Identifying the principal contributing traits for Commercial Cane Sugar yield at first clonal selection stage in Sugarcane (*Saccharum* spp.)

ABSTRACT

Commercial cane sugar (CCS) yield is the main target product in sugarcane, which depends on the sucrose content and cane yield related traits. Understanding the factors influencing CCS yield would help us to enhance the selection efficiency for sugar yield. A population of 1,080 seedlings derived from nine bi-parental crosses involving Indian Co canes and Co allied hybrids were evaluated in seedling stage. A subset comprised of 270 clones selected from the single stool stage were evaluated in the first clonal trial to identify major component traits of CCS yield in the early stages of selection. Investigations were undertaken in the population to explore the feasibility of employing PCA and regression analyses to provide a basis for selection criteria for identification of sugarcane genotypes that are higher in CCS yield. Comparative performance of families at ground nursery indicated that screening of seedling progenies should be towards the crosses with higher mean value of H.R. brix% and single cane weight to enhance the frequency of superior individuals in subsequent clonal generations. The results of correlation analysis at first clonal stage suggested that yield related traits including tillers at 120 days, single cane weight, number of millable stalks and cane yield are the most important characters that significantly influences the CCS yield. Principal component analysis revealed that four factors could explain approximately 80.40% of the total variation for CCS yield in the population studied. The first factor which accounted for 31.19% of the variation was strongly associated with juice brix%, sucrose%, H.R. brix%, purity% and CCS%, whereas the second component had a positive effect from single cane weight, juice weight and cane diameter explaining 21.74% of total variability. The third factor accounted for 18.97% of variability and had contribution from tillers at 120 days, number of millable stalks and cane yield. Stalk height was independent from other traits which accounted for 8.48% of variation in component 4. Stepwise regression analysis demonstrated that CCS%, cane yield and sucrose % had considerable high effects on commercial cane sugar yield which explained 85.53% (expressed as R^2) of variability in clones derived from bi-parental crosses between Indian Co canes.

Keywords: CCS yield; family performance; correlation; principal component analysis; stepwise linear regression

INTRODUCTION

Sugarcane varietal development programme is initiated by sexual means by evaluating thousands of original seedlings derived from true seed (fuzz) and then propagated by vegetative method. Sugarcane breeding relied on phenotypic selection by mass or family selection strategies. Estimating the potential of a cross to produce elite clones is essential for efficient use of breeding resources to realise improved genetic gain of the target trait. As the selection cycle of sugarcane usually involves a sequence of about 4 to 6 stages and takes about 8 to 10 years to complete from hybridization to release a variety (Scortecci et al. 2012), it is necessary to identify superior genotypes at initial stages to substantially reduce the time taken and monetary resources to release a new variety. Previous research showed that cane yields in small single-row plots are affected strongly by competition effects, therefore high weighting should be placed on sucrose content related traits at early stages of screening

(Jackson and Mc Rae, 2001). Identification of superior genotypes at first clonal stage (single-row, un-replicated plots) providing high weighting on relevant CCS yield associated traits would increase the frequency of obtaining genotypes with commercial value enhancing selection efficiency.

A study by Mebrahtom *et al*. (2016) revealed that cane yield, millable stalk height and millable stalk diameter are highly correlated with sugar yield through factor analysis. On the contrary, they also reported that cane yield, recoverable sucrose% and pol% contributed more towards the variability of sugar yield by multiple regression analysis. Plant breeders are seldom interested in reducing the number of traits, considering the response of direct selection for CCS yield may be unpredictable, unless there is better understanding on the nature of association between sugar yield and other traits. Keeping in view of the above information, the present study was conducted to determine the relationship between different agronomic traits with CCS yield. The information obtained may help to formulate the appropriate selection strategy to identify sugarcane genotypes for high sugar yield during early stages of selection.

MATERIALS AND METHODS

The experimental material consisted of 1,080 seedlings from nine biparental sugarcane families (**Table 1**). Fluff of all the nine crosses was sown during January, 2018 and the seedlings were raised in plastic trays in the mist chamber at controlled conditions of temperature (27°C) and humidity (80%). One hundred and twenty healthy seedlings from each cross were selected at random at forty-five days after sowing and transferred to polythene bags (10 x 10 cm) and kept in shade net house for hardening and further establishment. Sufficient care was taken to maintain 120 healthy seedlings in each cross by gap filling prior to transplanting in ground nursery. The hybrid seedling progenies derived from intervarietal crosses obtained from the economic breeding programme at ICAR-SBI, Coimbatore, represents combinations between high sucrose Co canes and Co allied hybrids (sucrose content ranging between 18.00 to 21.50%) were evaluated in two rows of 6m in length and 0.9m width in a RCBD with two replications during 2018-19 in the ground nursery.

In the ground nursery (stage I), five quantitative traits which includes number of millable stalks/ genotype, cane diameter, cane height, single cane weight, clump yield and for quality H.R. brix % were recorded for individual seedlings. Progenies performing better than the cross average for cane yield traits and HR brix% combining appreciable field stand were selected from ground nursery for evaluation in first clonal trial (stage II). 270 genotypes were evaluated in a RCBD with two replications adopting a plot size of 1row x 6m x 0.9 m along with five check varieties (Co 85004, CoC 671, Co 86032, Co 99004 and Co 0403) during 2019-20 and 2020 -21. Data were recorded for two plant crop harvests (2019-2021) on twelve CCS yield attributing traits includes tillers at 120 days, H.R. brix % at 240 days, at 360 days of crop age NMS (000/ ha), cane height, cane diameter and single cane weight, cane yield, juice weight, juice brix %, sucrose %, purity % and CCS %. Quality components were assessed through juice analysis at 12th month of crop age.

Table 1. List of crosses effected to generate base population for identifying principal component traits of CCS yield

Cross no.	Cross combination
1	Co 10033* x Co 13007*
2	Co 85002* x Co 8209*
3	Co 87044* x Co 86011*
4	Co 86011* x CoT 8201**
5	Co 8371* x Co 86011*
6	Co 86002* x CoC 671**
7	CoM 0265** x Co 0314*
8	Co 11015* x Co 775*
9	Co 62198* x Co 8353*

* - Co canes : Elite clones identified for cane yield and juice quality from the Pre Zonal Varietal Trial conducted at ICAR -SBI, Coimbatore. The prefix Co represents the location Coimbatore where the crosses are made
** - Co allied hybrids : Elite clones developed from the crosses made at the National Hybridization Garden (NHG) by the sugarcane research stations and selected in the respective locations.

Cane yield was calculated by weighing the cane yield without trash in kilograms in each genotype from a row of six metre length and converted into tons per hectare by using the following formula.

$$\text{Cane yield } (t\ ha^{-1}) = (\text{Yield in Kg/plot x 10000})/(\text{plot size x1000})$$

The estimate on CCS yield is derived from the product of cane yield (t/ha) and sugar recovery percent (CCS%) at harvest.

$$CCS\ yield\ (t/ha) = \frac{Cane\ yield\ (t/ha)\ X\ CCS\ \%\ at\ 360\ days}{100}$$

Pair-wise Pearson correlation analysis was used to determine the relationship between the CCS yield and its associating factors as suggested by Steel and Tori (1980). Principal component analysis was performed to quantify the contribution of individual attribute to the total variation of sugar yield using SPSS ver.16 software. Stepwise linear regression analysis was utilized to remove non-effective characters and to determine the variable accounting for majority of total variation by using Minitab Software ver.19.

RESULTS AND DISCUSSION

Comparative performance of families for cane yield traits and HR brix% at stage I

The potential of a cross to produce elite progeny for a trait could be accurately predicted by the cross mean of that trait (Chang and Milligan, 1992). In this study, of the nine crosses evaluated four families *viz*. Co 86011 x CoT 8201, Co 86002 x CoC 671, Co 85002 x Co 8209 and Co 11015 x Co775 exhibited higher number of clones above the cross average for HR brix % and single cane weight (**Table 2**). Results from stage I evaluation highlighted that the above four families (**Fig.1**) were most promising harbouring superior clones for cane yield and juice quality and indicative of obtaining high frequency of elite genotypes. Breeding value of the parental clones relied heavily on the percent of clones from a cross that are advanced to subsequent selection stages. The heritabilities are relatively high for many traits on a family basis indicating that selection is effective (Skinner *et al*. 1987). Improved genetic gain for brix yield through specific selection index in seedling stage has been reported in three sugarcane general collections (Bakshi Ram *et al*. 1997). A review on selection strategies in sugarcane breeding programmes have mentioned that stalk weight and juice brix as important traits because they represent principal components of sugar yield (Shanthi *et al*. 2014).

Elite clones identified at stage II evaluation

Elite clones in the first clonal trial with their CCS yield contributing parameters are presented in **Table 3**. The clone C8-996 from the cross Co 86002 x CoC 671 ranked first in terms of cane yield and CCS yield exhibiting an improvement of 11.45 % and 5.54 % over the popular check variety Co 86032. In terms of sucrose content, C8-996 and C11-1271 recorded a maximum of 22.83% and 22.19 % sucrose content with 5.20% and 2.25% of improvement over the best check variety CoC 671. Top ranking clones for CCS yield came from four families *viz*., Co 86002 x CoC 671, Co 11015 x Co 775, Co 86011 x CoT 8201 and Co 85002 x Co 8209. Results here indicated that selection of individual seedlings with high value of HR brix% and single cane weight at seedling stage would constitute an elite pre-breeding pool resulting in more selections for high CCS yield in the subsequent clonal generation. This observation reassures the earlier report made by Walker (1965) that brix is a better selection criterion because of its high repeatability between the seedling and clonal generations. Studies undertaken in the progenies derived from sugarcane bi-parental crosses revealed single cane weight as a highly repeatable trait in early clonal stages (Shanthi *et al*. 2008).

Table 2. Family performance for yield traits and HR brix% at ground nursery

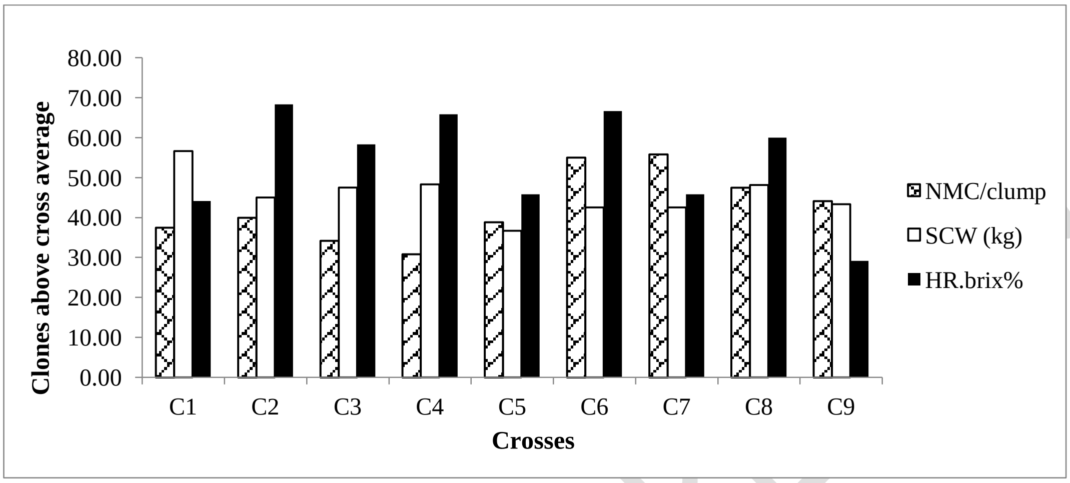
Cross Number	Cross	Number of millable canes/clump	Cane height (cm)	Cane diameter (cm)	Single cane weight (kg)	H.R. brix (%)
C1	Co 10033 x Co 13007	7.20±2.17	235.04±45.12	2.57±0.40	1.25±0.46	17.08±2.76
C2	Co 85002 x Co 8209	6.28±1.78	225.50±32.06	2.58±1.86	1.04±0.35	21.38±1.83
C3	Co 87044 x Co 86011	4.63±2.05	213.70±37.85	2.65±0.37	1.25±0.84	19.85±2.19
C4	Co 86011 x CoT 8201	8.01±1.70	221.11±37.46	2.57±0.38	1.02±0.46	20.14±0.97
C5	Co 8371 x Co 86011	5.96±1.86	222.50±38.69	2.79±0.32	1.19±0.39	17.80±2.04
C6	Co 86002 x CoC 671	5.10±2.01	216.85±33.50	2.51±1.66	0.94±0.34	21.75±2.43
C7	CoM 0265 x Co 0314	8.99±2.86	208.66±31.17	2.40±0.39	1.18±0.37	17.68±2.30
C8	Co 11015 x Co 775	7.56±1.79	208.06±37.28	2.69±0.37	1.27±0.58	20.02±0.37
C9	Co 62198 x Co 8353	7.01±2.84	199.14±35.92	2.60±0.43	1.06±0.42	16.68±2.27

Table 3. Elite clones ranked for CCS yield at first clonal evaluation

Clones	Parentage	Sucrose (%)	Cane yield (t/ha)	CCS yield (t/ha)	Ranking
C8-996	Co 86002 x CoC 671	22.83	111.32	16.22	1
C8-1271	Co 11015 x Co 775	22.19	101.61	15.79	2
C8-1287	Co 11015 x Co 775	21.74	114.18	15.45	3
C6-785	Co 86002 x CoC 671	21.76	102.67	15.42	4
C4-499	Co 86011 x CoT 8201	21.74	129.65	15.40	5
C6-792	Co 86002 x CoC 671	21.68	130.65	15.37	6
C2-382	Co 85002 x Co 8209	21.17	84.62	15.03	7
C2-396	Co 85002 x Co 8209	21.04	101.87	14.91	8
C8-1016	Co 11015 x Co 775	21.05	124.20	14.81	9
C4-504	Co 86011 x CoT 8201	20.85	108.52	14.80	10
C6-1138	Co 86002 x CoC 671	20.80	130.53	14.77	11
C6-795	Co 86002 x CoC 671	20.84	106.26	14.71	12
C2-401	Co 85002 x Co 8209	20.64	90.85	14.53	13
C8-1297	Co 11015 x Co 775	20.67	74.02	14.54	14
C4-508	Co 86011 x CoT 8201	20.75	124.75	14.43	15
	Checks				
	Co 86032	20.85	99.88	15.37	

	CoC 671	21.70	89.74	13.71	

Figure 1. Proportion of clones above cross mean for yield traits and H.R.brix%



Correlation analysis

In sugarcane, unlike other crops, commercial cane sugar yield is a desirable character that combines cane yield and commercial cane sugar percent in juice. Genetic gain for commercial cane sugar yield is expected in sugarcane populations only when there is simultaneous improvement in total cane yield as well as sucrose percent in juice. Understanding the association between the component traits is essential to bring about improvement in CCS yield due to the presence of GxE interaction as well as undesirable negative association between component traits in earlier stages of selection. The results on correlation analysis between CCS yield and other component traits associated with cane yield and juice quality traits are presented in **Table 4**. In the present investigation, highly significant and positive correlation was observed between cane sugar yield and cane yield (r = 0.825). Among the CCS yield component traits, cane yield were found to have strong influence towards CCS yield which would improve the genetic gain of the complex trait as been suggested in earlier studies (Javed et al. 2001; Panhwar et al. 2003). The results are in conformity with the findings of Singh *et al*. (2003), Gowda *et al*. (2016) and Krishna and Kamat (2017). In addition to the cane yield, stalk number, stalk weight and stalk height been used as indirect selection criteria for higher CCS yield. In our study, number of millable stalks (r = 0.659), tillers at120 days (r = 0.557), single cane weight (r = 0.513), and cane height (r = 0.331) were positive and significantly associated with CCS yield which were in accordance with the reports of Krishna and Kamat (2017). High positive correlation between CCS yield with number of tillers and number of millable stalks was also observed by Gowda *et al*. (2016). There was a positive but non-significant association of CCS yield with juice quality traits which includes H.R .brix % (r = 0.027), juice brix % (r = 0.118), sucrose % (r = 0.126) and purity % (r = 0.033). Our results corresponds to that of Singh *et al*. (2003) who reported non-significant positive correlation of juice brix %, juice sucrose %, purity % and CCS % with CCS yield. CCS% registered a significant and positive correlation with sugar yield, indicating that CCS% alone positively influences sugar yield among the juice quality characters at first clonal stage of evaluation. The results are in agreement with the findings of Krishna and Kamat (2017). Correlation analysis revealed that, tillers at 120 days, single cane weight, number of millable stalks and cane yield are the major components which have direct influence on cane sugar yield. Therefore, selection for these traits during early clonal evaluation will result in a significant improvement on sugar yield. Investigations to enhance selection efficiency for sugar yield in early clonal generation by Chandrakant *et al*. (2007), revealed that selection based on yield related traits viz. single cane weight, shoot population at 120 days, number of millable canes and quality traits needs to be delayed to later generation until the trait stabilizes.

Principal component analysis (PCA)

The results of principal component analysis extracted four factors which explained 80.40 % of variation for the twelve traits studied (**Table 5**). Principal component 1 (PC 1) with eigen value of 3.744 contributed about 31.196% of variability and its corresponding eigenvectors including juice brix % (0.934), sucrose % (0.934), CCS % (0.832), HR brix % (0.717) and purity % (0.434) and were named as sugar factor. Similarly, Zhou *et al*. (2015), reported 270 days Brix, 300 days Brix and 300 days sucrose content as major eigenvectors and its variance contribution rate was 31.11% in PC1. Another study by Al-Sayed *et al*. (2012) revealed that total soluble solids% and sucrose% accounted for 28.17% of the total variability in PC2. El-Geddawi *et al*. (1992) found richness%, sucrose%, brix% and purity% constituted first factor and suggested that they are major components of sugar yield. When considering PC 2 which accounted for 21.744% of the variance with eigen value of 2.609 included single cane weight (0.911), juice weight (0.890), and cane diameter (0.737) as major eigenvectors and named as cane weight – juice weight factor. Observations by Zhou *et al*. (2015) indicated that stalk diameter, leaf length, leaf width and leaf sheath length as major contributors of PC2, which accounted for 17.86% of total variability and named as stalk diameter - leaf factor. PC 3 with eigen value of 2.277 explained 18.979% of variability with high loadings from number of millable canes (0.960), tillers/120 days (0.929), cane yield (0.784) and named as millable cane – cane yield factor. Al-Sayed *et al*. (2012) recommended stalk weight, stalk thickness and number of millable stalks as major eigenvectors in factor I, which explained 34.89% of the total variability. Cane height (0.746) is the major eigen vector and named as cane height factor in PC4 which explained 8.481% of variability with eigen value of 1.018. This finding is in conformity with that of Zhou *et al*. (2015) as they reported cane height as an independent eigen vector with its variance contribution rate 10.29% in PC4. Further, they recommended that it was controlled by independent genetic factors. Factor analysis for the measured traits indicated that yield traits *viz*., number of millable stalks (0.960), cane yield (0.948), tillers at 120 days (0.912) and single cane weight (0.897), and considering quality sucrose % (0.929), juice brix % (0.903) and CCS % (0.816) had the highest communality and consequently the high relative contribution towards CCS yield. Principal component analysis sorted out the twelve cane yield and sugar yield characters into four main principal components. Based on the results of principal component analysis of this study involving clones derived from bi-parental crosses involving commercial hybrids popularly known as Co canes, it is suggested that the selection at early clonal stages should primarily consider sucrose factor for improvement in CCS yield followed by yield parameters which corroborated with the results of Zhou *et al*. (2015). High cane sugar yield can possibly be obtained by selecting individual clones with a high and positive selection response for these seven parameters *viz*., tillers/120 days, single cane weight, number of millable stalks, cane yield, juice brix %, sucrose % and CCS %.

Stepwise linear regression analysis

In order to identify vital component traits that contributes to variation and to remove non-effective characters on sugar yield, stepwise regression analysis was conducted and the results are presented in **Table 6**. Highly significant values of the partial regression coefficients for CCS%, cane yield and sucrose % justifies the maximum contribution of these three variables to the variation of sugar yield in this population developed from crosses involving Indian commercial hybrids. High value of R^2 (85.53%) indicates that the traits chosen for the study explained maximum variation and could be attributed to those aforementioned three parameters. Therefore, the prediction equation for sugar yield estimated by considering CCS%, cane yield and sucrose % is as follows:

$$\text{Sugar yield} = -14.068 - 0.005\ (\text{cane height}) + 0.101\ (\text{sucrose \%}) + 1.053\ (\text{CCS \%}) + 0.126\ (\text{cane yield})$$

The values of the Variation Inflation Factor (VIF) for the three accepted variables was less than 10 indicating no effect of multicollinearity. Keeping in view of significant R^2 and low VIF values indicated the validity of the proposed regression model and the effectiveness of these traits for enhancing the selection efficiency for realising high sugar yield at early clonal stages in a sugarcane population. The results of regression analysis in our study revealed that CCS%, cane yield and sucrose % are the most important traits to enhance selection efficiency of sugarcane breeding program and for obtaining clones with high sugar yield. Similar results were obtained by Mebrahtom *et al*. (2016) who stated that cane yield, recoverable sucrose % and pol% contributed more to sugar yield which accounted for 99.5% of the variability. Earlier investigations by Al-Sayed *et al*. (2012) to estimate the relative contribution of sugar yield traits have concluded that sucrose %, stalk weight and number of millable stalks were the most effective characters which explained 82.7% of the total variation of sugar yield.

Table 4. Correlation coefficients for the traits associated with cane yield and juice quality at first clonal stage

Traits	Cane height (cm)	Cane diameter (cm)	Single cane weight (kg)	NMS (000/ ha)	Cane yield (t/ha)	Juice weight (kg)	H.R. Brix (%)	Juice brix (%)	Sucrose (%)	Purity (%)	CCS (%)	CCS yield (t/ha)
Tillers/ 120days	0.165	-0.206**	-0.170*	0.904**	0.602**	-0.222**	-0.160*	-0.086	-0.103	-0.104	-0.122	0.557**
Cane height (cm)		0.047	0.388**	0.153	0.401**	0.258**	-0.092	-0.170*	-0.179*	-0.255*	0.109	0.331*
Cane diameter (cm)			0.529**	-0.202**	0.231**	0.500**	-0.127	-0.037	-0.031	-0.008	-0.073	0.196*
Single cane weight (kg)				-0.168*	0.586**	0.805**	-0.129	-0.171*	-0.164	-0.077	-0.148	0.513**
NMS (000/ha)					0.683**	-0.239**	-0.152	-0.070	-0.084	-0.102	-0.082	0.659**
Cane yield (t/ha)						0.420**	-0.206**	-0.166	-0.169*	-0.130	-0.176	0.825**
Juice weight (kg)							-0.015	-0.055	-0.043	0.054	-0.048	0.391*
H.R. brix (%)								0.565**	0.531**	0.142	0.438**	0.027
Juice brix (%)									0.983**	0.417**	0.732**	0.118
Sucrose (%)										0.508**	0.755**	0.126
Purity (%)											0.405**	0.033

***, ** Significant at 0.05 and 0.01 probability levels respectively**

Table 5. Summary of factor loadings for the CCS yield contributing traits of four principal components (PCs)

Principal components	1	2	3	4	Communality
Variables		**Eigen vectors**			
Factor I					
H.R.brix(%)	**0.717**	-0.135	-0.201	0.250	0.635
Juice brix(%)	**0.934**	-0.041	-0.013	-0.170	0.903
Sucrose (%)	**0.934**	-0.016	-0.014	-0.238	0.929
Purity (%)	0.434	0.125	0.006	-0.670	0.653
CCS (%)	**0.832**	-0.041	-0.039	-0.143	0.816
Factor II					
Single cane weight	-0.094	**0.911**	-0.005	0.242	0.897
Cane diameter	-0.092	**0.737**	-0.110	-0.183	0.597
Juice weight	0.025	**0.890**	-0.084	0.124	0.715
Factor III					
Tillers/120 days	-0.080	-0.200	**0.929**	0.059	0.912
Number of millable stalks	-0.055	-0.184	**0.960**	0.044	0.960
Cane yield	-0.100	0.530	**0.784**	0.205	0.948
Factor IV					
Cane height	-0.015	0.284	0.284	**0.746**	0.684
Eigen values	3.744	2.609	2.277	1.018	
% of variance	31.196	21.744	18.979	8.481	
Cumulative %	31.196	52.940	71.919	80.400	

Table 6. Regression parameters estimated through stepwise regression

Regression parameters	Regression coefficient (b)	Standard error (SE)	Probability level (P value)	Variance inflation factor (VIF)
CCS%	1.053**	0.047	0.000	2.35
Cane yield	0.126**	0.001	0.000	1.22
Sucrose %	0.101**	0.037	0.008	2.38
Cane height	0.005*	0.001	0.011	1.22
Intercept	-14.068			
Model sig.	<001			
R^2	85.61			
Adjusted R^2	85.53			

*, ** Significant at 0.05 and 0.01 probability levels respectively.

CONCLUSION

Genetic analysis describing trait relationships for commercial cane sugar yield in a first clonal sugarcane population has not been extensively studied, and would be useful in establishing effective selection priorities in early stages of sugarcane breeding programmes. The inferences drawn from the present study is based on the nine bi-parental crosses between Indian Co canes.

Initial screening at ground nursery stage should be targeted primarily towards H.R brix% and single cane weight due to its high repeatability across selection stages followed by other cane yield traits. Therefore, it is suggested to screen the seedlings rigidly for HR brix% and single cane weight as it would serve as a good selection criterion for predicting the superiority of genotypes in the clonal generations. The results on correlation analysis pointed out that prioritization of quantitative traits associated with sugar yield *viz*., tillers at 120 days, single cane weight, number of millable stalks and cane yield would be effective in improving sugar yield. As the number of independent variables influences a particular dependent variable, correlation may be inadequate and in such situations multivariate analysis enables breeder to decide on indirect selection criteria. Sugar yield being a complex trait, multivariate analysis can be used to reduce a large number of correlated variables to a small number of independent main factors. Principal component analysis demonstrated that high sugar yield can possibly be obtained by selecting individual clones with high values for tillers at 120 days, single cane weight, number of millable stalks, cane yield, juice brix%, sucrose% and CCS%. The results on step wise linear regression analysis concluded that CCS%, cane yield and sucrose % contributed significantly to sugar yield. Based on the above analyses, it is suggested that tillers at 120days, single cane weight, number of millable stalks, cane yield, sucrose% and CCS% are the most important traits to be given due weightage in early stages of sugarcane breeding programmes that enhances the likelihood of selecting potential clones for high sugar yield.

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