



Evaluation of parental value of selfs in sugarcane (*Saccharum* spp.) breeding

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Abstract

Use of selfs as parents in breeding programme was assessed. Genetic divergence estimated among eighteen selfs, six each derived from three proven parents viz., Co 7201, Co 775 and Co 1148 using twenty three sugarcane specific STMS primers revealed high within-parental group genetic similarity (0.86 in Co 7201 group, 0.84 in Co 1148 group and 0.85 in Co 775 group) and moderate genetic similarity among the groups. Sixteen more divergent ($SI \leq 0.60$) combinations were identified, which were not used in breeding programme. These diverse crosses could be used to exploit heterosis in sugarcane. Progenies from crosses effected involving diverse selfs of Co 1148 and Co 775 and between the original parents were evaluated. For Brix, the progenies from the selfs of Co 775 as female parent showed a marginal improvement (0.72 %) over those of Co 775 \times Co 1148 cross, indicating that selfs of Co 775 were not better for improving Brix, unlike selfs of Co 1148 that showed mean improvement of 5.17 % over Co 1148 \times Co 775. The magnitude of improvement in NMC and cane height was more in the selfs of Co 775 over that of Co 775 when used as female parent, whereas the selfs of Co 1148 were better as parents for juice quality, cane diameter, cane length and single cane weight. Use of diverse selfs of Co 775 and Co 1148 selected with maximum expression of the desired trait could be successfully utilized as parents in genetic improvement programmes and demanded more studies on the appropriate use of selfs to improve the accuracy of sugarcane breeding. The results of the study also assume significance in light of practical application of molecular tools at field situations in the complex crop of sugarcane.

Key words: Sugarcane, selfs, genetic diversity, cross performance, genetic gain

Introduction

As sugarcane breeding is highly unpredictable due to high levels of heterozygosity and polyploidy, parents are often selected based on selection rate of seedlings advanced till the final stage of selection [1]. Better ways of understanding parental worthiness through classical and molecular means were explored. Inbreeding

was one such method to expose and eliminate deleterious alleles in commercial breeding clones. In sugarcane the purpose of the inbreeding approach and the application of the system are somewhat different from those from seed-propagated crops, where the primary purpose of the inbred line is to preserve a particular genotype, which simply and effectively is ensured in sugarcane by vegetative propagation [2]. Though inbreeding is not considered as an effective procedure for releasing commercial types in sugarcane due to inbreeding depression, the methodology was successful for evolving elite donor inbred stocks for specific characters through stage-wise selfing and directional selection and to outcross the elite selfs with noble canes/commercial hybrids, to rapidly concentrate the genes for sugar content [1] and to produce reliable donors with predictable breeding behaviour [3].

However, breeding for developing inbreds was not pursued with interest by sugarcane breeders due to problems in selfing including sterility and incompatibility and slow rate of success in terms of varieties. Inbreeding, especially selfing could be more appropriately applied to assess the parental worth of a clone and to develop parents in breeding programmes. Work in these lines at Sugarcane Breeding Institute during the past two decades has contributed to several selfs and generated information of the breeding potential of several commercial canes and interspecific derivatives [4]. Quantification of genetic similarity among inbred progenies of sugarcane revealed that different parental clones behaved differently to selfing with regard to the mean and range for juice quality of progeny [5]. In this study, the extent of genetic divergence among the selfs developed from three proven parents was estimated using sugarcane specific Sequence Tagged Microsatellite Sites (STMS), and genetic gain of selected inbreds over those involving parents was estimated in order to assess the usefulness of selfs as parents in varietal development programmes.

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Materials and methods

Eighteen selfs derived from three proven parents, viz., Co 7201, Co 1148 and Co 775 were considered to estimate genetic divergence. These clones were second and third generation inbreds of Co 775 (775-9-167, 775-9-169, 775-2-16-170, 775-2-16-175, 775-2-16-179 and 775-2-16-180), first generation inbreds of Co 7201 (7201-134, 7201-136, 7201-138, 7201-149, 7201-153 and 7201-157) and fourth generation inbreds of Co 1148 developed from 1148-13-11-2 (1148-13-11-2-237, 1148-13-11-2-239, 1148-13-11-2-248, 1148-11-11-2-250, 1148-13-11-2-251 and 1148-13-11-2-255).

A total of 23 sugarcane specific STMS primers developed from the Genbank sequences containing microsatellite repeats were used for detecting the genetic polymorphism. The specific primers used were NKSCSSR 2, 5, 8, 12, 17, 20, 21, 23, 25, 27, 29, 30, 31, 40, 46, 51, 52, 56, 57, 63, 64, 66 and 69. The nucleotide sequences of the primers NKSCSSR 2-57 are available in the e web site www.nrcpb.org/STMS.html

Table 1. STMS primers used to quantify genetic diversity among the inbred derivatives of two commercial hybrids of sugarcane

Sl.No.	STMS Primer tested	Primer sequence		PIC values
		Forward (F)	Reverse (R)	
1	NKSCSSR 63	gattggaacat gggattgg	ggagaccctcctt ccttcagt	0.73
2	NKSCSSR 64	atgaaggcaga tgcattgaga	atctggcgaaca cgagagtc	0.67
3	NKSCSSR 65	aacatgccac cattcccata	ttgtgccacaca cacacac	0.55
4	NKSCSSR 69	atgggagtggg actgaagag	aaattaaaccc cggagcag	0.44

and those of NKSCSSR 63, 64, 65 and 69 along with polymorphism information content (PIC) are given in Table 1.

PCR Amplification and electrophoresis

DNA from young leaves of the above parental clones was isolated using CTAB method [6] and quantified by ethidium bromide staining after agarose gel electrophoresis using known concentration of λ DNA. The samples were diluted to get a final concentration of 20ng / μ l for PCR amplification. The PCR was performed in a thermal cycler (Gene Amp PCR System 9700, ABI) using a 10 μ l reaction mix consisting of final concentration of 20ng template DNA, 1 pmol each of forward and reverse primers, 0.5 units Taq polymerase, 1x Taq DNA polymerase buffer, 2mM dNTPs and 1.75mM MgCl₂. The basic cycling profile was 5 min at 94°C followed by 30 cycles of 30 seconds at 94°C, 30 seconds at the annealing temperature standardized for each primer (ranging from 51°C to 59°C) and 50 seconds extension at 72°C and a final

extension of 5 minutes at 72°C. PCR products were resolved through polyacrylamide gel electrophoresis (PAGE) on 7.5% non- denaturing polyacrylamide gels with 0.8% cross linker using 0.5 \times TBE buffer in a vertical electrophoresis apparatus (Hofer SE 600 Ruby) and stained with ethidium bromide. The gels were visualized under UV light in a gel documentation system (Fluorochem™ 5500, Alpha Innotech) to detect polymorphism.

Band scoring and analysis

Bands visualized on PAGE gels were sized using 50bp ladder and scored for their presence as 1 and absence as 0. Binary matrices consisting of 0s and 1s were analyzed to obtain Jaccard's similarity coefficients (SI) among the parental clones using NTSYS-pc software (Version 2.0), Exeter Biological Software, Setauket, NY). Jaccard coefficients were clustered using the clustering program, selecting the Unweighted Pair Group Method with Arithmetic average (UPGMA) algorithm in NTSYS-pc [7].

Evaluation of crosses

Selected inbreds of Co 775 and Co 1148 from the above study were used in hybridization program. Crosses were made between the inbreds of Co 775 and Co 1148 and between the original parents viz. Co 775 and Co 1148 in reciprocal combinations. Seedlings from these crosses were raised in ground nursery and all healthy seedlings were planted in first clonal trial in plot of 3 metre length spaced 90 cm apart. Those crosses with a minimum of thirty five progenies were considered to evaluate cross performance. Data on juice Brix %, cane length, cane diameter, single cane weight and number of millable canes (NMC) were estimated in all the progenies from eight crosses at 360days of crop age. Mean cross performance was estimated and percent improvement over the original crosses was calculated as difference in the mean of cross involving the inbred as parent and the original parents divided by original parent, expressed in percentage for the above five characters.

Results and discussion

Potential of selfs as parents was assessed to derive an improved set of progenies. For this, genetic divergence of the selfs was estimated to harness the best use of genetic diversity and the divergent types were used in hybridization in order to test whether the inbreds would serve as better parents in sugarcane improvement programmes.

Quantification of genetic similarity among the inbreds using STMS markers

Twenty three STMS primers were employed to estimate genetic diversity among eighteen inbreds, six each

derived from three proven parents viz. Co 7201, Co 775 and Co 1148. Microsatellites or simple sequence repeats are widely used as genetic markers and the primers designed from the sequences flanking specific microsatellite loci (STMS) have indicated the power of

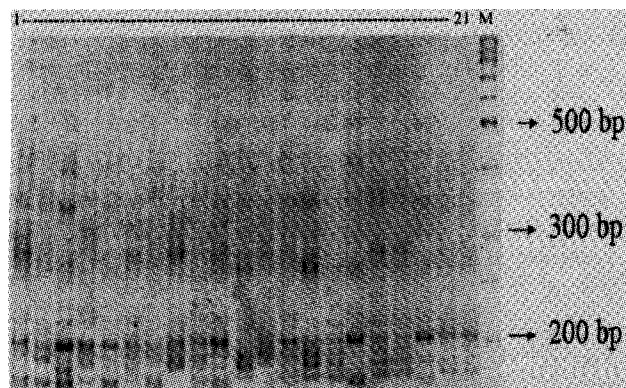


Fig. 1. Genetic polymorphism among eighteen selfs of Co 775, Co 7201 and Co 1148 and the parents revealed with the primer NKSCSSR 20. Lane 1: Co 7201, 2: 7201-134, 3: 7201-157, 4: 7201-136, 5: 72011-138, 6: 7201-149, 7: 7201-153, 8: Co 1148, 9: 1148-13-11-2-240, 10: 1148-13-11-2-250, 11: 1148-13-11-2-251, 12: 1148-13-11-2-237, 13: 1148-13-11-2-239, 14: 1148-13-11-2-252, 15: Co775, 16: 775-165, 17: 775-170, 18: 775-176, 19: 775-166, 20: 775-175, 21: 775-180; M: 50 bp ladder

this marker system in quantifying genetic diversity in sugarcane [8] and in identifying cane varieties [9,10]. The polymorphism detected among the inbreds with the primer NKSCSSR 20 is shown in Fig. 1. A total of 232 markers generated were used to estimate similarity indices among the selfs that showed a wide range of variation (Table 2). Mean similarity index among all the lines was 0.74 and that for each parental group was 0.86 (within Co 7201), 0.84 (within Co 1148) and 0.85 (within Co 775). Overall there were nine combinations with high similarity ($SI \geq 0.90$); four in Co 775 group, three in Co 7201 group and two in Co 1148 group, while no intergroup combination showed high similarity. This observation revealed an increase in homogeneity with inbreeding and highlighted the advantage of repeated inbreeding to develop homozygous lines in the complex polyploid species of *Saccharum*. Similarly, the low variability realized through inbreeding supported the view that selfing was an unattractive breeding approach to varietal development in sugarcane [2]. Sixteen combinations were the most divergent ($SI \leq 0.60$), of which fourteen involved the inbreds from Co 7201 and Co 775, providing molecular evidence to the success of the cross combination Co 7201 \times Co 775 that has generated several commercial hybrids. These diverse crosses, so far not attempted in varietal development programs in a large scale, show promise in the development of cultivars with high

heterotic vigor and for exploring the possibility of crossing between diverse inbreds as one of the breeding approaches in sugarcane. Microsatellite analyses of inbreds with high expression of a desirable trait would facilitate choosing divergent parents for use in sugarcane improvement. This finding supported Stevenson [2] who strongly expounded the value of inbreeding for developing parents in sugarcane varietal development.

Selfs as parents in breeding programme

Diverse combinations involving the inbreds of Co 775 and Co 1148 were identified for use in breeding programmes. Seven such crosses that produced more number of progenies (> 35 numbers) were evaluated along with two parental combinations i.e. Co 775 \times Co 1148 and its reciprocal. The crosses evaluated and the mean performance of the crosses is given in Table 3. The reciprocal crosses involving Co 775 and Co 1148 showed marked differences in performance. Mean values of Brix and cane diameter were better in crosses involving Co 775 as female parent, while NMC and cane length, two major yield components, were higher when Co 1148 was used as female parent. Considering the fact that Co 775 is a better juice quality parent and Co 1148 is notable for its high yield, the results gave enough reasons to believe that a clone with better expression of the trait used as female parent would be beneficial for better genetic gains. Previous investigations also indicated reciprocal differences in sugarcane for juice quality traits [3, 11] and maternal effects for stalk number, stalk length and refractometer solids [12].

Comparative performance of original parents and the selfs of both Co 775 and Co 1148 is provided in Table 4 and Figure 2. Selfs of Co 775 produced progenies with higher mean Brix than that of Co 775 as female parent and were better than Co 1148 \times Co 775 progenies. While the selfs of Co 775 registered a marginal improvement of 0.72% over the cross Co 775 \times Co 1148, the selfs of Co 1148 showed 5.17% improvement over Co 1148 \times Co775 for Brix. This indicated that selfs of Co 775 were no better parents than Co 775 in improving Brix unlike the selfs of Co 1148 which were better than the original parent. It has been reported that cane thickness is a character that showed high amount of inbreeding depression [1]. In this study, the mean of the progenies from the selfs of Co 775 registered a reduction in cane thickness of -7.8%. The progenies derived from the selfs of Co 1148 behaved better for the character with an improvement of 6.17 %. Similarly single cane weight also showed a positive effect in the progenies derived from selfs with 9.78% and 6.67% improvement in Co 775 and Co 1148 group respectively. Mean cane length of the progenies involving Co 775 as female parent

Table 2. Similarity indices between each pair of 18 selfed progenies and three parents based on markers generated from 19 sugarcane specific STMS primers

	Co 7201	7201-S ₁ -134	7201-S ₁ -157	7201-S ₁ -136	7201-S ₁ -133	7201-S ₁ -149	7201-S ₁ -153	Co 1148	1148-S ₄ -240	1148-S ₄ -250	1148-S ₄ -251	1148-S ₄ -237	1148-S ₄ -239	1148-S ₄ -252	Co 775	775-S ₂ -165	775-S ₃ -170	775-S ₃ -176	775-S ₂ -166	775-S ₃ -175	775-S ₃ -181
Co7201	1.00	0.87	0.81	0.84	0.82	0.85	0.88	0.83	0.78	0.83	0.80	0.78	0.73	0.80	0.67	0.66	0.60	0.62	0.65	0.61	0.64
7201-134		1.00	0.86	0.86	0.82	0.89	0.91	0.80	0.80	0.82	0.77	0.79	0.76	0.68	0.70	0.62	0.68	0.66	0.65	0.59	0.63
7201-157			1.00	0.90	0.86	0.85	0.85	0.82	0.82	0.78	0.79	0.81	10.74	0.75	0.64	0.63	0.62	0.62	0.64	0.62	0.57
7201-136				1.00	0.90	0.85	0.82	0.84	10.82	0.78	0.79	0.74	0.75	0.75	0.57	0.60	0.58	0.55	0.53	0.55	0.59
7201-133					1.00	0.85	0.84	0.84	0.83	0.78	0.77	0.85	0.78	0.81	0.79	6.81	0.73	0.66	0.57	10.60	0.59
7201-149						1.00	0.85	6.87	0.83	0.84	0.85	0.81	0.81	0.79	6.66	0.63	0.63	0.62	0.64	0.62	0.62
7201-153							1.00	0.81	0.84	0.81	0.80	0.79	0.81	0.74	0.70	0.65	0.65	0.68	0.65	0.63	0.65
Co1148								1.00	0.72	0.79	0.91	0.89	0.85	0.82	0.64	0.64	0.68	0.70	0.65	0.63	0.63
1148-13-11-2-240									1.00	0.89	0.88	0.83	0.81	0.77	0.73	0.67	0.72	0.74	0.71	0.67	0.71
1148-13-11-2-250										1.00	0.85	0.94	0.86	0.84	0.74	0.66	0.68	0.67	0.69	0.70	0.63
1148-13-11-2-251											1.00	0.88	0.83	0.85	0.74	0.63	0.71	0.69	0.65	0.65	0.64
1148-13-11-2-237												1.00	0.78	0.88	0.71	0.63	0.68	0.65	0.64	0.63	0.62
1148-13-11-2-239													1.00	0.84	0.69	0.60	0.65	0.63	0.64	0.59	0.64
1148-13-11-2-252														1.00	0.72	0.68	0.69	0.65	0.68	0.63	0.68
Co 775															1.00	0.83	0.85	0.82	0.85	0.86	0.87
775-9-165																1.00	0.85	0.82	0.83	0.84	0.79
775-2-16-170																	1.00	0.90	0.85	0.88	0.85
775-2-16-176																		1.00	0.81	0.90	0.85
775-9-166																			1.00	0.87	0.91
775-2-16-175																				1.00	0.92
775-2-16-180																					1.00

Mean of Co 7201 group = 0.86; Mean of Co 1148 group = 0.84; Mean of Co 775 group = 0.85

Table 3. Mean performance of crosses in plant and ratoon crops involving Co 1148 and Co 775 and their selfs

Sl. No.	Cross	No. of progeny	NMC		Cane diameter (cm)		Cane length (cm)		Single cane weight (kg)		Brix %	
			mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
1.	Co 775 × Co 1148	35	10.9	8.98	2.59	0.23	131.1	43.1	0.67	0.20	19.90	1.70
2.	775-9-169 × Co 1148	45	14.2	8.00	2.43	0.23	140.0	31.0	0.55	0.23	20.22	2.00
3.	775-9-167 × Co 1148	35	14.8	6.33	2.61	0.35	176.5	32.8	0.79	0.18	19.26	1.35
4.	775-9-179 × Co 1148	39	11.6	9.32	2.56	0.23	160.3	27.3	0.75	0.24	19.77	1.88
5.	775-2-16-179 × 1148-13-11-2-251	36	16.2	7.90	2.48	0.25	177.5	33.4	0.70	0.16	20.46	1.67
6.	Co 1148 × Co 775	35	16.1	14.36	2.32	0.40	143.8	31.7	0.60	0.18	18.54	3.05
7.	1148-2-13-3-11 × Co 775	56	14.6	8.56	2.45	0.20	151.9	32.4	0.68	0.27	19.80	2.12
8.	1148-13-11-2-239 × Co 775	42	14.9	9.51	2.47	0.29	178.6	38.1	0.59	0.21	18.59	3.16
9.	1148-13-11-2-255 × Co 775	36	17.1	7.40	2.50	0.29	174.4	43.9	0.66	0.23	19.47	1.95

was the lowest with 131.1 cm against that of Co 1148 (148.8 cm). Selfs of Co 775 were the better parents with an improvement of 21.5% in the progenies, while Co 1148 derived selfs showed 13.1% improvement. NMC is a character that showed maximum variation between the reciprocal crosses of Co 775 and Co 1148, Co 775 being the poor parent and Co 1148 the better parent. The selfs of Co 775 definitely had an advantage with a mean of 14.0 canes/plot and percent improvement of 28.44% over Co 775, while the Co 1148 derived selfs showed a marginal decline of 3.73% in the progeny mean over that of Co 1148.

A comparison of cross performance was made

between the two groups of selfs derived from Co 775 and Co 1148. The magnitude of improvement in NMC and cane height was more in the selfs of Co 775 (NMC = 14.0 and cane height = 159.3 cm) over that of Co 775 (NMC = 10.9 and cane height = 131.1 cm) when used as female parent. In contrast, the selfs of Co 1148 showed a slight reduction in progeny performance (NMC = 15.5 and cane height = 168.3 cm) over that of Co 1148 as female parent. This observation is particularly important as the sucrose rich, moderate yielding parent like Co 775 could produce better selfs for the yield components viz. NMC and cane height unlike those derived from the high yielding clone, Co 1148. It could be possible that maximum

Table 4. Group mean and percent improvement of crosses involving selfs of Co 775 and Co 1148 over original crosses

Groups	Brix %	Cane diameter (cm)	Single cane weight (kg)	Cane length (cm)	NMC (cm)
Co 775 × Co 1148	19.54	2.69	0.67	131.1	10.9
Co 775 selfs × Co 1148	19.68	2.48	0.70	159.3	14.0
Co 1148 × Co 775	18.56	2.27	0.60	148.8	16.1
Co 1148 selfs × Co 775	19.52	2.41	0.64	168.3	15.5
% Improvement in Co 775 selfs	0.72	-7.81	4.48	21.5	28.44
% Improvement in Co 1148 selfs	5.17	6.17	6.67	13.1	-3.73

quality, cane diameter, single cane weight and cane length could be made use of in sugarcane varietal development through use of the selfs selected with the maximum expression of the desired traits as parents. An earlier study led to the development and selection of selfs superior to corresponding parents for internode length through progressive selfing and production of inbred derivatives with high *gca* effects [3]. It was also reported that for effective use of the inbreeding method progeny tests might be made during inbreeding stages to ensure that heterotic loci are maintained. Similarly characters dependent upon hybrid vigour might be ignored and attention concentrated upon primary components of yield in an attempt to fix simple homozygous dominant alleles for the desirable traits and eliminate undesirable alleles. Inbreds homozygous for above mentioned characters are useful in top-crosses

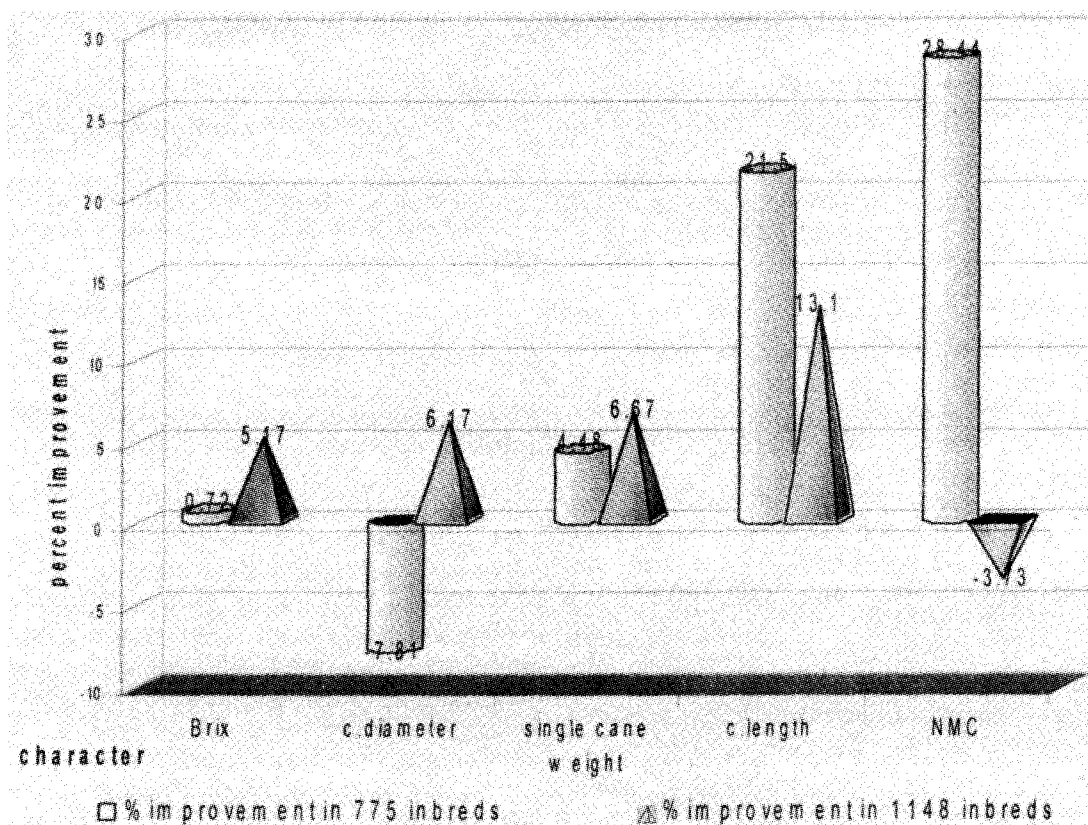


Fig. 2. Percent improvement in the progeny of inbreds as parents over that of the original parents

expression for juice sucrose is attained in Co 775, which could not be further improved upon through selfing, while there is more scope for improving NMC and cane height. Similarly, Co 1148 showed the maximum level of expression for NMC, with more scope for improving juice quality through selfing. This advantage of the selfs of Co 775 over Co 775 for cane length and NMC and selfs of Co 1148 over Co 1148 for juice

enabling selection for yield [1]. This study, in light of these findings could demonstrate the use of selfing as a pre-breeding methodology for developing parental lines for use in sugarcane improvement. Intensified studies in this direction, adopting molecular tools in selfing programmes are expected to refine sugarcane breeding with better precision and success rate.

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