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RESEARCH ARTICLE

Evaluation of intra-specific hybrids of *Saccharum officinarum* for yield, quality and incidence of Yellow leaf disease

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Abstract
Improvement of parental clones of *Saccharum officinarum* L. through repeated cycles of intra-specific hybridization and selection was undertaken at ICAR-Sugarcane Breeding Institute (SBI), Coimbatore during 1987 to 1996. Thirty one clones selected from six polycrosses involving 13 parental clones of *S. officinarum* constituted cycle 1 (C1) population. Intermating among fourteen C1 clones followed by selection resulted in identification of 33 clones in cycle 2 (C2) population. Fourteen clones selected in C2 and C1 were inter-crossed. From the progenies, 50 clones were selected to constitute cycle 3 (C3) population. As the original parental clones of *S. officinarum* had low number of millable canes (NMC) and poor cane yield, in each cycle of selection emphasis was given to select progenies with high NMC, single cane weight and cane yield. The C1, C2 and C3 population, totalling 114 (designated as population-improved *S. officinarum* clones or PIO clones) were evaluated at ICAR-SBI Research Centre, Agali during 2015-16 and 2016-17 for NMC, single cane weight, cane yield per plot, brix%, sucrose% in juice and resistance to yellow leaf disease (YLD). Generation-wise improvement in the targeted traits was estimated. Significant improvement in NMC /ha (37.18 per cent), single cane weight (16.67 per cent) and cane yield (61.51 per cent) was achieved in two cycles of selection. Ten YLD-free improved clones with acceptable agronomic traits were identified for further use. They are: PIO 94-847, PIO 94-875, PIO 99-633, PIO 00-513, PIO 00-547, PIO 00-638, PIO 00-757, PIO 00-769, PIO 00-772 and PIO 00-840. These improved PIO clones would serve as new parental gene pool in the inter-specific hybridization programmes and for diversifying the genetic base of commercial sugarcane varieties.

Keywords: Sugarcane; Intra-specific improvement; *S. officinarum*

Introduction

Varietal improvement in sugarcane across the World had been achieved through inter-specific hybridization between cultivated sugarcane *Saccharum officinarum* L. and wild species *S. spontaneum* L. followed by repeated backcrossing with the cultivated forms to improve agronomical traits of the hybrids. A large number of species-clones of sugarcane including *S. officinarum* (757 clones), *S. robustum* (145 clones), *S. barberi* (42 clones), *S. sinense* (30 clones), *S. spontaneum* (>1500 clones) are maintained in the World Collection of sugarcane germplasm at ICAR-Sugarcane Breeding Institute (ICAR-SBI), Research Centre, Kannur, India (Chandran 2010). However, only 15 clones of *S. officinarum,* two clones of *S. barberi* (Chunne and Saretha) and two *S. spontaneum* (Coimbatore local and Java), one clone of *S. sinense* (Kansar) had been utilized in the commercial varietal development programmes so far due to lack of flowering as well as synchronization of flowering and other practical difficulties associated with inter-specific hybridization. Intra-specific improvement of major *Saccharum* species prior to inter-specific hybridization had been suggested to achieve faster gains in inter-specific hybridization (Dunckelman and Breaux 1971; Walker 1987). Improved species-clones developed through repeated cycles of intra-specific hybridization and selection is expected to provide a generation-wise improvement in the targeted characters in the species concerned. The improved clones will serve as an elite and broader gene pool compared to the original species clones when used in inter-specific hybridization and would also pave way for achieving faster gains in productivity. Attempts to develop improved populations of *S. officinarum* through repeated cycles of intra-specific hybridization and selection were made during 1980s at ICAR-SBI (Nair et al. 1998). Emphasis was given to improve cane yield attributes as most of the parental clones in this species possess relatively higher sucrose content but are lower in cane yield. Improvement achieved in the targeted traits of intra-population improved *S. officinarum* in three cycle of selection is presented in this article.

Materials and Methods

1. Development of improved S. officinarum (PIO) clones: Six polycrosses involving 13 typical and atypical clones of *S. officinarum* from the World Collection (designated as C0 population) were made during 1987 to 1989 at ICAR-SBI, Research Centre, Kannur, Kerala (Table 1). The typical clones of *S. officinarum* such as AGS, 28 NG 89, 28 NG 93, 57 NG 203, 57 NG 62, NC 17, NG 77-142, Saipan G and Seleri had somatic chromosome number of 2n =80 whereas the atypical clones had chromosome number ranging from 2n=108 to 116 (2n=108 in IJ 76-564, 114 in IJ 76-418 and 116 in Chapina and IJ 76-501) (Sreenivasan and Nair 1991). The atypical clones are considered as natural hybrids of *S. officinarum*. Polycrosses were employed in the study for utilising more number of parents with lesser number of crosses and also to improve seed set, which is generally poor in the biparental crosses of *S. officinarum*. Two hundred and thirty-one seedlings generated from these crosses were evaluated for yield and quality traits at ICAR-SBI, Coimbatore during 1991-1992 and 31 clones were selected on the basis of number of millable canes (NMC), cane yield and sucrose % to constitute cycle 1 (C1) population (Table 1).

Six crosses were made during 1990 to 1993 using 12 clones selected in C1 cycle (Table 2). Parents

Table 1. Parentage of cycle 1 population of *S. officinarum* (PIO)

Sl. No.	Crosses	No. of seedlings evaluated	No. of progenies selected	Selected progenies to constitute cycle 1 population
1	28 NG 93 x (NC 17, 28 NG 89, 57 NG 203, IJ 76-501)	76	4	PIO 90-64, -90, -99, -102
2	57 NG 62 x (AGS, IJ 76-501, NG 77-142)	19	6	PIO 88-100, -101, -102, -103, -104, -110
3	Chapina x (AGS, IJ 76-501, NG 77-142)	30	5	PIO 88-88, -1704, -1715, PIO 90-438, -440
4	Saipan G x (NC 17, 57 NG 203, 28 NG 89)	8	3	PIO 90-227, PIO 91-391, -437
5	IJ 76-418 x (IJ 76-564, AGS, IJ 76-501, NG 77-142)	10	7	PIO 88-79, -81, --84, 136, -1784, -1785, -1819
6	Seleri x (NC 17, 28 NG 89, 57 NG 203, IJ 76-501)	88	6	PIO 90-142, -162, -177, -188, -196, -202
	Total	231	31	

Table 2. Parentage of cycle 2 population of *S. officinarum* derived from C1 x C1 crosses

Sl. No.	Crosses	No. of seedlings evaluated	No. of progenies selected	Selected progenies to constitute cycle 2 population
1	PIO 88-1715 x PIO 88-81, -103, -1704, -1819	15	5	PIO 91-79, -178, -190, PIO 93-397, -829
2	PIO 88-81 x PIO 91-391	13	3	PIO 94-428, -447, -517
3	PIO 88-84 x PIO 88-81, -103, -1704, -1819	14	2	PIO 91-304, -572
4	PIO 90-202 x PIO 88-1785	18	10	PIO 94-352,-357, -361, -375, -378, 742, -608, -694, -702
5	PIO 90-202 x PIO 90-227	27	6	PIO 94-847, -851, -855, -875, -890, -891
6	PIO 90-64 x PIO 90-188	9	7	PIO 93-100, -104, -107, -165, -183, -729, -779
	Total	96	33	

for inter-crossing were chosen based on flowering and pollen fertility. Ninety six seedlings generated were evaluated at Coimbatore. Out of which 33 clones were selected to constitute cycle-2 (C2) population.

One hundred and fifty nine progenies from eight crosses made during 1993 to 1996 using 14 clones from C2 and C1 cycles were evaluated for yield and quality traits. From these, 50 clones were selected to constitute cycle-3 (C3) population (Table 3).

2. Evaluation of PIO clones: At each stage of selection emphasis was given on selecting clones with higher NMC and stalk weight. Selections from C1, C2 and C3 cycles, totalling 114 improved *S. officinarum* clones (designated as population improved *S. officinarum* or PIO clones) were planted on 18 Feb 2015 at SBI, Research Centre, Agali (Kerala) in plot of size- 2 rows of 5 m length per clone x 0.9 m row spacing, and replicated twice. Data on number of millable canes (NMC), single cane weight (kg), cane yield (t/ha), brix% and sucrose% in juice were recorded at harvest (12th month). Agali (Kerala) is a hot spot for sugarcane yellow leaf disease (YLD). The natural incidence of YLD in the PIO clones was recorded. The pooled mean data of each cycle were analysed for measures of variation following standard statistical procedures (Sharma 2006). Elite clones selected during 2015-16 season based on yield, juice quality and YLD incidence were re-planted during 2016-17 season (2 row per entry x 6 m L x 0.90 m row spacing) at Agali Centre for evaluation.

Results and Discussion

The range, mean and coefficient of variation in PIO clones at each cycle of selection and per cent improvement over cycle for different traits is presented in Table 4.

Cycle 1 population: The mean of C1 population (Table 4) for NMC was 55,291 /ha, single cane weight was 0.84 kg, cane yield was 45.91 t/ha, brix% was 14.67 and sucrose % was 12.46. The progenies of the cross 28 NG 93 x (NC 17, 28 NG 89,57 NG 203, IJ 76-501) were mostly thin stalked, susceptible to either YLD or mosaic or ring spot or rust. Bud sprouting was common in the progenies of the cross 57 NG 62 x (AGS, IJ

Table 3. Parentage of C3 population of *S. officinarum* derived from C1 x C2 and C2 x C2 crosses

Sl. No.	Nature of Crosses	Crosses	No. of seedlings evaluated	No. of progenies selected	Selected progenies to constitute cycle 3 population
1	C1 x C2	PIO 88-101 x PIO 91-178	5	3	PIO 96-716, -724, -741
2	C1 x C2	PIO 88-103 x PIO 91-178	46	17	PIO 00-744, -749, -757, -758, -762, -764, -769, -772, -774, -787, -794, -798, -813, -814, -840, -845, -846.
3	C1 x C2	PIO 88-81 x PIO 94-428	13	2	PIO 96-352,-435
4	C1 x C2	PIO 88-1704 x PIO 91-79, PIO 94-428	10	9	PIO 96-427, -428, -443, PIO 98-295, -297, PIO 99-633, -633A, -666, -671
5	C1 x C2	PIO 88-1715 x PIO 94-352	15	6	PIO 98-204, PIO 00-671, -638 -726, -732, -740
6	C1 x C2	PIO 90-177 x PIO 94-447	17	4	PIO 96-70, -421, -423, -426
7	C1 x C2	PIO 90-202 x PIO 94-447	41	5	PIO 00-513, -538, -547, -548, -581
8	C2 x C2	PIO 94-357 x PIO 93-829	12	4	PIO 98-364, -397, -573, PIO 00-682
		Total	159	50	

76-501, NG 77-142). The NMC in the progenies of the cross Saipan G x (NC 17, 57 NG 203, 28 NG 89) were relatively higher (62,963 /ha) than the C0 population (46,905 /ha). Sucrose% was moderate (13.43) in this cross. The progenies of the cross IJ 76-418 x (IJ 76-564, AGS, IJ 76-501, NG 77-142) were mostly thin, short and with splits on internode. The incidence of YLD was noticed in the population of this cross. Although sucrose content was moderate (13.55%), the NMC was low (48,413 /ha). Sucrose content was better (15.41%) in the progenies of Seleri x (NC 17, 28 NG 89, 57 NG 203, IJ 76-501) though NMC was lower (43,122 /ha). Except the clone PIO 90-202, all the progenies of this cross showed incidence of YLD.

Cycle 2 population: Among the C2 population, higher millable canes were recorded (91,534 /ha) in the cross PIO 88-81 x PIO 91-391. However, the incidence of YLD was seen in the cross. Another C2 cross PIO 88-84 x (PIO 88-81, -103, -1704, -1819) recorded high NMC (62,698 /ha) but most of the progenies had zig-zag stalks and were affected by leaf rust. In three crosses *viz*., PIO 90-202 x PIO 88-1785, PIO 90-202 x PIO 90-227 and PIO 90-64 x PIO 90-188, the incidence of YLD was not noticed. In the cross PIO 90-64 x PIO 90-188, the sucrose % was higher (14.1 6%). The mean NMC in this cross was 66,780 /ha. In the cross PIO 90-202 x PIO 90-227, the sucrose % was 13.28 and NMC was 91,799 /ha. The pooled

Table 4. Range, mean, CV% for selected traits in PIO clones at each cycle of selection and % improvement.

Cycle of selection	Variability statistics	NMC /ha	Single cane weight (kg)	Cane yield (t/ha)	Brix % at 12 m	Sucrose % at 12m
C1 (30 clones)	Range	34921-100794	0.34-1.36	31.54-82.15	10.12-18.67	7.81-16.64
	Mean	55291	0.84	45.91	14.67	12.46
	CV%	29.48	32.20	24.79	17.59	19.51
C2 (32 clones)	Range	34127-113492	0.34-1.59	27.22-129.00	10.45-19.40	8.66-17.37
	Mean	69918	0.89	63.45	15.38	13.06
	CV%	28.48	34.40	47.98	16.97	19.15
C3 (51 clones)	Range	42063-118254	0.49-1.25	31.19-109.13	10.12-20.22	8.03-18.11
	Mean	75848	0.98	74.15	16.19	13.63
	CV%	28.67	21.72	36.45	15.89	17.83
Total (113 clones)	Range	34127-118254	0.34-1.59	27.22-129.00	10.12-20.22	7.81-18.11
	Mean	69898	0.92	64.97	15.50	13.11
	CV%-	31.29	28.54	43.02	16.96	18.82
% improvement in different cycles of selection						
C1 to C2		26.45	5.95	38.21	4.84	4.90
C2 to C3		8.48	10.11	16.86	5.27	4.36
C1 to C3		37.18	16.67	61.51	10.36	9.39

mean of all C2 population for NMC was 69,918/ha which was 26.45 per cent improvement over C1 mean (Table 4). The mean single cane weight of C2 population was 0.89 kg which was 5.95 per cent improvement over C1 mean. Cane yield ranged from 27.22 to 129.00 t/ha with a mean of 63.45 t/ha which was 38.21 per cent improvement over C1 clones. Juice brix % and sucrose % in C2 population were 15.38 and 13.06, respectively. There was improvement of 4.84 per cent for brix % and 4.90 per cent for sucrose content in C2 population over C1.

Cycle 3 population: The number of millable canes in cycle 3 population varied from 42,063 to 1,18,254 /ha with an average of 75,848 /ha which was 8.48 per cent improvement over C2 and 37.18 per cent improvement over C1 mean (Table 4). The mean single cane weight was 0.98 kg and it was 10.11 per cent improvement over C2 and 16.67 per cent improvement over C1 population. The cane yield in C3 ranged from 31.19 to 109.13 t/ha with an average yield of 74.15 t/ha. The C3 population exhibited 10.70 t/ha higher yield than C2 (or 16.86 per cent improvement) and 28.24 t/ha (or 61.51 per cent improvement) higher yield than C1 population. The mean brix % and sucrose % in C3 was 16.19 % and 13.63 %, respectively and it was 5.27 and 4.36 per cent higher over C2 and 10.36 and 9.39 per cent higher over C1 population. A notable observation in C3 population was that greater number of clones was free from natural incidence of YLD. Two crosses viz. PIO 88-103 x PIO 91-178 and PIO 90-202 x PIO 94-447 yielded YLD free progenies with high NMC and high cane yield.

Table 5. List of YLD- free PIO clones suggested as parents in inter-specific hybridization for the improvement of agronomic traits in sugarcane

Sl No	Elite PIO Clones	Selection cycle	Pedigree	NMC/ ha	SCW (kg)	Cane yield (t/ha)	Brix % 12m	Sucrose % 12m
1	PIO 94-847	C2 (C1 x C1)	PIO90-202 x PIO 90-227	84921	1.25	106.15	17.32	14.82
2	PIO 94-875	C2 (C1 x C1)	PIO90-202 x PIO 90-227	89683	1.20	107.62	16.62	13.35
3	PIO 99-633	C3 (C1 x C2)	PIO88-1704x PIO91-79,PIO 94-428	107937	1.15	94.13	18.15	17.03
4	PIO 00-513	C3 (C1 x C2)	PIO90-202 x PIO 94-447	91270	1.05	95.83	16.00	12.57
5	PIO 00-547	C3 (C1 x C2)	PIO90-202 x PIO 94-447	80159	1.20	96.19	20.02	16.97
6	PIO 00-638	C3 (C1 x C2)	PIO88-1715x PIO 94-352	89683	1.15	93.13	18.87	14.65
7	PIO 00-757	C3 (C1 x C2)	PIO88-103 x PIO 91-178	89683	1.25	102.10	18.35	15.52
8	PIO 00-769	C3 (C1 x C2)	PIO88-103 x PIO 91-178	105556	1.00	101.94	18.87	15.63
9	PIO 00-772	C3 (C1 x C2)	PIO88-103 x PIO 91-178	86508	1.18	102.08	17.77	14.96
10	PIO 00-840	C3 (C1 x C2)	PIO88-103 x PIO 91-178	87302	1.00	103.49	19.55	17.30
Mean of 10 elite PIO clones				**91270**	**1.14**	**100.27**	**18.15**	**15.28**
Mean of 12 original parental clones (C0 population) (data recorded at Kannur Centre*)				**46905**	**0.92**	**42.83**	**16.42**	**14.09**

* *Sreenivasan and Nair (1991)*

Observations on the natural incidence of YLD in PIO clones indicated that out of thirty C1 clones three clones namely, PIO 88-81, PIO 88-136 and PIO 90-202 were free from the disease (data not shown). Of the thirty-three C2 clones six clones *viz.* PIO 93-100, PIO 93-729, PIO 93-779, PIO 94-352, PIO 94-694 and PIO 94-847 remained free from YLD. Out of fifty C3 clones eighteen were free from YLD. They were PIO 96-741, PIO 98-573, PIO 99-633, PIO 99-671, PIO 00-513, PIO 00-538, PIO 00-547, PIO 00-581, PIO 00-638, PIO 00-671, PIO 00-757, PIO 00-758, PIO 00-764, PIO 00-769, PIO 00-772, PIO 00-774, PIO 00-814 and PIO 00-840 were free from YLD. Evidently, higher proportion of C3 clones were YLD free. Field observations during the second year (2016-17 season) confirmed that these clones were free from natural incidence of YLD which offer scope for identifying true resistance in improved population of *S. officinarum* through intensive screening. Viswanathan et al. (2016) reported few YLD free / asymptomatic clones in *S. officinarum*.

A perusal of data presented in Table 4 shows that the mean of the targeted traits viz. number of millable canes, single cane weight, stalk weight and cane yield increased considerably in three cycles of hybridization and selection (C1 to C3). In respect of NMC and cane yield, greater selection gain was achieved in two cycles of intermating and selection (C1 to C2) and in the third cycle of intermating and selection (C2 to C3) only marginal improvement could be achieved. The CV was high for NMC (Table 4). Variability was maintained without reduction though there has been progressive increase in the mean NMC from C1 to C3. The high CV for NMC highlighted the potential for selecting superior clones within the population which is vital for yield improvement in sugarcane (Nagarajan et al. 2000). In case of single cane weight, progressive improvement could be discernible up to three cycles of recurrent selection (C1 to C3). The CV for single cane weight was high but it reduced marginally from C1 to C3. For cane yield, CV increased from C1 to C2 with pronounced reduction in C3. Therefore, by taking into account the variability in the population and per cent improvement over selection cycle it may be stated that the targeted recurrent selection is expected to achieve larger selection gains for improving traits like NMC, single cane weight and cane yield in *S. officinarum* and three cycles recurrent selection may be treated as threshold level to achieve better selection gain. Walker (1987) suggested 2 to 3 cycles of recurrent selection for improving parental clones of *Saccharum* species.

As regard to quality traits (brix and sucrose), the variability in the base population was moderate and it did not increase appreciably after intermating and selection. Consequently, the per cent improvement from C1 to C3 was moderate, i.e. 10.36% for brix and 9.39% for sucrose content at 12 months. In other words, improvement per selection cycle was 3.45 % for brix and 3.13 % for sucrose.

Yellow leaf disease (YLD) has emerged as a serious threat to sugarcane productivity in the country and the YLD-free PIO clones identified may prove useful in developing YLD-resistant varieties. Ten YLD free elite PIO clones with acceptable agronomic traits were selected from three cycles of hybridization and selection (Table 5). These ten clones showed a mean of 91,270 millable canes/ha, 1.14 kg single cane weight and 100.27 tonnes cane yield per hectare. The thirteen original parents (C-0) used to generate the C-1 hybrids could not be grown along with the PIO hybrids at Agali Centre due to quarantine and other issues. Hence, the published data on these clones form the Kannur Centre (Sreenivasan and Nair 1991) was utilized for comparison. In comparison to the mean of C0 population improvement of 44,365 millable canes per hectare, 0.22 kg single cane weight and 57.44 tonnes cane yield per hectare was noticed in the mean of ten elite clones. As many of the parental clones of *S. officinarum* clones are shy tillering type with low cane yield the data presented above exemplify achievement of targeted improvement for these traits. These clones would therefore serve as improved parental gene pool in inter-specific hybridization programmes.

Of the 10 elite PIO clones two clones namely, PIO 99-633 and PIO 00-769 recorded >1.0 lakh millable canes per hectare (1,07,937/ha and 1,05,556 /ha, respectively) and three clones namely, PIO 00-757, PIO 00-769 and PIO 00-840 exhibited high cane yield combined with acceptable sucrose level. All the PIO clones are clonally maintained at the ICAR-SBI Research Centre, Agali (Kerala) and are currently being utilized in the breeding programmes (Nagarajan et al. 2000; Anonymous 2019). Crosses involving improved PIO clones such as PIO 88-110 x PIS 44 and PIO 88-94 x IND 82-319 were reported to yield superior progenies for cane yield (Nagarajan et al. 2000).

Conclusion

The importance of intra-specific improvement of Saccharum species prior to interspecific hybridisation had been discussed by various authors (Brown et al. 1969; Tew 1987; Walker 1987). It had been suggested that through repeated cycles of intraspecific hybridisation and selection, generation-wise improvement in important agronomic characters could be brought about in the species-germplasm, while maintaining a high level of genetic diversity. Our results showed that by repeated cycles of hybridisation and selection significant improvements in yield traits could be achieved in S. officinarum, with marginal gains in juice quality attributes. Development of intra-specific population in basic species like Saccharum officinarum is impeded by inherent problems like poor and irregular flowering and lack of good seed set. Consequently, there had been hardly any attempts to develop intraspecific populations of the species in India, barring a study initiated in1988 at the Sugarcane breeding Institute (Anonymous 1988; Nair et al. 1998). Evaluation of the hybrids developed after three cycles of intraspecific hybridisation and selection showed progressive improvement in yield-traits over the cycles. The % improvement from cycle-1 to cycle-2 was higher compared to that from cycle-2 to cycle-3, indicating that the improvement gets reduced as the generation advances. However, seven out of the ten superior hybrids identified in the study were from Cycle-3 suggesting that there is scope for improvement even beyond cycle-3. The ten YLD-free PIO clones identified from the study with better yield-traits can serve as superior species-germplasm for developing inter-specific hybrids of sugarcane with better productivity and a diverse genetic base.

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