RESEARCH ARTICLE

STUDIES ON ECONOMIC TRAITS IN SUCCESSIVE GENERATIONS OF SELFING IN SUGARCANE (SACCHARUM SPP.)

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Abstract

Selfing is one of the classical methods with demonstrated merits to understand parental worthiness, achieving homozygosity and for removing the deleterious genes from the genome. The selfed progenies of S₁, S₂ and S₂ generation of sugarcane clones viz., Co 775, Co 99008, Co 0304, Co 2000-10, Co 0112 and MS 68/47 and S_{e} and S_{a} progenies of Co 1148 were studied for homozygosity in successive generations of selfing, level of inbreeding depression and transgressive segregation for important economic traits. A narrow level of variation for HR Brix and wide variation for number of millable canes and moderate level of variation for cane thickness was observed in S, S, and S, generations. The genotypes with least variation among the selfed progenies in preceding generation of selfing did not show the same response in succeeding generations of selfing indicating that the genotypes behave differently in successive generations of selfing during the process of attaining homozygosity. The inbreeding individuals of 1148-13-11-2-237 and 1148-S4-247 depicted the least variation for the traits studied in later generations of selfing. Some of the inbreeding individuals from early generation of selfing of Co 775, MS 68/47, and Co 0112 for number of millable canes, Co 99008, MS 68/47 and Co 0112 for HR Brix and MS 68/47, Co 0112 for cane thickness surpassed their respective parents in their performance. HR Brix was the only trait which was least affected by inbreeding depression and greater level of inbreeding depression was observed for cane thickness. Though inbreeding was observed in early generation of selfing for number of millable canes, no inbreeding depression was observed for the trait in succeeding generations of selfing. Inbreds with higher level of homozygosity in the later generation of selfings viz., S_6 -2-59 and S_6 -2-60, S_6 -2-63 and S_6 -2-73 and S_6 -2-66 and S_6 -2-69 of Co 1148 developed in this study may be utilized for hybrid seed production in sugarcane.

Keywords: sugarcane, self, variation, homozygosity, inbreeding depression, transgressive segregation

Introduction

The present-day sugarcane is a derivative of different species of Saccharum complex and related genera and hence was considered as to have wider genetic base than other crop plants (Roach and Daniels, 1987). Among the different methods of breeding crop plants, selfing is the most intense form of inbreeding (Mettler and Gregg, 1973). In sugarcane, level of selfing was reported to be in the range of 0-80% depending on the specific cross combination, the direction

of the crossing (Hogarth, 1980), pollen fertility and self-incompatibility mechanism operating within the parents. *Saccharum officinarum* though a polyploidy, predominantly formed bivalents at meiosis and the large number of seedlings were obtained on selfing indicating that there was no incompatibility system operative resulting in self-sterility. In case of *Saccharum spontaneum* which is also highly self-fertile, no loss of vigour was observed on selfing but superior types were obtained (Babu and Ethirajan 1962). Panje and

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Ethirajan (1959) studied inbreeding in Saccharum spantaneum with various forms from India and some of the clones from Spontaneum Expedition Scheme (SES) collection and remarked that most striking feature in the selfed progenies was no loss of vigour as a result of the inbreeding. Similar to the basic species, inter-specific hybrids also performed well the inbreeding without loss of vigour in the progeny. Stevenson (1953) demonstrated that some population derived from the inter-specific hybrids did not inbreeding show any appreciable loss of vigour and they behaved like wild ancestors. Self-pollination did not produce a general loss in yield traits such as soluble acids, stalk number and stalk diameter (Silva and Goncalves 2011).

Inbreeding has been used as method of sugarcane improvement in many ways and it is one of the classical methods to understand the potential worthiness of parents. Selfing exposes and eliminates the deleterious alleles in commercial breeding clones and rapidly concentrates the genes for sugar content. In some populations from selfed S.spontaneum derivatives, there were distinct improvements over the parents in sucrose content of juice and later it was concluded that inbred derivatives with appreciable improvement in juice quality could be produced by successive selfing of certain forms of S.spontaneum and such selfed derivatives were selected as basic material for progressive inbreeding programme (Stevenson, 1965). Ethirajan et al. (1978) recognized the potential present in the inbred selection to establish a gene pool for breeding of high quality sugarcane. Further Cassalet et al. (1996) mentioned that it is possible to segregate different traits in a particular variety without vigor losses by means of selfing and identify disease free plants from susceptible parents. Ferreira et al. (2005) in their study on effects of inbreeding and selection of clones in families obtained through bi-parental, polycrossings and selfing suggested an alternative form of using S₁ clones as donors in the breeding process of reciprocal recurrent selection. The seedling population in which one or both parents were inbreds gave an overall selection percentage at the field nursery stage higher than those populations in general programme. Clonal evaluation of selfs of commercial varieties indicated an improvement in sucrose content with successive cycles of selfing (Shanthi and Alarmelu 2011). Inbreeding also created required variation and helps in exploiting additive genetic variance in the complex polyploidy genome of sugarcane (Durai and Hemaprabha 2016). This method of breeding is being utilized in identifying 22 Coimbatore canes (Co canes) including the recently identified Co 15002 (polycross derivative of self of Co 1148), genetic stock (SBI 1148-11-13-2-255) and commercial varieties like CoLk 94184 (self of CoLK 8001) in India.

The production of inbreds and their use in hybrid production is well demonstrated in cross pollinated diploid crop like maize. Though inbreeding approach in the complex polyploidy genome of sugarcane has been considered as controversial, superior genotypes can be obtained through self-pollination. Repeated selfing of sugarcane clones for few generations guided by molecular marker profiling and progeny testing is one of the approaches to develop inbreds and identifying homozygous individuals (Ram et al. 2015). Development of homozygous parental lines with good combining ability is important to develop heterotic hybrids in sugarcane with high yield, quality and resistance to major stresses that can be directly used in commercial planting. The isozyme analysis of hybrid clone and inbred derivatives have shown a higher level of genetic similarity of inbreds in contrast to the respective parental clones indicating that the level of heterozygosity in this complex polyploidy crop could be lowered by selfing (Hemaprabha et al. 2003). Parental potential was assessed through selfing by a criterion that a good parent for quality improvement would be one that generated selfs with high mean, low SD and narrow range (Hemaprabha and Singh 2007).

Considering the potential worthiness of inbreeding and the current research to identify homogenous lines in sugarcane, present study was conducted in the modern sugarcane varieties i.e. interspecific hybrids with the objectives to estimate the level of variation in different inbreeding populations of sugarcane *viz.*, S_1 , S_2 , S_3 , S_6 and S_7 in order to explore the possibilities of getting relatively homogenous inbreds on repeated selfing which can be used further in sugarcane hybrid seed production and to quantify the inbreeding depression / transgressive segregation among the traits of breeding importance over different generations of selfing.

Materials and Methods

Breeding material of the present study comprised six parents viz., Co 775, Co 99008, Co 0304, Co 2000-10, Co 0112 and MS 6847. The selfing cycle was initiated with selfing of parents during the year 2008 and a total of 350 S₁ inbreeding individuals were developed which were forwarded from ground nursery to clonal stage. Among the S₁ inbreeding population, nine flowering selfs (99008-91, 99008-113, 775-21, 775-22, 775-27, 775-28, 775-45, 0304-127 and ms68/47-3) with more than 20 % pollen fertility were further selfed to obtain 457 S_2 populations. These S_2 population of Co 775 (775-27-311 and 775-45-406) and MS 68/47 (ms 68/47 -3-12) were selfed to get S₂ individuals which were carried to the clonal stage. Similarly the clones 1148-13-11-237, 1148-13-11-2-252, 1148-S₄-242-5 and 1148-S₄-242-7 were selfed to get four S_6 inbreeding families of 185 individuals. Further eight inbreeding individuals *viz.*, 1148-13-11-2-237-69, 1148-13-11-2-252-15, 1148-13-11-2-252-47, 1148-13-11-2-252-55, 1148-S₄-242-5-54, 1148-S₄-242-7-118, 1148-S₄-242-7-146, 1148-S₄-242-7-121 in S₆ generation were selfed to get 173 S₇ inbreeding individuals. All the inbreeding individuals in clonal stage were evaluated during 2016-17 in a single row trial of plot size of 5.4 m² (1r x 6m x 0.9m) and the parents were replicated in each block. Inbreeding populations were studied along with their respective parents for three important economic traits *viz.*, number of millable canes (NMC), cane thickness (CT) and HR Brix.

Standard statistical techniques were followed to estimate different parameters of variation. Inbreeding depression / transgressive segregation was estimated as per the following formula.

Inbreeding depression (IBD) $\% = (F_1 - F_2) / F_1 \times 100$

where

 F_1 = Mean of the clone I (parental performance)

 F_2 = Mean of the generation of clone I (progeny performance)

For molecular analysis of advanced generation inbreds for their homozygosity, plant sample was collected from eleven S_{τ} selfed progenies viz., 1148-S₆-2-59, 1148-S₆-2-60, 1148-S₆-61, 1148-S₆-63, 1148-S₆-2-66, 1148-S₆-2-69, 1148-S₆-2-73, 1148-S₆-81, 1148-S₆-82, 1148-S₆-88 and 1148-S₆-89 along with their immediate parent $1148-S_{6}-2$ and the grandparent Co 1148. DNA was isolated from young leaf tissue by C-TAB method (Doyle et al. 1987) and purified. The quantity and quality were checked using Nanodrop Spectrophotometer (ND-1000). PCR reaction was carried out with a total volume of 10 µl reaction mix consisting final concentration of 20 ng template DNA, 25 pmol each of forward and reverse primers, 0.3unit Taq polymerase (Merck, India), lx Taq Buffer (Merck, India) and 100 µM dNTPs (Merck, India).

The PCR cycles consisted of 2 minutes at 95°C followed by 30 cycles of 1 minute at 94°C, 40 seconds at the annealing temperature standardized for each primer (ranging from 55°C to 58°C), 40 seconds extension at 72°C and a final extension of 7 minutes at 72°C. The PCR reaction was performed in a thermal cycler (Mastercycler Pro S, Eppendorf, Germany) The amplified products

of microsatellite analysis were separated on 8 per cent denaturing polyacrylamide gels and micro satellite bands were resolved by silver staining procedures.

For molecular data analysis the amplified bands were scored for presence (1) or absence (0) in all inbreds used and the data were used to calculate the Jaccard's similarity coefficient

Primers	Forward primer(5'3')	Reverse primer(5'3')	Temp
NKS1	TGGCATGTGTCATAGCCAAT	CCCCAACTGGGACTTTTACA	58
NKS3	CGTGTTCCTCTTCAACAACG	TGCTTCGCTATATATGGGTTCA	58
NKS8	GTGACAGCGGCTTGTTCAG	TTAAACACGCAGCCATTCAG	58
NKS9	CTTTCAGTGGCCATCTCCAT	GAATGCGCAGGGATAGGATA	58
NKS31	AACCACCACTCATCGTCCTC	CACCGAGTTCCCATTGTTCT	58
NKS34	CGTCTTGTGGATTGGATTGG	TGGATTGCTCAGGTGTTTCA	58
NKS57	CGAGCCTCCCTCCATAGATT	ACCACCACCAACCTCATCTC	55
NKS61	TTGGACATGGCAAGTCTTTG	AGGAACCTCCCAAGAACACA	55

List of Primers used

(JSCs) using NTSYS- pc software (Version 2.0). Using molecular marker data, degree of dissimilarity between two genotypes, as the direct description of allelic variation was derived as per Nei and Li (1979). Genetic similarity (GS) that represents the proportion of molecular markers common between the two individuals being compared was performed by comparing the number of rows of marker fragments of (apparently) similar size separated by electrophoresis. Genetic distances between each pair of lines were estimated as D = 1 -GS. For genetic diversity analysis, Jaccard's coefficient (Jaccard 1908) was used considering it appropriateness with when using a co-dominant marker like SSR. The pair wise Jaccard index is calculated by the following equation: GSij = Nij/ [Ni + Nj+ Nij] where Nij is the total number of bands common to lines i and j, and Ni and Nj are the number of bands only present in i and j, respectively.

Results and Discussion

The various economic traits viz., NMC,CT and HR Brix were affected differently in the selfed progenies and hence these selfed progenies exhibited dissimilar level of variation (Fig. 1) and some of them showed increased improvement over parental clones. Among the three characters studied, very narrow level of variation was observed for HR Brix in the selfed progenies of Co 775. Almost a similar trend was noticed in all S_1 progenies of other genotypes and also in S_2 and S₂ generations. Narrow range observed for HR Brix may be due to the fact that sucrose content in sugarcane is additive in nature and highly heritable with relatively lesser level of environmental effects. Ethirajan et al. (1978) also reported greater phenotypic stability for sucrose content among the selfed progenies of Co 775, Co 1148, CP 44-101, NCo 310, Co 285 and Co 312. NMC recorded wide variation and for cane thickness moderate level of variation were observed. Though the genotypes

Characters		S ₁	S	2	S	3
Number of millable	0.66	0.80	0.49	0.65	0.49	0.63
canes	Co 99008	Co 2000-10	0304-127	775-27	775-27-311	68/47-3-12
(NMC)						
Cane thickness	0.16	0.63	0.14	0.53	0.11	0.48
	Co 2000-10	Co 0112	99008-91	775-27	775-45-406	68/47-3-12
H.R. brix	0.10	0.19	0.09	0.17	0.07	0.16
	Co 775	MS 68/47	99008-91	775-27	775-45-406	775-27-311
			and 99008-			
			113			

Table 1. Co-efficient of variation observed in early generation inbreds

differed in their selfing ability, they produced progenies in identical manner. These results were in agreement with Durai et al. (2012) wherein it was observed that high level of genetic similarity among the parents may be due to the repeated use of few set of parents in developing varieties which are themselves related as parents.

The co-efficient of variation in S_1 for NMC ranged from 0.66 (self of of Co 99008) to 0.80 (S_1 selfs of Co 2000-10). The variation in S_2 for this cane character varied from 0.49 (selfs of 0304-127) to 0.65 (selfs of 775-27). In S_3 it varied from 0.49 (selfs of 775-27-311) to 0.63 (selfs of 68/47-3-12). The co-efficient of variation for CT varied from 0.16 (selfs of Co 2000-10) to 0.63 (selfs of Co 0112) in S_1 and ranged from 0.14 (selfs of 99008-91) to 0.53 (selfs of 775-27) in S_2 . The variation

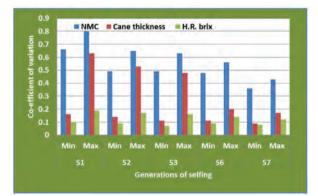


Fig. 1. Trend of variation in the different generations of inbred populations

in S_3 for CT was in the range of 0.11 (selfs of 775-45-406) to 0.48 (selfs of 68/47-3-12). The co-efficient of variation for HR Brix was found between 0.10 (selfs of Co 775) and 0.19 (selfs of MS 68/47) in S_1 and it ranged from 0.09 (selfs of 99008-113 and 99008-91) to 0.17 (selfs of 775-27) in S_2 . The variation in S_3 for HR Brix was in between 0.07 (selfs of 775-45-406) and 0.16 (selfs of 775-27-311). (Table 1).

The range of variation in S_6 for NMC was from 0.48 (1148-13-11-2-237) to 0.56 (1148- S_4 -242-7). This was reduced to 0.36 (1148-13-11-2-252-47) to 0.43 (1148- S_4 -242-7-118) in S_7 . As observed in the case of NMC, the variation for CT reduced from S_6 (0.11 in1148- S_4 -242-7 to 0.20 in 1148-13-11-2-252) to S_7 (0.09 in 1148-13-11-2-237-29 to 0.17 in 1148-13-11-2-252-47). The S_6 inbreeding individuals of 148-13-11-2-237 showed the lowest level of variation for HR Brix (0.09) and among them 1148- S_4 -242-7 showed the highest level of variation (0.14). This variation was reduced to the range of 0.08 (1148- S_4 -242-7-121) to 0.12 (1148-13-11-2-252-47) in S_7 (Table 2).

The genotypes exhibiting the least variation in S_1 did not show-lowest variation in S_2 and S_3 which indicated the fact that the different genotypes behave differently in attaining the homozygosity on selfing. Among the different Co canes studied, Co 99008 for NMC, Co 2000-10 for CT and Co

Characters	S ₆		S ₇	
NMC	0.48	0.56	0.36	0.43
	1148-13-11-2-237	1148-S4-242-7	1148-13-11-2-252-47	1148-S4-242-7-118
Cane	0.11	0.20	0.09	0.17
thickness	1148-S4-242-7	1148-13-11-2-252	1148-13-11-2-237-29	1148-13-11-2-252-47
H.R. brix	0.09	0.14	0.08	0.12
	1148-13-11-2-237	1148-S4-242-7	1148-S4-242-7-121	1148-13-11-2-252-47

Table 2. Co-efficient of variation observed in later generation inbreds

775 for HR Brix had the least variation among their selfed progenies for the respective traits and may be considered for developing homogenous inbred population. Thirteen inbreeding individuals 0304-127 in S_1 and 775-27-311 in S_2 1148-13-11-2-237 in S_6 and 1148-13-11-2-252-47-1 in S_7 for NMC and 99008-91 in S_1 and 775-45-406 in S_2 , 1148- S_4 -242-7 in S_5 and 1148-13-11-2-227- S_6 -1 in

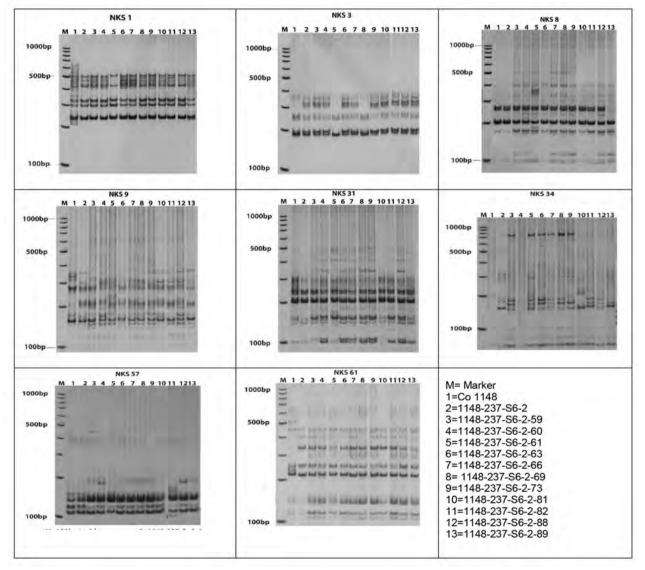


Plate 1. Molecular variation in later generation inbred progenies of Co 1148

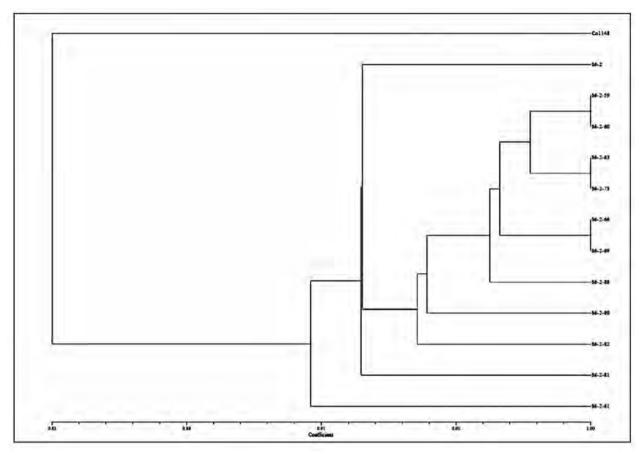


Fig.2. Dendrogram showing genetic similarity among advanced generation inbreds of Co 1148

 S_6 for cane thickness and 99008-113 and 99008-91 in S_1 and 775- S_2 -12 in S_2 1148-13-11-2-237 in S_6 and 1148- S_4 -242-7-121 in S_7 for HR Brix were identified to produce relatively uniform progenies for the above traits. The earlier generation inbred 775- S_2 -5 with least variation for CT and HR Brix and the later generation inbred 1148-13-11-2-237 for NMC and HR Brix may considered for further selfing and developing inbreds which may be further utilized in hybridization to have homogenous progenies.

Among the four advanced generation inbreeding individuals showing the least variation for the traits studied viz., 1148-13-11-2-237 (for NMC) and HR Brix), 1148-13-11-252-247 (for NMC), 1148-13-11-2-237-29 (for CT) and 1148-S₄-242-7 (for CT and HR Brix) showed the lowest variation for the traits studied. Two parents *viz*.,

1148-13-11-2-252-247 and 1148-13-11-2-237-29 showed higher variation in their S_7 progenies for any one of the traits studied. Among the remaining two inbreds, S_7 progenies of 1148-13-11-21-237 with sufficient number of individuals have been taken for molecular analysis of homozygosity. The molecular analyses of parents and inbred derivatives have shown a higher level of genetic similarity of inbreds in comparison to the respective parents (Plate 1 and Fig 2). Hemaprabha et al. (2000) also identified genotypes which showed higher levels of genetic similarity among the selfed progenies explicating the potential of some parents in giving a near homozygous population with limited selfing.

From the molecular analysis of S_7 progenies, it is revealed that S_6 -2-59 and S_6 -2-60, S_6 -2-63 and S_6 -2-73 and S_6 -2-66 and S_6 -2-69 were highly similar to each other while other inbreds *viz.*, S_6 -2-88, S_6 -2-89, S_6 -2-82, S_6 -2-81 and S_6 -2-61 formed distinguished cluster indicating heterozygosity among the inbreeding individuals in the population. Similar report on similarity between inbred lines in maize was reported by Shehata et al. (2009). Relatively homozygous individuals at S_5 to S_7 generation of selfing identified in the study may be further selfed to get homozygous individuals or can be utilized directly in the crossing programme as donor parents to assess the combining ability and in identification of good combiners for hybrid seed production technology in sugarcane.

Transgressive segregation on selfing in sugarcane

The progeny mean and parental mean and the level of inbreeding depression (DE) and transgressive segregation (TS) in different generations of selfing is given in the Table 3. The parental mean and population mean were found the same for 0304-127 and its progenies for NMC, 99008-113 and its progenies for cane thickness in S₂ and 775-45-406 and its progenies for HR Brix in S₃ generation. More number of transgressive segregants were obtained in Co 775, MS 68/47 and Co 0112 for NMC, in Co 99008, MS 68/47 and Co 0112 for HR brix and in MS 68/47 and Co 0112 for cane thickness. Among the 86 inbreds of Co 775, 20 had high number of NMC than the parent. Similarly, eight of Co 0112, five of MS 68/47, three each of Co 99008, CoM 0265 and two each of Co 94012, Co 95012 and Co 0402 were found to have better NMC than their respective parents. Four inbreds of Co 775, two inbreds of Co 99008 and one each of Co 0304 and MS 68/47 recorded significantly higher HR Brix than their parents (Data not shown). Similar observation in some of the selfed families of SP 88-754 and SP 84-202 were reported by Ferreira et al. (2005).

Inbreeding depression (IBD) on selfing in sugarcane

Inbreeding has been described as an extreme form of genetic assortative mating (Allard, 1960) and allogamous species are expected to have higher level of IBD. However it was demonstrated that selfing in sugarcane results in loss of vigour but not always (Stevenson, 1959). All the parents exhibited IBD for cane thickness and HR Brix and not for NMC in S₁ The IBD for NMC varied from 2.00 (Co 2000-10) to 14.87 % (Co 0304) in S₁and no IBD was observed among the S₁ progenies of Co 775, Co 99008 and Co 0112. However, in S_2 other than 775-22, all the families exhibited IBD that ranged from 1.50 (775-45) to 61.47 % (775-27). Though IBD was observed in few S₁ families and all the families in S_2 except 775-22, the inbreeding depression for NMC was inhibited in such a manner that no inbreeding depression was observed for NMC at S₃ generation of selfed populations of Co 775 and MS 68/47. Inbreeding in general is due to loss of favourable heterozygote combinations and IBD effect on a trait depends on the proportion of directional dominance in the trait. More number of transgressive segregants was observed for NMC which suggests further selfing to achieve homozygosity in this trait. This may be due to the fact that life history traits usually show less IBD since these characters were preferred over other traits during the selection or evolution process (Wright et al. 2007).

IBD for cane thickness varied from 0.73 (Co 2000-10) to 28.92 (Co 99008) in S_1 and 3.75 (68/47-3) to 20.7 (0304-127) in S_2 and more number of families showed IBD for cane thickness (14). Silva and Goncalves (2011) also had the opinion that selfing resulted in reduction of average stalk diameter while studying the 26 parental clones from Brazil. Further less number of transgressive segregation in cane thickness may possibly be caused by the low frequencies of favourable

		Number of millable canes		С	Cane thickness			HR brix		
Generation	Genotype	ProM	PM	IBD (%)	ProM	PM	IBD (%)	ProM	PM	IBD (%)*
	Co 775	14.47	13.67	Negative	2.40	2.97	19.19	18.4	20.67	10.98
	Co 99008	19.33	15.92	Negative	2.04	2.87	28.92	18.56	21.53	13.79
C 1	C0 0304	13.34	15.67	14.87	2.70	3.23	16.41	20.06	21.53	6.83
S1	Co 2000-10	14.70	15.00	2.00	2.71	2.73	0.73	17.11	17.33	1.27
	Ms 68/47	13.00	13.52	3.85	2.97	3.00	1.00	14.12	16.87	16.30
	Co 0112	15.52	15.33	Negative	2.60	2.65	1.89	15.61	20	21.95
	775-21	7.43	13.00	42.85	2.73	2.6	Negative	20.38	20.00	Negative
	775-22	11.35	8.00	Negative	2.68	2.3	Negative	21.31	17.80	Negative
	775-27	6.55	17.00	61.47	2.45	2.7	9.26	19.91	23.60	15.64
	775-28	8.00	10.00	20.00	2.48	2.6	4.62	21.03	21.00	Negative
S2	775-45	7.88	8.00	1.50	2.55	2.8	8.93	21.76	20.80	Negative
	99008-91	10.00	14.00	28.57	2.27	2.8	18.93	22.71	24.00	5.38
	99008-113	9.35	11.00	15.00	2.3	2.3	0.00	22.69	23.00	1.348
	0304-127	7.00	7.00	0.00	2.38	3.0	20.7	22.06	23.00	4.09
	68/47-3	6.68	8.00	16.50	2.31	2.4	3.75	21.7	21.00	Negative
S3	775-45-406	18.0	17.00	Negative	2.50	2.4	Negative	20.20	20.20	0.0
	775-27-311	20.2	16.00	Negative	2.40	2.6	7.69	18.80	18.20	Negative
	68/47-3-12	16.2	16.00	Negative	2.40	2.3	Negative	19.80	20.80	4.80

Table 3. Inbreeding depression and transgressive segregation in S₁, S₂ and S₃ inbred populations

IBD= Inbreeding depression; ProM= Progeny mean ; PM= Parental mean; the word 'Negative' indicate transgressive segregants in positive direction

alleles for these traits. Clones with higher parental mean had severe IBD for cane thickness. However the most promising results were obtained in 775-21 with higher parental mean and produced lesser level IBD for cane thickness. These results were in agreement with Cassalet et al. (1996) and Silva and Goncalves (2011) and the effect may be due to retention of the superiority of parents in successive generations of selfing by some of inbreeding individuals.

IBD for H.R brix varied from 1.27 (Co 2000-10) to 21.95 (Co 0112) in S_1 and 1.35 (99008-113) to 15.64 (775-27) in S_2 . In S_2 generation, HR brix was the trait which showed very less IBD than other traits. The IBD for H.R brix was reduced from maximum of 21.95 % in S_1 to 4.80 % in S_3 .

Hogarth (1980) and Ferreira et al. (2005) also observed that brix was the less affected trait by the IBD. Some of the clones with higher parental mean had less IBD for HR Brix and cane thickness due to inheritance of excellent agronomic traits of the parents by these inbreds. Hogarth (1980) who analyzed some diallel crossings stated that the trait brix was not affected by IBD indicating that the genetic variance for brix is additive.

Conclusion

Transgressive segregants identified in the earlier generation of inbreeding may be utilized to develop trait specific inbreds by repeated selfing guided by molecular profiling of the progenies for generation advancement and selecting for positive extremities for the traits of breeding importance. Inbreds with higher level of homozygosity in the later generation of selfings *viz.*, S_6 -2-59 and S_6 -2-60, S_6 -2-63 and S_6 -2-73 and S_6 -2-66 and S_6 -2-69 of Co 1148 developed in this study may be utilized for hybrid seed production in sugarcane after assessing their combining ability, breeding values and seed setting percentage in the inbred cross combinations. This study also revealed the fact that it is possible to develop inbred population with high sucrose and also identify superior inbred donors for improvement of yield traits *viz.*, NMC and CT in future breeding programmes.

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