibre % and brix % in PC₁ were positive. The corresponding Eigen vectors of PC₂ also showed that quality traits (brix % and sucrose %) and cane yield characters (NMC, SCW, cane yield/clump) and fibre % were grouped together. The present results suggests that the traits viz., NMC, SCW and cane height are the primary traits to be considered in clonal selection to improve fibre content followed by brix and sucrose % and thereby to develop genotypes with multipurpose utility.

Cluster analysis (Euclidean distance) grouped 36 *S. robustum* interspecific hybrids into 10 distinct clusters (Figure 1), where two clusters contained a single genotype. Cluster I was distinct from cluster X (13.065), VI (13.061), IV (11.070) and II (12.680). The smallest distance was observed between cluster II and III (5.241). The clones, RO 15073 (RxO 1), OR 15066 (OxR 2), RO 15012 (RxO 5), RO 15019 (RxO 10), OR 15081 (OxR 9), BC 15084 (BC 4), OR 15043 (OxR 3), RO 15027 (RxO 12) and BC 15055 (BC 8) were identified as energy canes with more than 20 % fibre and 15 % juice brix; RO 15033 (RxO 2), RO 15029 (RxO 8) and RO 15041 (RxO 3) as energy canes with more than 25 % fibre are recommended for further genetic manipulation and utilization. The study also envisages the evaluation of these high biomass genotypes under larger plot size for their use as energy canes in cogeneration. Crossing between genotypes of clusters with large distance and significantly different for yield and quality traits following the information about indirect effects of traits on fibre % and sucrose will increase efficiency of breeding programs in selection of clones for multiple purposes.

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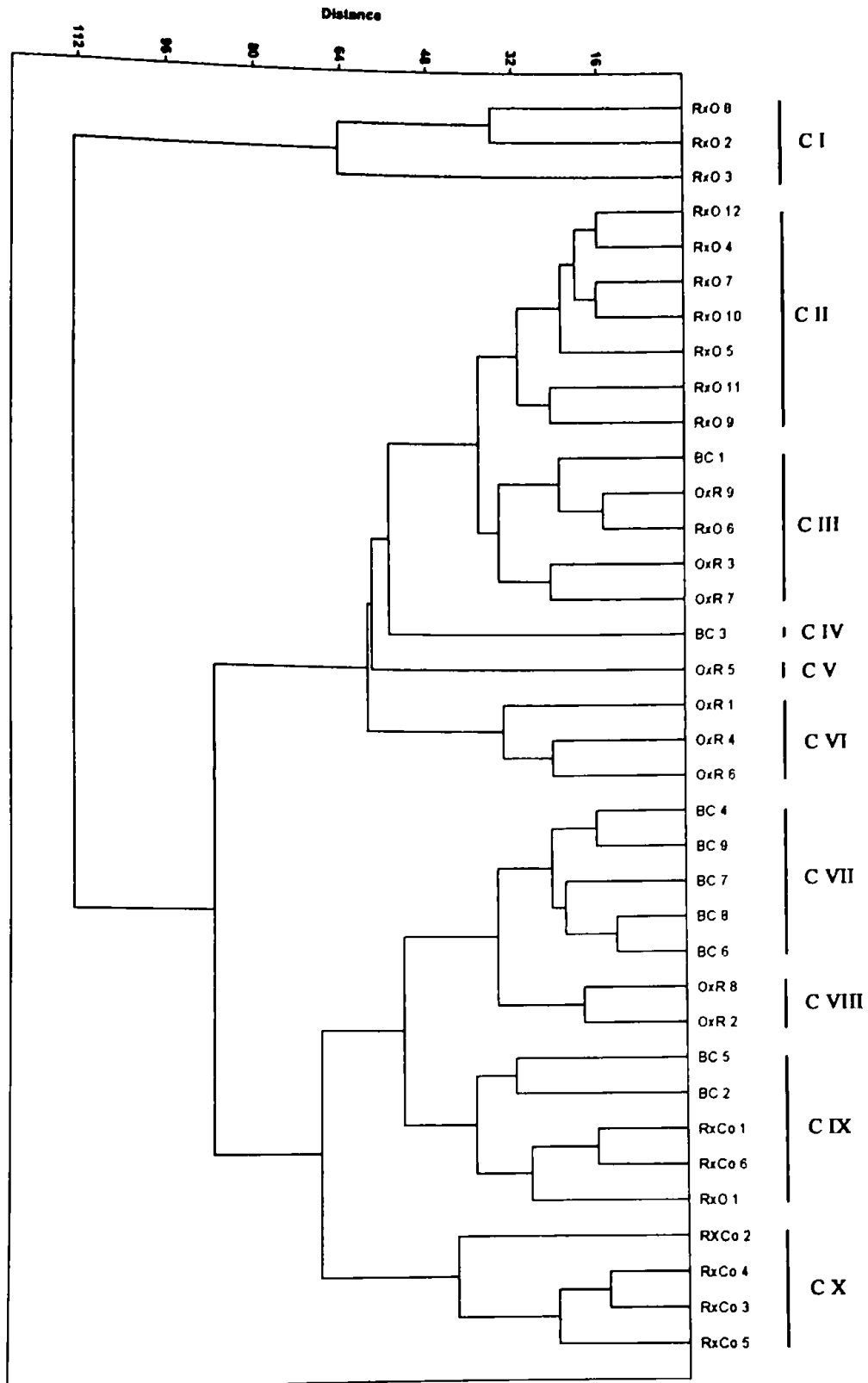


Figure 1. Dendrogram of cluster analysis for improved *S. robustum* hybrids (based on euclidean distances) for cane yield, quality and fibre %.



Table 1. Genotypic correlation coefficient between fibre % and cane yield & quality traits in improved *S. robustum* hybrids

Traits	Fibre %	NMC	Cane thickness (cm)	SCW (kg)	Cane height (cm)	Cane yield/clump (kg)	Brix %	Sucrose %	Purity %	CCS %
NMC	0.537**	1								
Cane thickness (cm)	0.172*	-0.090*	1							
SCW (kg)	0.439**	0.142*	0.049**	1						
Cane height (cm)	0.515**	0.034	0.268**	0.017**	1					
Cane yield/clump (kg)	0.716**	0.648**	0.105*	0.332**	0.291**	1				
Brix %	0.041*	-0.012	-0.003*	-0.063	-0.224**	-0.172**	1			
Sucrose %	-0.068	-0.054*	-0.040	-0.072*	-0.288*	-0.525*	0.916**	1		
Purity %	-0.113	-0.107	-0.011**	-0.227**	-0.085**	-0.104*	0.344**	0.979**	1	
CCS %	-0.080	-0.195**	-0.047	-0.038*	-0.021	-0.189	0.988**	0.643**	0.523**	1
Juice weight (g)	0.068*	-0.051	-0.074*	-0.035	0.0018*	0.035**	0.114*	0.043**	0.154**	0.244**

*, ** Significant at 5 % and 1 % probability respectively



Table 2. Eigen value, factor loads and contribution of first three PC Axes to variation in improved *S. robustum* interspecific hybrids

Variable	Eigen vectors		
	Pc1	Pc2	PC3
Eigen value	2.6174	1.3394	1.0124
Cumulative percent of total variation	33.40	58.90	76.40
NMC [*]	0.576	0.026	0.208
Cane thickness (cm)	0.189	-0.164	0.366
SCW (kg)	0.514	0.105	0.041
Cane height (cm)	0.429	-0.087	-0.023
Cane yield/ clump (kg)	0.585	0.146	0.110
Brix %	0.021	0.411	-0.029
Sucrose %	-0.106	0.304	-0.228
Purity %	-0.166	0.382	-0.134
CCS %	-0.037	0.204	-0.050
Juice weight (g)	0.149	0.169	-0.081
Fibre %	0.316	0.196	0.021

I-P11

GENOME MODIFICATIONS IN WIDE HYBRIDS INVOLVING *SACCHARUM* AND *ERIANTHUS*

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Intergeneric hybridization has been performed by plant breeders to improve the genetic base of cultivated crops to impart better resistance to biotic and abiotic stresses, better yield etc. In sugarcane, intergeneric hybridization was attempted since 1913 for introducing new features for improving the agronomic traits (Barber, 1916) and since then several successful attempts were made in producing intergeneric hybrids of *Saccharum*. *Erianthus* is considered as the most important wild genetic resource for sugarcane varietal improvement (D'Hont *et al.*, 1995; Cai *et al.*, 2005; Amalraj and Balasundaram, 2006). It can contribute many desirable agronomic traits such as high biomass, ratoonability, tolerance to drought and water logging, resistance to diseases and pests, etc. The combination of two genetically distant genomes in an intergeneric hybrid can result in genetic alterations such as chromosome elimination, sequence elimination, sequence modifications etc., especially when more number of diverse genomes from polyploid parents were combined.

The chromosome contributions by *E. arundinaceus* in *Erianthus* × *Saccharum* intergeneric hybrids and in successive generations of back cross hybrids with sugarcane clones as male parent were studied using biotin labelled *E. arundinaceus* probe (Maya Lekshmi *et al.*, 2016). Four generations of hybrids were analyzed using GISH and the results showed progressive elimination of *Erianthus*

II-O13

POPULATION STRUCTURE AND GENETIC DIVERSITY CHARACTERIZATION OF SACCHARUM INTERSPECIFIC HYBRIDS USING SSR MARKERS

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The degree of genetic diversity found in germplasm indirectly reflects the level of genetic progress achievable in future cultivars. Due to domestication bottlenecks and artificial selection, the amount and distribution of genetic variation in the genomes of modern cultivars is poorly understood. Hence a genetic resource management with an investigation of genetic diversity and the extent of genetic differentiation within and between populations along with an understanding of processes that maintain the variation is essential. The use of molecular markers to improve the utilization of genetic diversity from sugarcane germplasm based on population genetics and model based approach is reported by Mariana and Jorge Alberto 2015 and Esayas Tena et al., 2016. The present study aims to characterize the genetic diversity and genetic differentiation of interspecific hybrids generated involving improved *S. spontaneum* and improved *S. robustum* using SSR markers and then to characterize the genetic structure of the populations with model based approach utilizing the Structure software.

Thirteen biparental crosses involving improved *S. officinarum*, *S. robustum*, *S. spontaneum* and commercial were made during the crossing season of 2012, two hundred and eighty four interspecific hybrids (top performed) were evaluated for cane yield and quality traits in replicated field trial during the growing season of 2014 at Sugarcane Breeding Institute (ICAR), Coimbatore. Among these 67 clones which showed significant improvement over parents for phenotypic traits were analyzed using 28 microsatellite markers (SSR) to evaluate the genetic diversity and population structure. These clones represent four different populations; 20 were from *S. spontaneum* x *S. officinarum*, 16 from *S. robustum* x *S. officinarum*, 17 from *S. robustum* x Commercial and 14 from *S. spontaneum* x Commercial. Genomic DNA was extracted using CTAB method. Microsatellite primer sequences (22 NKS primers; SMC 863 GC, SMC 31 CUQ, SMC 597 GC, SMC 1039 GC, mSSCIR 54 and mSSCIR 66) were obtained from Genbank database [www.nrcpb.org/STMS.html] and NCBI. PCR amplified products were resolved on 8 % (w/v) polyacrylamide gel electrophoresis and bands were detected by silver staining.

Allele frequency, number of alleles (Na), effective number of alleles (Ne) percentage of polymorphic bands (PPB), gene diversity or expected heterozygosity, He (Levene 1949), Shannon's Information Index of Diversity, I (Lewontin 1972), inbreeding coefficient and Nei's genetic distance were computed with POPGENE 1.32 (Yeh et al., 1997). Polymorphism information content (PIC) was estimated using the program POWERMARKER Ver3.25 (Liu and Muse 2005). Analyses of molecular variance and Fst (genetic differentiation) between populations were performed with the software Arlequin 3.5 (Excoffier et al. 2005). Wright (1951) fixation index (Fst) of the total populations and pair wise Fst among all-pairs of populations were computed using FSTAT software (Goudet 2002) and significance was tested based on 10000 bootstraps. Gene flow was estimated using indirect method based on the number of migrants per generation (Nm) as $(1 - Fst) / 4 Fst$. To assess genetic structure,



model based approach was utilized with Structure ver 2.3.4 software (Pritchard et al. 2000). The actual number of subpopulation (K) was identified with a parameter set up at a burn-in period of 10,000 Markov Chain Monte Carlo iterations and 100,000 run length and with an admixture model following Hardy-Weinberg Equilibrium (HWE) and correlated allele frequencies method. Each k value was run for 10 times. The optimal K was determined using Structure Harvester (<http://taylor0. biology. ucla. c du/ structure Harvester/>) following the simulation method of Evanno et al., 2005.

Genotyping of 67 *Saccharum* interspecific hybrids (which includes 34 genotypes of *S. spontaneum* cytoplasm and 33 of *S. robustum* cytoplasm) using 28 SSR markers revealed 227 polymorphic loci with an average of 8.1 alleles per locus. Rare alleles (genotype specific, frequency < 0.05) comprised 18.5% and abundant alleles (frequency > 0.75) comprised 51.6% of all detected alleles. The maximum species specific (3) and genotype specific allele (6) were identified at SMC 863 GC and NKS 34 respectively; 19 unique alleles for 13 genotypes were detected. Six primers deviated from HWE. The gene diversity and PIC values ranged from 0.427 (SMC 31 CUQ) to 0.711 (NKS 28) and from 0.456 (SMC 31 CUQ) to 0.816 (NKS 28), with averages of 0.589 and 0.614 respectively. The effective number of alleles (N_e) per primer varied from 1.574 to 3.096 and frequency of the major allele was in range of 0.44-0.75 with a mean of 0.51. Comparison of diversity statistics of genotypes separately for *S. spontaneum* and *S. robustum* is presented in Table 1. The values of gene diversity (GD) were higher in genotypes of *S. spontaneum* x *S. officinarum* followed by *S. robustum* x *S. officinarum*. The average values of PIC calculated over all the loci showed moderate difference in two species (0.598 in *S. spontaneum* and 0.403 in *S. robustum*). Analysis on private alleles revealed 13.13% of private alleles in *S. spontaneum* and 14.52% in *S. robustum* showed allele frequency greater than 12%. The pair-wise values for unbiased genetic distance (GD) and genetic identity (GI), population differentiation (F_{st}) between *S. spontaneum* and *S. robustum* showed moderate genetic differentiation between the genotypes of both species (Table 2 and Table 3). Population differentiation was slightly higher for genotypes of *S. robustum* than in *S. spontaneum*; correspondingly higher level of gene flow existed in *S. robustum* ($N_m = 0.962$) than in *S. spontaneum* ($N_m = 0.503$). The hierarchical distribution of molecular variance by AMOVA revealed highly significant genetic differentiation among the species; 16 % of the total variation was between the species, while 86 % was among individuals within the species.

The Bayesian model-based approach to determine population structure in genotypes from two species (*S. robustum* and *S. spontaneum*) of sugarcane with STRUCTURE software revealed three sub-populations ($K=3$), Figure 1. *S. robustum* x *S. officinarum* genotypes (16) and 3 from *S. robustum* x Co cane were grouped in sub-population 1 (CI); *S. spontaneum* x *S. officinarum* genotypes (20) and 2 from *S. spontaneum* x Co cane were grouped in sub-population 2 (CII); sub-population 3 (CIII) consisted of 36 genotypes (generated involving Co canes). The estimates of pair wise Nei's unbiased genetic distance (GD) and population differentiation (F_{st}) for three sub-populations inferred by structure analysis also ensures low gene flow (N_m) in genotypes grouped in CI ($N_m = 0.416$) and CII ($N_m = 0.378$) with large genetic variation. All genotypes generated involving commercial clones were grouped in one cluster CIII with high gene flow ($N_m = 0.784$) revealed that major portion of genetic variance has been exploited for cultivar improvement. The findings suggest exploitation of populations CI and CII through prebreeding which will contribute genes for important traits in breeding population with a broad genetic base. The SSR primers reported in the present study detecting private alleles >12% frequency can be used to discriminate *S. spontaneum* and *S. robustum* hybrids in



marker assisted backcross selections. The present study sheds light on the genetic diversity, population structure and gene flow of interspecific hybrids involving improved *S. spontaneum* and *S. robustum*; and a source of new germplasm for crop improvement.

Table 1. SSR diversity for 227 microsatellite loci in four populations of sugarcane interspecific hybrids utilizing improved parents

Population	Sample size	Na	Ne	I	GD	%P
<i>S. robustum</i> x <i>S. officinarum</i>	16	2.819	1.715	0.612	0.529	94.27
<i>S. robustum</i> x Co cane	17	2.08	1.425	0.588	0.436	88.54
<i>S. spontaneum</i> x <i>S. officinarum</i>	20	2.649	1.657	0.769	0.664	95.07
<i>S. spontaneum</i> x Co cane	14	2.331	1.511	0.606	0.526	89.60
Mean		2.469	1.577	0.6437	0.5387	91.87

Na = number of different alleles; Ne = effective number of alleles; I = Shannon index; GD = genetic diversity according to Nei (1978); and %P = percentage of polymorphic loci

Table 2. Unbiased Nei's genetic distance (below diagonal) and identity (above diagonal) among and between populations

Populations	<i>S. robustum</i> x <i>S. officinarum</i>	<i>S. robustum</i> x Co cane	<i>S. spontaneum</i> x <i>S. officinarum</i>	<i>S. spontaneum</i> x Co cane
<i>S. robustum</i> x <i>S. officinarum</i>	0.000	0.857	0.604	0.741
<i>S. robustum</i> x Co cane	0.155	0.000	0.618	0.864
<i>S. spontaneum</i> x <i>S. officinarum</i>	0.411	0.382	0.000	0.836
<i>S. spontaneum</i> x Co cane	0.259	0.147	0.185	0.000



Table 3. Pair-wise FST values of four populations of sugarcane interspecific hybrids

Populations	<i>S. robustum</i> x <i>S. officinarum</i>	<i>S. robustum</i> x Co cane	<i>S. spontaneum</i> x <i>S. officinarum</i>	<i>S. spontaneum</i> x Co cane
<i>S. robustum</i> x <i>S. officinarum</i>	0.000			
<i>S. robustum</i> x Co cane	0.075	0.000		
<i>S. spontaneum</i> x <i>S. officinarum</i>	0.248	0.125	0.000	
<i>S. spontaneum</i> x Co cane	0.106	0.098	0.084	0.000

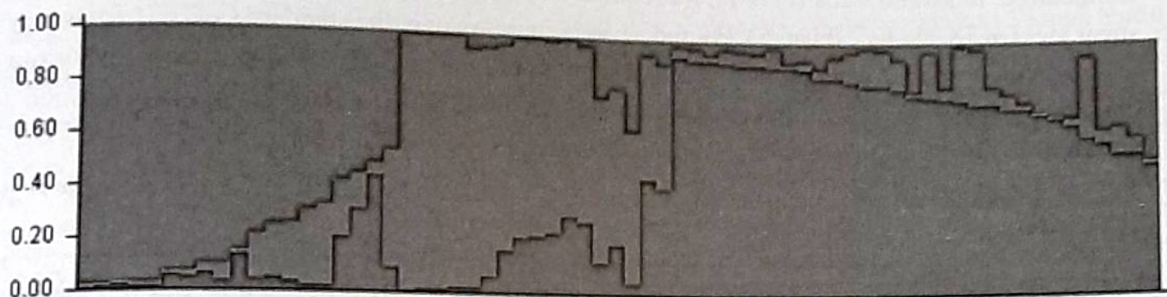


Figure 1. Model based ancestry of 67 Sugarcane interspecific hybrids based on 28 SSR markers. The K value is based on maximum of adhoc measure ΔK based on Evanno et al., 2005. Bar length represent the membership probability of accessions belonging to different subgroups and y-axis show coefficient of membership/assignment.

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I-P31

EVALUATING THE POTENTIAL OF SUGARCANE FAMILIES BY DTOPSIS

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Family selection has been adopted in sugarcane breeding programs as an indirect method of selection of superior clones in different stages of selection. Generally breeders choose or select clones from the whole population or first select the most promising families and then select the best clones within these best families. The basis for family selection is not to produce superior families with commercial value but rather to identify families with a higher frequency of superior clones and neglect the poor families to improve breeding efficiency. Hence, a method for identification of promising families with the maximum potential for producing economically viable genotypes would be of great interest and usefulness to the sugarcane breeders. The Dynamic Technique for Order Preference by Similarity to Ideal Solution (DTOPSIS) was used for evaluating rural economies by many factors and this method has been widely used in evaluation of crops like tomato, tobacco etc. In sugarcane, it was used in the evaluation of cane cultivars and sugarcane clones. The possibility of using DTOPSIS to identify superior sugarcane families by using comprehensive index was attempted in the present study. The families were evaluated in plant and ratoon crop of ground nursery, first clonal and second clonal stages from 2012-2016 at ICAR-SBI, Coimbatore. Observations were recorded in 18 families and four standards on four traits viz., number of millable canes (NMC), cane height, cane diameter and HR brix % at 330 days. Weight coefficients of 0.30, 0.30, 0.10 and 0.30 were given to the traits NMC, cane diameter, cane height and brix % respectively. Comprehensive index (C_i) was calculated considering the four traits simultaneously and used to test the distance of each family to the best family (Table 2). C_i values were significantly correlated to the selection rate at the two stages indicating that the elite clones were selected from families with higher C_i values. The method can be used to determine the elite families to increase the selection efficiency by considering many traits simultaneously. Among the families studied, the families with more NMC/clump were Co 0312 x Co 98010, Co 0312 x Co 0209 and 81V48 x Co 0209. The progenies of Co 10031 x Co 99006 had thick canes (3.21cm) followed by CoM 0265 x CoT 8201 (3.14 cm), Co 98010 x Co 0209 (2.98cm) and Co 86032 x CoN 10072 (10.91 cm). Brix % was the maximum in Co 10033 x Co 06002 (21.45%) and Co 0312 x Co 0209 (20.36%). The standard Co 99004 was the tallest with 271.25 cm. The method identified the ideal cross with 22.54 % brix, 13.34 NMC/clump, 3.21cm cane thickness and plant height of 271.25 cm. The selection rate at stage I (ground nursery) varied from 2.00-7.14 % with an overall selection rate of 5.09 %. The selection rate ranged from 0-5.71 % at stage II (first clonal trial) with an overall selection of 3.06 %. In second clonal stage, no clones were selected from six families which had low values of C_i . It ranged from 0.1278 to 0.7558 among the families. Eleven families had higher C_i values than the standards and



four families Co 86032 x CoN 10072, Co 0312 x Co 98010, Co 0312 x Co 0209, Co 86011 x Co 94008 had clones advanced to final stage of selection. From the families with low C_i , no clones were selected.

Selection rate (SR) at clonal population I (SR_1), clonal population II (SR_2) and comprehensive index (C_i) for 18 crosses

Cross No	Crosses	Sample size	SR_1	SR_2	C_i
1	U 09053 x Co 94008	122	2.46	0.82	0.1278
2	Co 0312 x Co 94008	200	5.50	4.00	0.4590
3	Co 86032 x CoN 10072	140	7.14	5.71	0.6535
4	CoM 0265 x CoT 8201	81	6.18	4.23	0.6235
5	Co 98010 x Co 0209	180	5.56	4.44	0.5583
6	Co 0312 x Co 98010	210	4.76	2.38	0.7558
7	81V48 x Co 0209	100	4.00	3.00	0.6445
8	Co 0312 x Co 0209	100	6.00	4.00	0.7161
9	Co 86011 x Co 94008	120	5.83	4.17	0.6544
10	Co 11019 x Co 06002	100	2.00	2.00	0.2400
11	07-305 x ISH 176	140	7.86	5.71	0.3316
12	Co 06022 x Co 05007	150	2.00	0.00	0.1659
13	Co 06022 x CoN 10072	186	4.30	1.08	0.2003
14	Co 86032 x Co 94008	210	3.33	1.43	0.1883
15	Co 11020 x Co 06002	180	6.67	3.89	0.4298
16	Co 99004 x Co 05007	124	5.65	0.81	0.2206
17	Co 10031 x Co 99006	118	5.93	3.39	0.5057
18	Co 10033 x Co 06002	124	6.45	4.03	0.5407
<i>r</i>			0.4802*	0.5838*	

*the correlation of SR with C_i was significant at $P=0.05$

The C_i values were significantly correlated with the selection rate. It showed a positive correlation ($r = 0.4802$ and $r = 0.5838$) with the selection stages respectively. Three families viz., U09053 x Co 94008, Co 06002 x Co 05007 and Co 99004 x Co 05007 with low selection rate of 0.82%, 0% and 0.81% respectively showed low C_i . The C_i values and selection rate indicated that the families Co 0312 x Co 98010, Co 0312 x Co 0209, Co 86011 x Co 1148, Co 86032 x CoN 10072, 81 V 48 x Co 0209, CoM 0265 x CoT 8201 as potential ones with more recombinants. Family selection increases the genetic gain for yield and its related traits and superior recombinants are expected from elite families.



Family selection followed by individual plant selection has more advantages and it will be useful to assess the breeding potential of parents. With the current scenario of limited availability and optimum use of resources, comprehensive index can be used as a reliable estimate to identify potential crosses and reject the inferior crosses. Selection based on NMC, cane height is important for maximum improvement in cane yield and the study suggests families with high C_i values are close to the ideal family from which elite clones can be selected for yield traits and brix % improvement.

II-P32

MIXED MODEL ANALYSIS IN SUGARCANE VARIETAL EVALUATION PROGRAMME IN INDIA

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Genetic improvement in economic yield is a key of success in every breeding programme. The All India Coordinated Research Project on Sugarcane AICRP(S) has been playing a pivotal role in development of improved, location-specific sugarcane varieties for five agro-climatic zones viz., Peninsular, East Coast, North West, North Central and North East zones of India since 1970. Since then a large number of improved varieties have been identified through the AICRP trials, which are currently under cultivation in most parts of the country and have contributed in improving the sugarcane productivity. One of the major coordinated efforts right from the inception of the AICRP on sugarcane has been the Zonal Varietal Trials which are conducted under the Crop Improvement discipline at about 40 research stations located in five sugarcane agro-climatic zones across the country. It is necessary to assess the impact of breeding programme on genetic improvement of sugarcane over the years. In this paper, attempt has been made to assess the genetic improvement in elite clones of sugarcane evaluated in zonal varietal trials conducted during 1989 to 2015. A secondary aim is to develop a mechanism for evaluating the efficiency of these programmes based on the estimated variance components. This will help in restructuring sugarcane variety evaluation programmes in our country.

Mixed model analysis was used to long term data of sugarcane cane yield recorded in zonal varietal trials conducted in five zones of the country and analyzed for genetic improvement in cane yield over the years commencing from 1989 to 2015. Mixed models offer a powerful and flexible tool for analysis of longitudinal data. In longitudinal studies, a growth curve model based on a linear mixed model including two random effects (intercept and slope) which are normally distributed with an independent Gaussian error is probably the most routinely used to study change over time of a quantitative outcome. Proposed and applied two type of models for comparing the statistics of different criteria of choosing the most reliable and important factors of the programme (Year, Crop, Maturity and Zone). In this study, we considered variables, Zone, Crop, Maturity, Zone*Crop, Zone*Maturity and Zone*Crop*Maturity, as fixed and year as random in first mixed effect model. In second mixed model we have taken all the first, second and third order interactions of different factors (Table 1 & 2). In this case, Year, Year*Zone, Year*Crop, Year*Maturity, Year*Zone*Crop, Year*Zone*Maturity, Year*Crop*Maturity were taken as random and Zone, Crop, Maturity, Zone*Crop, Zone*Maturity, Zone*Crop*Maturity were as fixed. Where - zone (Four zones of AICRP(S)), Crop





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