ARTICLE

Identification of promising true sugarcane seed families to explore the possibility of direct commercial cultivation

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Abstract: *True sugarcane seed (TSS) families offer economic, environmental, and practical advantages over clonal varieties. Identification of well-germinating and high-yielding TSS families to replace sett/settling planting is a prerequisite for direct commercial cultivation. Promising TSS families are identified through family selection, an efficient, proven, and cost-effective approach. A total of 139 crosses, with varying sets of 22, 51, and 66 crosses were produced in 2018-19, 2019-20, and 2020-21, respectively. Among the 66 TSS families evaluated, 18F02, 18F27, 19F25, 19F46, 20F10, and 20F11 outperformed the popular clonal check Co 86032, with moderate to low variability, within an acceptable range for cane (number of millable canes per clump ≥ 5.0; single cane weight ≥ 1.0 kg) and sugar (Brix% ≥ 18.0%; Sucrose% ≥ 15.0%) yield traits, accompanied by good fluff germination and seedling establishment parameters. These results implied the possibility of exploiting promising true seed-based families for commercial sugarcane cultivation.*

Keywords: Saccharum*, family selection, acceptable range, TSS families, fluff germination*

INTRODUCTION

Sugarcane (*Saccharum* spp.) is a complex polyaneuploid crop belonging to the Poaceae family, with lengthy breeding and selection cycles. Sugarcane is the second largest economically viable agro-industry based crop next only to cotton, providing valuable by-products such as bagasse, molasses, fodder (green cane tops), and more. Globally, India emerged as the world's largest producer of sugar in 2022-23, with 36.88 million metric tonnes, surpassing Brazil. Upper peninsular India is a high cane and sugar-yielding zone, significantly contributing to Indian sugar production. In contrast, the lower peninsular region is favorable for profuse flowering and true seed setting, making it a key area for sugarcane cultivation and seed production. Sugarcane is cultivated on about 5 million hectares in India, with an annual requirement of 14.42 million tonnes of cane as planting material, even though nearly 55% of the area is occupied by ratoon cane (Bakshi Ram et al. 2015). In the current scenario of increased cost of sugarcane cultivation, largely due to costly and bulky setts/settlings, and the outbreaks of various biotic stresses have become significant factors contributing to clonal

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varietal decline. The practice of monocropping of very few sugarcane clones across large, diverse agro-ecological regions in the country intensifies these issues. The impact of clonal deterioration due to the red rot epidemic was evident in past sugarcane varieties such as CoC 671 and CoJ 64, and more recently Co 0238, resulting in significant economic losses. Hence, seedlings derived from true sugarcane seeds (TSS) should be considered an alternative and novel approach to replace vegetative setts, thereby avoiding the problems associated with clonal sugarcane cultivation.

Sugarcane cultivation through TSS families helps overcome the drawbacks associated with clonally propagated setts, such as the cost of setts/settlings, transportation, seed treatment, admixtures, and bulkiness. TSS-based cultivation saves a huge amount of seed cane, which can be directed to sugar production, and it reduces problems associated with systemic and sett-transmitted diseases. Especially fungi and viruses are considerably reduced, as TSS carries far fewer pathogens. Furthermore, the lifespan of such TSS families is expected to be longer, due to their variable reactions against insects and pathogens, and that acts as a buffer against pest and disease outbreaks. Additionally, the required quantity of TSS (fluff) can be easily packed, stored (-18 °C), and transported to facilitate large-scale seedling nursery production for faster varietal replacement. This approach of using true seeds/seedlings can reduce the cost of sugarcane cultivation. This breakthrough can be achieved through various approaches, such as intermating near homozygous inbred clones developed through repeated selfing (Pathy et al. 2023). Another method is to develop polyhaploid homozygous clones through anther culture (Bakshi Ram et al. 2015). Indeed, family selection is a proven and cost-effective approach for assessing superior clones (individual clone selection) derived from established commercial parents for varietal development programmes (Simmonds 1996, Kimbeng and Cox 2003, Mbuma et al. 2017).

In the current study, this well-established, efficient, and proven family selection approach is employed to identify promising TSS families with high mean values and an acceptable range of variability for farmer-desired (cane yield) traits [number of millable canes per clump (NMC/C) ≥ 5.0, cane girth (CG) ≥ 1.5 cm, single cane weight (SCW) ≥ 1.0 kg] and industry-essential (sugar yield) traits [Brix% ≥ 18.0%, Pol% ≥ 15.0%, commercial cane sugar (CCS%) ≥ 10.5%], along with higher fluff germination (FGP ≥ 1.0%) and better seedling establishment parameters. In sugarcane breeding programs, most current parental clones are commercially established or advanced elite clones, exhibiting considerable uniformity due to their common ancestry. These hybrids typically share a narrow genetic base, often resulting from crosses involving repeated parents, which increases the likelihood of producing genomes with similar compositions, despite substantial phenotypic variation (Mahadevaiah et al. 2021, Margarido et al. 2022). However, due to poly aneuploidy, there is still sufficient genetic variability within families for both cane and sugar yield traits. With this background, the study aimed to identify promising TSS families with higher fluff germination, better seedling establishment, and high mean yield, along with an acceptable range of variability for farmer-desired and industry-essential traits, to enable commercially viable seedling nursery production and subsequent cultivation. This study is unique and the first of its kind in its approach, as it explores the possibility of true-seed derived sugarcane families for direct commercial cultivation.

MATERIAL AND METHODS

Planting material and experimental conditions

A total of 139 TSS families, with sets consisting of 22, 51, and 66 crosses, were produced during the years 2018, 2019, and 2020, respectively. Among them, 15, 26, and 23 crosses (irrespective of the type of cross) were produced at the National Hybridization Garden, Sugarcane Breeding Institute, Coimbatore, India (lat 11° 00′ 58″ N, long 76° 58′ 16″ E), during the flowering period (Nov-Dec) in 2018, 2019, and 2020, respectively. In parallel, a total of 40 general collections (GCs), with varying sets of 3, 13, and 24 GCs, were produced at the Agricultural Research Station (ARS), Sankeshwar (Peninsular Zone, lat 16° 14′ N, long 74° 30′ E). Additionally, a total of 35 GCs, with varying sets of 4, 12, and 19 GCs, were produced at the ARS, Mugad (lat 15° 26′ 39″ N, long 74° 54′ 46″ E), during the post-flowering period (Dec-Jan) of 2018, 2019, and 2020, respectively (Supplementary Figure 1). During the month of May of each cropping season, the collected fluff was sown in a shaded nursery under controlled environmental conditions, with temperatures maintained between 30-35 °C and high humidity levels between 80-85%. This ensured an optimal environment for fluff germination with faster and healthy seedling growth. Simultaneously, settlings of commercial varieties such as CoC 671, Co 09004, CoSnk 09211, Co 86032, CoSnk 09227, CoSnk 09293, and MS 13081 were raised in seedling trays for comparative analysis.

Experimental design

The experiment was set up using an augmented design-II (Federer and Searle 1976), with a plant spacing of 1.20 m × 0.60 m and a row length of 6.00 m. The experiment was distributed across three, five, and five blocks, with 7, 4, and 5 families in each augmented block, respectively, to evaluate 66 TSS families (≥ 40 seedlings) consisting of varying sets of 21, 20, and 25 crosses during the 2019-20, 2020-21, and 2021-22 cropping seasons, respectively, at ARS, Sankeshwar, Karnataka, India. In the ground nursery experiment, each family was represented by 40 random seedlings in a four-row plot with 10 seedlings per row. Additionally, in every block, all seven commercial checks represented by one row each consisting of 10 settlings across all three cropping seasons. The remnant seedlings from the 66 crosses (≥ 40 seedlings), along with seedlings from the remaining 73 crosses (< 40 seedlings) were transplanted into the extended blocks of respective ground nurseries for comparative analysis of germination and seedling establishment parameters. The seedlings and settlings were transplanted into the ground nursery after 45-55 days of nursery growth, based on seedling vigour. The entire package of recommended practices was followed.

Data collection

The weight of fluff sown per cross in grams was recorded before sowing the TSS families in the shaded nursery. After evenly spreading true seeds on a well-prepared seedbed, the number of seedlings germinated (NSG) per cross was recorded at 45 days after sowing, and the FGP was calculated following Singh and Singh (2021). Number of progenies surviving (NPS) per cross was recorded at harvest and seedling survival percentage (SSP) was calculated using the formula:

$$\text{Fluff germination percentage (FGP)} = \frac{\text{Number of seedlings germinated}}{\text{Weight of fluff sown (g)} \times 250} \times 100$$

$$\text{Seedling survival percentage (SSP)} = \frac{\text{Number of progenies surviving}}{\text{Number of seedlings transplanted}} \times 100$$

$$\text{Percentage of unacceptable progeny (PUP)} = \frac{\text{Number of unacceptable progeny}}{\text{Number of progenies surviving}} \times 100$$

$$\text{Percentage of acceptable progenies (PAP)} = \frac{\text{Number of acceptable progeny}}{\text{Number of progenies surviving}} \times 100$$

$$\text{Percentage of population selected (PPS)} = \frac{\text{Number of selectable segregants}}{\text{Number of progenies surviving}} \times 100$$

At harvest, the study recorded the number of unacceptable progeny (NUP) per cross to determine the percentage of unacceptable progeny (PUP). Unacceptable progenies were identified based on visual criteria, such as slower establishment and reduced vigour (Kimbeng and Cox 2003). Additionally, the number of acceptable progenies (NAP) per cross was recorded, indicating progenies that satisfied specific criteria for cane yield (NMC/C ≥ 5.0 and CG ≥ 1.50 cm) and with visual scoring comparable to commercial clonal checks. The percentage of acceptable progenies (PAP) in a cross was then calculated. Furthermore, the percentage of the population selected (PPS) per cross was also determined, based on the number of segregants selected (NSS) for advancement at harvest, considering their superiority over popular checks for yield traits (SCW, CG, Brix%, Pol%, and CCS%), as well as cane features. The data on all remaining seedlings transplanted in the extended blocks of the respective ground nursery were also recorded for comparative analysis among TSS families for seedling establishment parameters. The parentage of all TSS families for each year, along with details regarding the fluff collection location (FCL) and seedling establishment parameters, are listed in Supplementary Table 1. Traits such as NMC/C, CG (cm), and cane height (CH) (cm) were recorded from all the individually tagged progeny populations (TSS families), along with commercial clonal checks, across three cropping seasons at 360 days after transplanting (DAT). For analysis of industry-essential traits, ten random progenies from each of 66 TSS families (family size ≥ 40) were sampled, following the methodology outlined by Leite et al. (2009). The average of three millable canes per clump (progeny) was used to determine SCW. The composite juice extracted from three millable canes per progeny was analysed for Brix% using a Brix hygrometer and for Pol% using a polariscope. Additionally, the CCS% was estimated, following the protocol outlined by Meade and Chen (1977). Cane yield (CY) (t/ha) of each progeny was estimated by multiplying the NMC/C and SCW. Commercial cane sugar yield (CCSY) (t ha^{-1}) was calculated as CCSY = CCS% × CY (t ha^{-1}). For commercial clonal checks, five random samples comprising three random millable canes per clump from each commercial check in every block were assessed at harvest across all three cropping seasons for comparison of yield traits.

Table 1. Comparative performance of promising TSS families for germination and seedling establishment parameters in s sugarcane seedling nursery

Sl. No.	Year	FCL	FC	TSS families (Parentage)	WFS	NSG	NSS	FGP	SSP	PUP	PAP	PPS
1	2019-20	CO	18F02	Co 86032 × CoVC 14061	8.00	125	117	6.25	93.60	7.69	92.31	49.57
2		CO	18F09	CoM 6806 (GC)	17.00	400	389	9.41	97.25	8.74	91.26	29.31
3		CO	18F14	CoVC 14062 (GC)	14.00	275	257	7.86	93.45	4.67	95.33	15.95
4		CO	18F27	Co 11015 (GC)	8.00	122	118	6.10	96.72	6.78	93.22	31.36
5		M	18F41	Co 87015 (GC)	18.00	246	198	5.47	80.49	8.08	91.92	31.82
6	2020-21	CO	19F07	CoVC 14062 × CoT 8201	28.00	340	256	4.86	75.29	15.63	84.38	35.16
7		CO	19F12	Thirumadhuram × CoPant 97222	36.00	303	259	3.37	85.48	19.31	80.69	22.01
8		CO	19F25	CoVC 14062 (GC)	10.00	179	141	7.16	78.77	5.67	94.33	15.60
9		CO	19F26	ISH 69 (GC)	27.00	280	198	4.15	70.71	13.64	86.36	17.68
10		SNK	19F46	Co 85002 (GC)	18.00	302	219	6.71	75.52	6.85	93.15	20.55
11	2021-22	CO	20F10	NB 94-545 × Co 775	56.00	314	268	2.24	85.35	9.97	90.07	12.69
12		CO	20F11	MS 68/47 × CoSe 92423	41.00	257	219	2.51	85.21	4.57	95.43	24.66
13		CO	20F12	Co 94007 × CoPant 97222	33.00	529	469	6.41	88.66	5.54	94.46	12.15
14		CO	20F13	CoVC 14062 × Co 1148	18.00	433	357	9.62	82.45	4.76	95.24	12.89
15		CO	20F16	Co 86032 × HR 83-144	23.00	240	231	4.17	96.25	6.06	93.94	10.39
16		CO	20F19	CoV 89101 (PC)	8.00	157	141	7.85	89.81	8.51	91.49	18.44
17		CO	20F24	CoSnk 05103 (GC)	58.00	264	225	1.82	85.23	12.89	87.11	14.22
18		SNK	20F46	Co 10027 (GC)	26.00	256	206	3.94	80.47	12.62	87.38	17.96
19		M	20F76	Co 09004 (GC)	14.00	189	174	5.40	92.06	5.17	94.83	34.48
20		M	20F107	CoN 17071 (GC)	38.00	491	461	5.17	93.89	4.99	95.01	18.66

FCL: fluff collection location – CO: NHG, SBI, Coimbatore; SNK: ARS, Sankeshwar; M: ARS, Mugad. FC: family code, GC: general collections (open pollinated crosses), PC: poly crosses, WFS: weight of fluff sown (gram) per cross, NSG: number of seedlings germinated per cross, NPS: number of progenies surviving per cross, FGP: fluff germination percentage, SSP: seedling survival percentage: PUP: percentage of unacceptable progenies, PAP: percentage of acceptable progenies, PPS: percentage of population selected per cross

Table 2. Analysis of variance for cane and sugar yield traits of sugarcane in seedling generation throughout the 2019-20 to 2021-22 cropping seasons

2019-20 cropping season										
Source of variation	**df**	**NMC/C**	**CG**	**CH**	**SCW**	**Brix%**	**Pol%**	**CCS%**	**CY**	**CCSY**
Treatment (ignoring blocks)	27	5.06**	0.19**	1970.15**	0.10**	8.26**	9.28**	5.72**	1093.34**	22.90**
Checks	6	5.43**	0.33**	7022.87**	0.24**	11.91**	11.24**	6.32**	2029.58**	36.38**
TSS families	20	3.83**	0.05**	508.56**	0.05**	3.53**	2.80**	1.65**	824.16**	15.40**
TSS families vs. Check	1	27.52**	2.26**	885.69**	0.33**	8.87**	7.12**	3.56**	859.52**	92.23**
Blocks (ignoring treatments)	2	0.76	0.02	31.14	0.01	0.22	0.08	0.02	200.90	3.28
Residuals	12	0.26	0.01	30.98	0.00	0.23	0.12	0.06	78.62	1.59
2020-21 cropping season										
Source of variation	**df**	**NMC/C**	**CG**	**CH**	**SCW**	**Brix%**	**Pol%**	**CCS%**	**CY**	**CCSY**
Treatment (ignoring blocks)	26	5.77**	0.16**	4267.72**	0.13**	5.71**	6.23**	3.77**	2151.89**	37.34**
Checks	6	4.90**	0.28**	12297.06**	0.27**	21.20**	20.07**	11.29**	3976.09**	74.18**
TSS families	19	1.57**	0.05**	1463.68**	0.05**	0.98**	1.68**	1.20**	836.89**	18.30**
TSS families vs. Check	1	50.83**	1.49**	9368.45**	0.81**	2.73**	9.65**	7.65**	16191.71**	178.00**
Blocks (ignoring treatments)	4	0.21	0.01	20.10	0.01	0.19	0.13	0.06	231.23	5.02
Residuals	24	0.26	0.00	9.01	0.00	0.09	0.06	0.04	216.63	4.01
2021-22 cropping season										
Source of variation	**df**	**NMC/C**	**CG**	**CH**	**SCW**	**Brix%**	**Pol%**	**CCS%**	**CY**	**CCSY**
Treatment (ignoring blocks)	31	3.80**	0.18**	1305.71**	0.10**	5.34**	5.48**	3.34**	1540.97**	30.26**
Checks	6	4.90**	0.34**	3719.54**	0.27**	21.2**	20.07**	11.29**	3409.11**	63.49**
TSS families	24	1.94**	0.04**	641.03**	0.05**	1.39**	1.99**	1.33**	941.70**	16.94**
TSS families vs. Check	1	41.72**	2.65**	2774.89**	0.42**	5.11**	1.63**	4.08**	4714.45**	150.70**
Blocks (ignoring treatments)	4	0.21	0.01	14.09	0.01	0.19	0.13	0.06	188.35	4.11
Residuals	24	0.26	0.00	7.47	0.00	0.09	0.06	0.04	172.54	3.17

* $P < 0.05$, ** $P < 0.01$, TSS: true sugarcane seed, NMC/C: number of millable canes per clump, CG: cane girth, CH: cane height, SCW: single cane weight, CCS%: commercial cane sugar percentage, CY: cane yield, CCSY: commercial cane sugar yield

Statistical analysis

The percentage of fluff germination and seedling establishment parameters were calculated for each TSS family using the formulas mentioned above. All recorded data for cane yield and juice quality parameters were statistically analysed using R software (version R-4.2.1) (www.r-project.org). The family means were compared (p = 5%) using Microsoft Excel (Microsoft-365). The promising families were identified based on their performance in terms of germination, seedling establishment parameters, and progeny performance for cane yield and juice quality traits.

RESULTS AND DISCUSSION

Analysing TSS families for fluff germination and seedling establishment parameters

Good germination of true seeds and, consequently, better seedling establishment parameters in sugarcane are essential for identifying promising TSS families suitable for direct commercial cultivation. In this regard, a total of 139 TSS families, with varying sets of 22, 51, and 66 crosses, resulted in the germination of 2284, 3300, and 5467 seedlings, with overall survival frequencies of 85.96%, 72.03%, and 79.62% at 360 DAT during the 2019-20, 2020-21, and 2021-22 cropping seasons, respectively (Supplementary Table 1). Notably, TSS families [*viz*., 18F02, 18F09, 18F14, 18F27, and 18F41 in 2019-20; 19F07, 19F12, 19F25, 19F26, and 19F46 in 2020-21; and 20F10, 20F11, 20F12, 20F13, 20F16, 20F19, 20F24, 20F46, 20F76, and 20F107 in 2021-22 (Table 1)] showed good fluff germination (FGP ≥ 1.0%) and better seedling establishment parameters (SSP ≥ 70%, PAP ≥ 80%, PUP ≤ 20%, and PPS ≥ 10%). Similar results have been reported by Sanghera and Jamwal

Table 3. Genetic variability of yield traits of sugarcane in seedling generations throughout the 2019-20 to 2021-22 cropping seasons

Trait	**NMC/C**[1]	**CG** (cm)	**CH** (cm)	**SCW** (kg)	**Brix%**	**Pol%**	**CCS%**	**CY** (t ha^{-1})	CCSY (t ha^{-1})
				2019-20 cropping season					
Mean	9.00	2.46	235.53	1.30	20.01	17.50	12.37	153.59	18.94
PV	3.83	0.05	508.56	0.05	3.53	2.80	1.65	824.16	15.40
GV	3.57	0.04	447.58	0.04	3.31	2.68	1.59	745.54	13.81
EV	0.26	0.01	60.98	0.01	0.23	0.12	0.06	78.62	1.59
h^2_{BS}	93.16	88.08	88.01	94.72	93.61	95.77	96.27	90.46	89.67
PCV%	22.39	8.84	9.57	17.32	9.39	9.56	10.40	18.69	20.71
GCV%	21.61	8.29	9.28	16.86	9.08	9.36	10.20	17.78	19.61
GAM	43.04	16.06	18.55	33.85	8.13	18.89	20.65	34.88	38.32
				2020-21 cropping season					
Mean	8.00	2.46	271.95	1.66	21.75	19.48	13.93	190.67	26.45
PV	1.57	0.05	1463.68	0.05	0.98	1.68	1.20	836.89	18.30
GV	1.30	0.04	1254.66	0.04	0.89	1.62	1.13	620.27	14.28
EV	0.26	0.01	209.02	0.01	0.09	0.06	0.07	216.63	4.01
h^2_{BS}	83.15	97.22	85.72	94.28	90.64	96.53	96.73	74.12	78.07
PCV%	14.90	9.43	14.07	13.27	4.56	6.66	7.86	15.17	16.18
GCV%	13.58	9.30	13.12	12.89	4.34	6.54	7.73	13.06	14.29
GAM	25.55	18.92	28.84	25.81	8.52	13.26	15.68	23.20	26.05
				2021-22 cropping season					
Mean	8.00	2.58	214.62	1.47	22.13	20.39	14.77	156.87	23.09
PV	1.94	0.04	641.03	0.05	1.39	1.99	1.33	941.70	16.94
GV	1.68	0.04	583.56	0.04	1.30	1.93	1.29	769.16	13.77
EV	0.26	0.00	57.47	0.01	0.09	0.06	0.04	172.54	3.17
h^2_{BS}	86.40	92.92	91.03	94.05	93.39	97.07	97.04	81.68	81.26
PCV%	17.99	8.09	11.80	14.66	5.33	6.92	7.80	19.56	17.82
GCV%	16.72	7.80	11.73	14.21	5.15	6.82	7.68	17.68	16.07
GAM	32.06	15.51	24.05	28.44	10.27	13.86	15.61	32.96	29.88

[1] See codes in Table 2. PV: phenotypic variance, GV: genotypic variance, EV: environmental variance, h^2_{BS}: broad sense heritability (%), PCV: phenotypic coefficient of variation. GCV: genotypic coefficient of variation, GAM: genetic advance over mean (%).

(2019a) and Sudhagar et al. (2023) for germination and seedling establishment parameters. These parameters are of prime importance in ensuring economically viable seedling nurseries that can effectively compete with current settlings nurseries.

To fulfil the requirements for commercial cultivation, the crosses should produce a minimal number of weak, thin seedlings (typically from selfed seeds) in the seedling nursery stage and a minimal number of low-yield progenies in the ground nursery stage. Hence, it is important to minimize the PUP (≤ 20%) by implementing effective pollination strategies. Furthermore, it is important to note that certain TSS families exhibited inadequate fluff germination under shaded nursery conditions (Supplementary Table 1). To enhance germination and seedling establishment parameters, it is also essential to optimize the hybridization process and fluff production in a favourable environment, with utmost care, particularly during the flowering and seed setting stages. This approach could be beneficial for improving these parameters.

Identifying promising TSS families for farmer-desired and industry-essential traits

Analysis of variance (Table 2) indicated significant mean sum of squares for all the traits across various sources of variation, indicating sufficient genetic variability in the population. Block effects were non-significant for all traits, ensuring homogeneity among evaluation blocks throughout the 2019-20 to 2021-22 cropping seasons. Similar observations were reported by Sanghera and Jamwal (2019b). Genetic variability parameters such as the genotypic and phenotypic coefficient of variation (GCV, PCV), broad sense heritability (h^2_{BS}), and genetic advance as a percentage of mean (GAM) were estimated from 21, 20, and 25 crosses in augmented block designs during the 2019-22 cropping seasons to aid in selection for trait improvement. Results indicated genetic variability among the evaluated families for cane yield

Table 4. Descriptive statistics of promising TSS families for yield traits in seedling generation of sugarcane in the 2019-20 cropping season

TSS family	Statistics	NMC/C[1]	CG (cm)	CH (cm)	SCW (cm)	Brix%	Pol%	CCS%
Co 86032 × CoVC 14061	mean	10.00*	2.32	242.05*	1.53*	21.75*	17.52	11.87
	range	5.00–28.00	1.52–3.09	99.90–320.12	1.23–2.30	18.35–24.10	16.05–21.47	10.90–15.31
	CV	37.80	14.46	16.12	16.59	9.37	10.47	12.83
CoM 6806 (GC)	mean	9.00	2.54*	233.42	1.17	19.53	17.36	12.37
	range	4.00–29.00	1.70–3.53	68.46–374.46	0.89–1.70	16.87–23.09	15.34–20.44	10.72–14.65
	CV	54.61	13.10	24.83	18.26	8.65	11.88	11.42
CoVC 14062 (GC)	mean	10.00*	2.59*	251.10*	1.45*	20.64	15.75	10.35
	range	4.00–32.00	1.60–3.77	83.23–367.73	1.06–2.30	16.34–23.60	14.92–20.34	9.04–14.28
	CV	76.28	14.71	20.30	37.25	9.78	9.73	12.08
Co 11015 (GC)	mean	8.00	2.35*	242.69*	1.59*	21.43*	19.31*	13.84*
	range	3.00–20.00	1.59–3.12	111.54–353.87	1.49–1.70	19.09–24.10	16.05–22.41	10.90–16.51
	CV	40.64	13.07	19.64	18.05	8.07	9.43	12.87
Co 87015 (GC)	mean	7.00	1.60	242.96*	1.40	21.94*	19.71*	14.11*
	range	5.00–27.00	0.46–2.59	77.47–354.14	1.24–2.33	18.92–23.93	16.92–22.36	12.09–16.30
	CV	52.08	22.87	22.04	16.85	7.09	9.11	9.39
Commercial checks (plants grown with vegetative settlings)								
Co 09004	mean	7.00	2.73	216.17	1.37	24.17	21.85	15.69
	range	6.00–8.00	2.35–2.95	192.00–238.00	1.25–1.85	23.92–24.53	21.64–22.12	15.55–15.87
	CV	14.29	11.16	10.54	15.13	1.33	1.13	1.05
Co 86032	mean	8.00	2.31	222.17	1.15	20.12	17.98	12.85
	range	5.00–9.00	2.20–2.73	173.69–232.00	1.10–1.40	19.97–21.03	17.05–18.93	12.45–13.07
	CV	28.57	10.88	14.11	12.11	2.61	2.92	2.47
Overall mean ± SD		9.00±1.88	2.46±0.32	235.53±30.24	1.30±0.24	20.01±2.21	17.50±2.28	12.37±1.80
Standard error		0.36	0.06	5.71	0.05	0.42	0.43	0.34
Critical difference @ 5%		1.38	0.20	14.97	0.14	1.28	0.93	0.67
CV %		6.14	2.91	2.34	3.85	2.29	1.87	1.90

* Significant at 5% level of probability over popularly grown clonal check Co 86032, SD: standard deviation, CV: coefficient of variation, GC: general collections, TSS: true sugarcane seed. [1] See codes in Table 2.

Table 5. Descriptive statistics of promising TSS families for yield traits in seedling generation of sugarcane in the 2020-21 cropping season

TSS family	**Statistics**	**NMC/C**	**CG** (cm)	**CH** (cm)	**SCW** (cm)	**Brix%**	**Pol%**	**CCS%**
CoVC 14062 × CoT 8201	mean	9.00*	2.57*	204.40	1.66*	23.76*	21.54*	15.49*
	range	5.00–21.00	1.40–3.77	65.00–336.00	1.09–2.01	21.50–25.02	18.46–23.74	12.93–17.42
	CV	42.38	14.50	27.35	24.30	4.65	6.83	7.92
Thirumadhuram × CoPant 97222	mean	11.00*	2.20	264.70*	1.40	22.71*	19.56*	13.72
	range	5.00–35.00	1.40–3.37	81.00–412.00	1.06–1.77	18.51–24.52	15.29–23.02	10.50–16.82
	CV	66.21	16.22	25.42	17.27	7.31	10.78	12.62
CoVC 14062 (GC)	mean	8.00	2.43*	289.31*	1.76*	21.88*	19.56*	13.74
	range	6.00–31.00	1.30–3.00	128.33–413.44	1.13–2.14	18.50–24.00	16.50–21.64	11.77–15.52
	CV	59.43	13.54	20.71	16.89	8.69	9.03	9.14
ISH 69 (GC)	mean	10.00*	2.43*	301.51*	1.45	20.69	17.63	12.31
	range	6.00–38.00	1.43–3.20	123.67–479.56	1.01–1.73	18.50–23.52	16.00–21.93	11.25–15.97
	CV	66.95	14.23	20.48	17.50	13.93	9.03	22.81
Co 85002 (GC)	mean	9.00*	2.51*	329.72*	1.68*	21.97*	19.72*	14.11*
	range	6.00–25.00	1.43–3.37	195.00–371.50	1.45–2.33	18.90–23.90	16.92–22.36	12.09–16.30
	CV	34.28	13.50	15.29	16.85	6.73	8.56	9.39
Commercial checks (plants grown with vegetative settlings)								
Co 09004	mean	6.00	2.81	277.87	2.04	24.18	21.85	15.69
	range	5.00–9.00	2.47–3.07	224.50–290.00	1.40–2.14	24.01–24.92	21.54–22.21	15.55–16.17
	CV	34.69	10.83	12.45	19.70	1.86	1.68	1.62
Co 86032	mean	7.00	2.33	254.46	1.49	21.10	19.01	13.59
	range	6.00–10.00	2.17–2.70	202.22–265.56	1.13–1.53	20.53–22.05	18.10–19.34	12.79–14.74
	CV	29.71	11.67	13.21	14.79	1.22	2.01	2.39
Overall mean ± SD		8.00±1.70	2.46±0.27	271.95±41.77	1.66±0.25	21.75±1.33	19.48±1.51	13.93±1.22
Standard error		0.33	0.05	8.04	0.05	0.26	0.29	0.23
Critical difference @ 5%		1.24	0.09	7.26	0.13	0.73	0.58	0.48
CV %		6.95	1.50	1.15	3.00	1.38	1.22	1.39

* Significant at 5% level of probability over popularly grown clonal check Co 86032, SD: standard deviation, CV: coefficient of variation, GC: general collections, TSS: true sugarcane seed. [1] See codes in Table 2.

and juice quality traits. The estimates for PCV were consistently higher than GCV in all traits, suggesting a greater effect of the environment on genetic variation (Table 3). Traits such as CY, CCSY, NMC/C, and SCW exhibited the highest PCV and GCV. These results corroborate findings by Kumar et al. (2018), and Sanghera and Jamwal (2019b). High heritability, along with high GAM, was observed for all traits except Brix% (Table 3), indicating predominance of an additive genetic effect in determining these traits. This also implies that selection for these traits will be effective for future cane improvement.

In the current investigation, the TSS families were primarily obtained through different mating systems, *viz*., biparental crosses (BPs), general collections (GCs), and poly crosses (PCs), involving popular, well-established commercial varieties, as well as advanced commercial or near commercial clones as proven parents. As a result, the resulting seedling population exhibited sufficient genetic variability in an acceptable range for cane yield and juice quality traits. In this context, along with good germination and seedling nursery parameters, two more essential criteria are used to identify promising TSS families. These criteria include “families with high mean values” and “families with an acceptable range and low CV”, especially for farmer-desired traits (NMC/C, CG, and SCW) and industry-essential traits (Brix%, Pol%, and CCS%). The family selection process also takes into account the overall appearance of the cane types, including features such as colour, detrashability (clasping of leaves) and clump stand of the progeny population of a TSS family, to explore its suitability for direct commercial cultivation.

To identify promising TSS families, seedlings derived from different types of crosses were compared with commercial clonal checks for cane yield and juice quality traits (Supplementary Tables 2, 3, and 4). The TSS families, *viz*., 18F09, 18F14, 19F26, 20F11 (Figure 1), 20F12, and 20F16, exhibited significantly higher mean values for farmer-desired traits within an acceptable range (NMC/C ≥ 5.00, CG ≥ 1.50 cm, and SCW ≥ 1.00 kg) compared to the popularly grown check Co 86032 (Tables 4, 5, and 6). Conversely, 18F41, 19F12, 20F46, 20F76, and 20F107 showed significantly superior performance in industry-essential traits within an acceptable range (Brix% ≥ 18%, Pol% ≥ 15%, and CCS% ≥ 10.50%) compared to the popularly grown check Co 86032 (Tables 4, 5, and 6). These findings are consistent with similar observations reported by Sanghera and Jamwal (2019a), Singh and Singh (2021), Sudhagar et al. (2023), and Pathy et al. (2023), where the crosses exhibited the highest variability. However, 18F02, 18F27, 19F07, 19F25, 19F46, 20F10, 20F13, 20F19, and 20F24 families exhibited significantly higher mean values

Table 6. Descriptive statistics of promising TSS families for yield traits in seedling generation of sugarcane in the 2021-22 cropping season

TSS family	Statistics	NMC/C	CG (cm)	CH (cm)	SCW (cm)	Brix%	Pol%	CCS%
	mean	10.00*	2.75*	253.97*	1.63*	22.50*	21.60*	15.93*
NB 94-545 × Co 775	range	7.00–22.00	1.80–3.60	72.59–351.06	1.27–2.13	21.01–24.52	19.22–23.50	13.88–17.32
	CV	25.45	13.34	29.49	18.82	5.92	7.44	8.19
	mean	11.00*	2.92*	245.98*	1.76*	21.05	19.07	13.71
MS 68/47 × CoSe 92423	range	8.00–23.00	2.13–4.53	60.67–384.22	1.02–2.96	18.00–23.02	15.11–21.26	10.47–15.42
	CV	24.98	14.02	30.25	17.38	6.99	10.48	12.17
	mean	9.00*	2.50	209.57	1.41*	21.95	20.02	14.43
Co 94007 × CoPant 97222	range	7.00–24.00	1.30–4.10	80.28–418.00	1.28–1.76	18.50–24.52	14.88–22.55	10.07–16.32
	CV	30.16	14.12	31.03	11.43	11.86	14.01	15.22
	mean	9.00*	2.31	237.57*	1.47*	22.86*	21.71*	15.94*
CoVC 14062 × Co 1148	range	6.00–31.00	1.60–3.77	66.00–356.50	1.08–2.24	20.51–24.52	18.50–23.50	13.27–17.32
	CV	29.83	14.63	58.58	15.35	6.00	8.83	10.59
	mean	9.00*	2.34	215.48*	1.44*	21.65	20.25	14.77
Co 86032 × HR 83-144	range	6.00–27.00	1.47–3.23	88.78–312.89	1.02–1.65	19.42–22.93	17.87–21.73	12.94–15.94
	CV	26.37	13.69	18.42	16.43	5.48	6.88	7.55
	mean	9.00*	2.50	212.98*	1.40	21.71	20.44	14.99*
CoV 89101 (PC)	range	5.00–30.00	1.33–3.43	82.50–311.50	0.82–1.67	17.92–24.43	15.60–22.79	11.00–16.60
	CV	30.81	14.71	21.90	16.26	9.17	10.87	11.74
	mean	9.00 *	2.54	212.13*	1.54*	22.44*	21.76*	16.12*
CoSnk 05103 (GC)	range	4.00–32.00	1.30–3.50	72.00–381.33	1.15–2.24	21.01–25.02	18.02–23.97	12.62–17.66
	CV	31.75	16.02	29.09	24.34	5.69	8.56	9.95
	mean	8.00	2.33	211.23	1.29	23.48*	21.84*	15.88*
Co 10027 (GC)	range	6.00–28.00	1.10–3.23	65.00–341.67	0.64–1.39	20.42–24.93	17.82–23.88	12.59–17.60
	CV	35.08	16.46	23.41	23.53	6.71	9.44	10.73
	mean	9.00*	2.43	209.98	1.30	23.68*	22.41*	16.42*
Co 09004 (GC)	range	5.00–19.00	1.47–3.67	92.56–338.00	1.25–2.09	22.42–24.93	21.30–23.97	15.64–17.69
	CV	29.42	14.70	23.69	16.91	4.27	4.10	4.17
	mean	8.00	2.40	214.87*	1.48	22.67*	21.22*	15.48*
CoN 17071 (GC)	range	6.00–18.00	1.57–3.33	80.00–349.44	1.05–1.87	20.01–25.02	17.39–23.90	12.26–17.59
	CV	21.90	14.85	22.88	18.70	6.82	8.23	9.04
Commercial checks (plants grown with vegetative settlings)								
	mean	6.00	2.91	233.83	1.80	24.68	21.84	15.54
Co 09004	range	5.00–8.00	2.73–3.17	220.00–288.33	1.36–1.97	23.87–25.03	21.64–22.12	15.40–15.75
	CV	19.09	7.46	15.45	17.90	1.91	1.19	0.91
	mean	7.00	2.49	205.11	1.27	21.60	19.97	14.49
Co 86032	range	5.00–8.00	2.02–2.67	185.00–235.00	1.05–1.57	20.49–21.97	19.10–21.13	13.75–15.00
	CV	17.57	13.68	12.22	19.34	1.06	1.81	1.23
Overall mean ± SD		8.00±1.54	2.58±0.28	214.62±26.38	1.47±0.23	22.13±1.39	20.39±1.52	14.77±1.22
Standard error		0.27	0.05	4.66	0.40	0.25	0.27	0.22
Critical difference @ 5%		1.24	0.13	6.61	0.13	0.73	0.58	0.48
CV %		7.21	2.03	1.24	3.43	1.36	1.19	1.36

* Significant at 5% level of probability over popularly grown clonal check Co 86032, SD: standard deviation, CV: coefficient of variation, GC: general collections, PC: poly cross, TSS: true sugarcane seed. [1] See codes in Table 2.

Figure 1. Field view of MS 68/47 × CoSe 92423 (20F11) seedling population, showing better germination and seedling establishment in the shaded nursery and exhibiting acceptable variability in the progeny population (sufficient uniformity) in cane yield traits at harvest.

within an acceptable range for both cane yield and juice quality traits compared to the popularly grown check Co 86032 (Tables 4, 5, and 6). The CV values reveal that families 18F02, 18F27, 19F25, 19F46, and 20F10 exhibited moderate to low variation among TSS families, whereas families 18F14, 19F12, 20F13, and 20F46 showed higher variability for most of the cane yield and juice quality traits. Similar results were reported by Silveira et al. (2016) in selecting energy cane families and Pathy et al. (2023) in selfed families derived from various sugarcane parental clones. Supplementary Figures 2, 3, and 4 illustrate TSS families, *viz*., 18F02, 18F14, 19F07, 19F12, 19F46, 20F19, and 20F24 based on CY and CCSY, exhibiting a significant yield advantage over the popular clonal check Co 86032, as well as over the best checks, CoSnk 09293 (CY) and Co 09004 (CCSY). These crosses were identified as the most promising among those evaluated for most of the traits studied. These findings are consistent with similar studies by Silveira et al. (2016) and Sudhagar et al. (2023).

Sugarcane cultivation presents challenges, such as poor planting material and transportation issues due to bulky seeds, slow varietal replacement rate, moderate yield, and biotic stress outbreaks. Cultivating sugarcane through TSS can reduce material requirements significantly, from truckloads to about 30-50 grams of true seed for planting a hectare (Bakshi Ram et al. 2015, Pathy et al. 2023). Identifying promising TSS families that do not compromise yield compared to clonal varieties is crucial. In this regard, family selection plays a pivotal role in identifying promising parents that can be used in commercial TSS production programs. This preliminary study indicates that the TSS families 18F02 (Co 86032 × CoVC 14061), 18F27 (Co 11015 GC), 19F25 (CoVC 14062 GC), 19F46 (Co 85002 GC), 20F10 (NB 94-545 × Co 775), and 20F11 (MS 68/47 × CoSe 92423) showed good germination and better seedling establishment parameters. They also outperformed the popularly grown clonal check Co 86032, in both farmer-desired and industry-essential traits, showing high mean values and acceptable ranges, along with lower CV values. Therefore, these parental combinations can be considered the most promising TSS families, as they fulfil the important requirements of possible commercial cultivation. However, further testing of these promising families in diverse locations over multiple years is necessary to assess their feasibility for true seed-based commercial sugarcane cultivation.

DATA AVAILABILITY

The datasets generated and/or analyzed during the current research are available from the corresponding author upon reasonable request.

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