Received: 05 Jan 2022 | Revised: 14 Mar 2022 | Accepted: 16 Mar 2022

Electronic Journal of Plant Breeding

Research Article

Genetic analysis in *Saccharum spontaneum* accessions of Maharashtra collection

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Abstract
In this study, 41 *Saccharum spontaneum* accessions collected from Maharashtra, India was characterized for 13 quantitative traits and 25 qualitative traits. The accessions were then planted in the field using a randomised block design with three replications. The quantitative traits were analysed using descriptive statistics, correlation, principal component analysis and cluster analysis. Phenotypic parameters *viz.,* plant height ranged from 74.30 to 239.60 cm, stalk diameter ranged from 2.20 to 4.80 cm and HR brix varies from 5.30 to 15.20%. Also found strong association between stalk diameter and HR brix content. In PCA analysis, the first four components exhibited more than one eigenvalue. All accessions were clustered into four groups using agglomerative cluster analysis. The Shannon weaver index was used to analyze the qualitative traits. The highest diversity among the traits was found for bud germpore (0.96), leaf dewlap colour (0.94), bud shape (0.93), and stalk colour- exposed to sun (090).

Keywords: Sugarcane, genetic resources, PCA Correlation

INTRODUCTION

Due to the use of a few parent species accessions and severe selection pressures in breeding programmes, sugarcane crop development is gradual (Berding and Roach, 1987; Nair *et al.,* 2002). Understanding and recording the pattern of genetic variability in *Saccharum* species germplasm has been hampered due to its complex genome, heterozygosity, polyploidy, and environmental influence. As a result, it is important to collect, characterise, conserve and use the novel variety of *Saccharum* species, notably *Saccharum spontaneum,* in order to increase the sugarcane production and productivity. *Saccharum spontaneum*, a wild sugarcane relative, has contributed in the production of improved sugarcane varieties. Sugarcane cultivars nowadays are complex hybrids derived mostly from inter specific crosses between *S. officinarum* L. and *S. spontaneum* (Mary *et al.,* 2006). *S. spontaneum's* importance in producing high productivity and disease and pest resistance has long been recognised (Panje, 1972). *S. spontaneum* has a wide range of morphologies and cytotypes. Because of the species' significance in sugarcane varietal improvement programmes, germplasm of *S. spontaneum* should be collected and protected from its distribution areas.

In Coimbatore, India, the ICAR-Sugarcane Breeding Institute (ICAR-SBI) is a forerunner in collection and is actively involved in breeding to enhance cultivars, that are resistant to abiotic and biotic environments. Despite the abundance of resources and genotypes available for crop improvement, managing and utilising a large number of genotypes in germplasm collections is one of the major challenges faced during selection and utilization in plant breeding programme. Hence, *Saccharum spontaneum* clones collected from Maharashtra states of India were characterized for quantitative and qualitative traits.

MATERIALS AND METHODS

The materials comprised of 41 *Saccharum spontaneum* accessions (**Table 1**) collected from Maharashtra, India during 2016. Initially all the forty-one accessions were raised in pots to quarantine for disease and pests. During 2017 the accessions were transplanted to the germplasm field at ICAR-SBI, Coimbatore.

The forty-one accessions planted in the field using a randomised block design with three replications. They were phenotypically characterized for 13 quantitative and 25 qualitative traits. During the end of grand growth stage, maturity stage and harvest stage the quantitative traits (plant height, stalk diameter, stalk length, leaf lamina length, leaf lamina width, leaf midrib width, leaf sheath length, internode number, internode length, root zone width, root eye row number, dewlap, Brix content) and the qualitative traits *viz*., leaf attitude, leaf sheath colour, leaf hairiness, dewlap colour, ligule shape, ligule hairs, stalk colour exposed, internode waxiness, internode shape, internode alignment, internode cross-section, node wax band, node swelling, root zone colour, root zone swelling, root eye alignment, bud shape, bud Size, bud cushion, bud germpore, bud groove, bud hairs, bud tip extension, growth ring colour, growth ring swelling data were recorded as per sugarcane descriptor developed by ICAR– SBI in collaboration with PPV & FRA (https://plantauthority.gov.in/sites/default/files/sugarcane_2.pdf) . In total, 38 morphological traits of the Maharashtra accessions were characterized around the year in the field.

Data management and analysis were carried out using PAST 3 (Hammer *et al.,* 2001). Correlations were obtained using the Pearson method. To reduce data dimensions for better visualization of accessions and traits, principal component analysis (PCA) and Cluster analysis were conducted for grouping the accessions using 13 quantitative traits. Variation in qualitative traits was quantified using Shannon-weaver diversity index (H') using the formula, H' = - Σpi (log2 pi)/log2 n Where Pi= frequency proportion of each descriptor states, n= number of states. Shannon diversity index has a value ranging from 0 to 1, where 0 indicates no diversity and 1 indicates the maximum diversity.

RESULTS AND DISCUSSION

All quantitative traits were studied using descriptive statistics (minimum, maximum, average mean, standard deviation, coefficient of variation) as stated in **Table 2**, with the exception of the number of nodal root eye rows, which is common in all accessions. Silva *et al.* 2005; Gouy, 2012; Ekpelikpezw *et al.* 2016 conducted similar investigations for characterization of quantitative traits in *Saccharum* species. The genotype IND 15-1713 had the highest mean value for plant height (239.60 cm) and stalk length (159.10 cm). The genotype IND 15- 1741 had the highest brix content (15.27 percent). The maximum stalk diameter was recorded in IND 15-1747 (4.80 cm). The genotype IND 15-1720 had the lowest plant height and leaf sheath length.

Stalk diameter, stalk length, internode number and internode length had strong correlation with plant height. Stalk length, internode length and leaf lamina width significantly correlated with stalk diameter. Internode number and length were closely correlated with stalk length. Sheath length was strongly related to the length of the leaf lamina. Brix had a positive relationship with stalk diameter (**Table 3**). Similar findings of a positive association between brix % and stalk diameter were found by Todd *et al.* 2014 and Tena *et al*. 2016. This study demonstrated a negative significant relationship between

Table 1. List of clones of *Saccharum spontaneum* used in the study

S.No.	Accession number	S.No.	Accession numbers	S.No.	Accession number
1	IND 15-1704	15	IND 15-1721	29	IND 15-1735
2	IND 15-1705	16	IND 15-1722	30	IND 15-1736
3	IND 15-1706	17	IND 15-1723	31	IND 15-1737
4	IND 15-1708	18	IND 15-1724	32	IND 15-1738
5	IND 15-1709	19	IND 15-1725	33	IND 15-1739
6	IND 15-1710	20	IND 15-1726	34	IND 15-1740
7	IND 15-1711	21	IND 15-1727	35	IND 15-1741
8	IND 15-1712	22	IND 15-1728	36	IND 15-1742
9	IND 15-1713	23	IND 15-1729	37	IND 15-1743
10	IND 15-1714	24	IND 15-1730	38	IND 15-1744
11	IND 15-1716	25	IND 15-1731	39	IND 15-1745
12	IND 15-1718	26	IND 15-1732	40	IND 15-1746
13	IND 15-1719	27	IND 15-1733	41	IND 15-1747
14	IND 15-1720	28	IND 15-1734		

Table 2. Descriptive statistics for different traits

Parameters	Minimum	Maximum	Mean	SD	CV (%)
PHT	74.30	239.60	126.05	35.89	28.48
STD	2.20	4.80	3.25	0.65	19.91
STL	53.30	159.10	93.14	31.48	33.80
INN	5.00	21.00	11.15	2.94	26.39
INL	5.10	13.70	8.00	2.17	27.15
RZW	0.30	0.80	0.44	0.10	22.55
LLL	45.10	111.70	68.92	16.27	23.61
LLW	0.40	0.80	0.53	0.12	23.45
MDW	0.10	0.20	0.13	0.05	35.77
SHL	8.60	22.90	13.79	4.01	29.09
DEW	0.10	0.40	0.21	0.05	22.25
BR	5.30	15.20	9.24	2.22	24.02

Plant height (PHT), Stalk diameter (STD), Stalk length (STL), Inter node numbers (INN), Internode length (INL), Root-zone width (RZW), Leaf lamina length (LLL), Leaf lamina width (LLW), Midrib width (MDW), Leaf sheath length (SHL), Dewlap width (DEW) and Brix% (BR)

Table 3. Genetic relationships among different traits in *Saccharum spontaneum* accessions

	PHT	STD	STL	INN	INL	RZW	LLL	LLW	MDW	SHL	DEW	BR
PHT	1.00											
STD	0.72**	1.00										
STL	0.93**	0.73**	1.00									
INN	0.53**	0.38*	0.57**	1.00								
INL	0.77**	0.62**	0.80**	0.19	1.00							
RZW	0.40*	0.22	0.34*	0.05	0.45*	1.00						
LLL	-0.12	-0.10	-0.27	-0.39*	-0.18	-0.01	1.00					
LLW	0.43*	0.61**	0.46*	0.10	0.50*	0.07	-0.05	1.00				
MDW	0.32*	0.29	0.25	0.13	0.19	-0.04	0.22	0.19	1.00			
SHL	0.28	0.20	0.16	-0.33*	0.27	0.13	0.66**	0.20	0.28	1.00		
DEW	0.30	0.25	0.30	-0.03	0.44*	0.39*	0.19	0.13	0.01	0.39*	1.00	
BR	0.12	0.33*	0.09	0.08	-0.08	0.05	-0.05	-0.01	-0.08	-0.10	-0.03	1.00

* and **, significant at $p < 0.05$ and 0.01, respectively.
Plant height (PHT), Stalk diameter (STD), Stalk length (STL), Inter node numbers (INN), Internode length (INL), Root-zone width (RZW), Leaf lamina length (LLL), Leaf lamina width (LLW), Midrib width (MDW), Leaf sheath length (SHL), Dewlap width (DEW) and Brix% (BR)

internode number, leaf lamina, and sheath length (Khan, 1995 and Tena *et al.,* 2016). The traits that had a highly significant positive association and direct effects on cane quality were plant height, stalk diameter, stalk length, internode number, and root zone width (Brix per cent). Patel *et al*. (1993) discovered that some qualities were positively related to cane yield factors. This shows that these characteristics could be employed as a selection criterion to boost cane yield.

Principal Component analysis (PCA) is a valuable method, which works by determining inter-relationship among variables (Sneedon, 1970). In this study, except for number root eye rows, which have two numbers in all accessions, principal component analysis was employed to assess variability among 12 quantitative traits **(Table 4).** The eigen values of the correlation matrix, which shows how total variance is partitioned by each component. The first four primary components (**Table 5**) in this study reveal that more than one eigen value was used in the analysis. The cumulative variance of four traits accounted for nearly 75 per cent of the total variance. Among the

Table 4. Eigen values and % total variance for principal components

Variables	Eigen value	% variance	Cumulative percentage
PHT	4.39	36.61	36.6
STD	2.20	18.32	54.9
STL	1.31	10.91	65.8
INN	1.10	9.20	75.0
INL	0.90	7.48	82.5
RZW	0.62	5.17	87.7
LLL	0.51	4.22	91.9
LLW	0.44	3.67	95.6
MDW	0.20	1.69	97.3
SHL	0.18	1.54	98.8
DEW	0.09	0.77	99.6
BR	0.05	0.43	100.0

Plant height (PHT), Stalk diameter (STD), Stalk length (STL), Inter node numbers (INN), Internode length (INL), Root-zone width (RZW), Leaf lamina length (LLL), Leaf lamina width (LLW), Midrib width (MDW), Leaf sheath length (SHL), Dewlap width (DEW) and Brix% (BR)

Table 5. Principal component in 12 quantitative traits

Variables	PC 1	PC 2	PC 3	PC 4
PHT	0.45	-0.04	0.03	-0.02
STD	0.40	-0.04	0.18	0.28
STL	0.45	-0.12	0.01	-0.07
INN	0.22	-0.42	0.14	-0.09
INL	0.41	0.05	-0.18	-0.19
RZW	0.21	0.09	-0.56	0.04
LLL	-0.07	0.57	0.17	0.18
LLW	0.29	0.04	0.24	-0.02
MDW	0.16	0.18	0.56	-0.17
SHL	0.14	0.58	0.10	0.05
DEW	0.21	0.30	-0.43	0.02
BR	0.06	-0.15	0.03	0.90
Eigen value	4.39	2.20	1.31	1.10
Variance percentage	36.6	18.3	10.9	9.2
Cumulative percentage	36.6	54.9	65.8	75.0

first four components PC1 contributed 36.6 per cent of the variation. Except for leaf lamina length and had a positive value for all of the attributes. As a result, PC1 was recommended for cane yield characteristics with high and positive values for plant height (PHT), stalk diameter (STD), stalk length (STL), internode length (INL), internode numbers (INN), root zone width (RZW) and leaf lamina width (LLW). PC2 was responsible for 18.3 per cent of the total variance, with positive results for internode length (INL), root zone width (RZW), leaf lamina length (LLL), leaf lamina width (LLW), midrib width (MDW), sheath length (SHL) and dewlap width (DEW). It indicates the highest value for leaf attributed traits in terms of improving biomass. PC3 and PC4 were responsible for 10.9 and 9.2 per cent of the total variance, respectively. Because PC4 has the highest positive value for brix (BR) percent, the components in it were used to improve cane quality characteristics. PCA for quantitative features in Saccharum species has been studied by Zhou *et al.* (2015); Rakesh *et al.* (2019) and Raza *et al.* (2017). According to Lukibisi and Lanyasunya (2010), the scree plot aids in detecting the relevant components. **Fig. 1.** depicts scree plot of all component eigen values. The components that appear before the break down have high

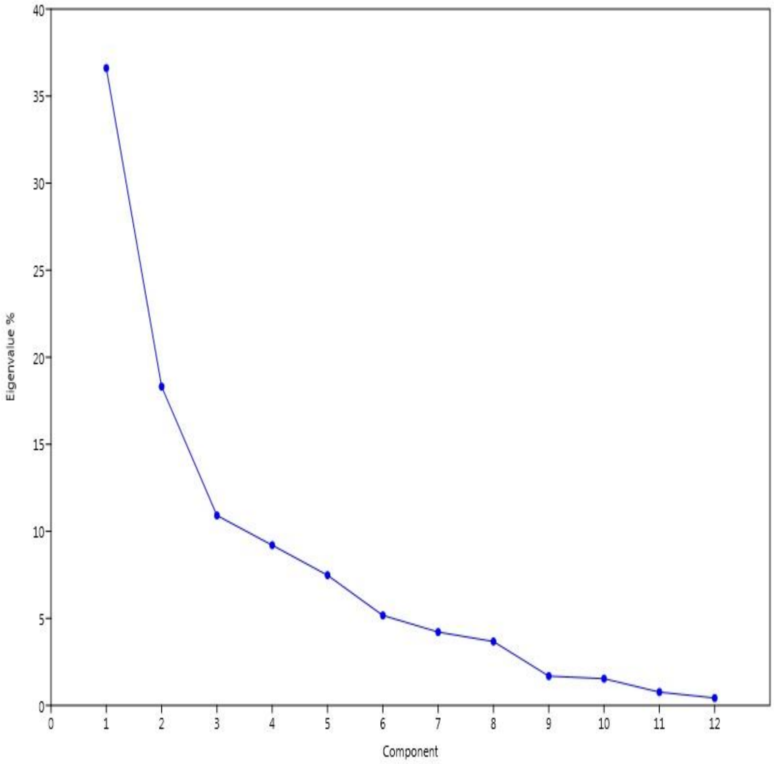

Fig. 1. Scree plot of all PCAs

eigen values of more than one, while the components that appear after the break have eigen values of less than one. As a result, the first four components are used to analyse and determine the diversity of accessions. **Fig. 2**. demonstrates that accessions from the biplot axis PC1 and PC2 had the highest plant height, Stalk diameter, Stalk length, Brix percent, whereas IND 15-1742, IND 15-1735, IND 15-1727, IND 15-1726 have the highest leaf contributing traits. IND 15-1708, IND 15-1713, IND 15-1746, IND 15-1739, IND 15-1719, IND 15-1725, IND 15-1737, and IND 15-1734 1007, which likewise had the highest variance among the other accessions, were all found on the convex of the hull. Smiullah *et al.* (2013) and Rakesh *et al.* (2019) also studied the genetic diversity in Saccharum species using PCA.

A dendrogram for 41 Saccharum spontaneum accessions was shown in **Fig. 3**. Based on agglomerative cluster analysis, Ward's method was used to group them into four groups. Three accessions (IND 15-1704, IND 15-1713, IND 15-1746) were grouped in cluster 1; twelve accessions (IND 15-1705, IND 15-1706, IND 15-1708, IND 15-1709, IND 15-1710, IND 15-1711, IND 15-1712, IND 15-1714, IND 15-1716, IND 15-1735, IND 15-1744, and IND 15-1747) were grouped in cluster 2; twenty accessions (IND 15-1718, IND 15-1719, IND 15-1720, IND 15-1723, IND 15-1724, IND 15-1725, IND 15-1728, IND 15-1729, IND 15-1730, IND 15-1731, IND 15-1732, IND 15-1733, IND 15-1734, IND 15-1736 and IND 15-1737, IND 15-1738, IND 15-1740, IND 15-1741, IND 15-1743 and IND 15-1745) were grouped in cluster 3 and six accessions (IND 15-1721,IND 15-1722,IND 15-1726,IND 15-1727,IND 15-1739 and IND 15-1742) were grouped in cluster 4. Cluster 1 had the highest values for plant height, stalk diameter, stalk length, internode length, root zone width, leaf lamina width, sheath length, and dewlap width (**Table 6**). As a result, accessions from this group could be used to improve cane yield-related features and root zone width would help for yield improvement in ratoon crops (Yang *et al*., 2021). Cluster 2 had the largest internode numbers and brix content, which might be used to improve cane quality criteria. On the other hand, Cluster 4 has the highest value for leaf midrib width, which could be used to increase leaf biomass. Tena *et al.* (2016) used cluster analysis to characterise 21 quantitative variables in 400 accessions that were classified into twenty groups. Rakesh *et al.* (2019) studied cluster analysis for nine quantitative features in twenty-four sugarcane varieties and divided them into three groups.

In order to create a base collection over a period of time, phenotypic traits have become increasingly important in genetic diversity analysis and genotype discrimination (Bhattacharjee *et al.,* 2007; Li *et al.,* 2011;

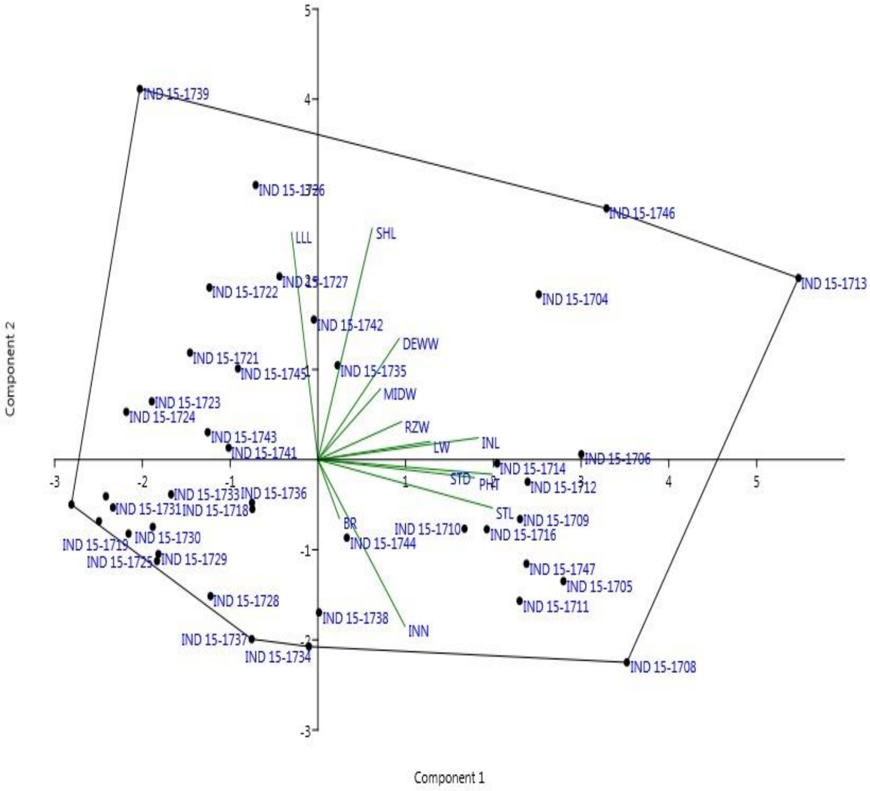

Fig. 2. Bioplot representing 41 acessesions along the axes PC1 and PC2

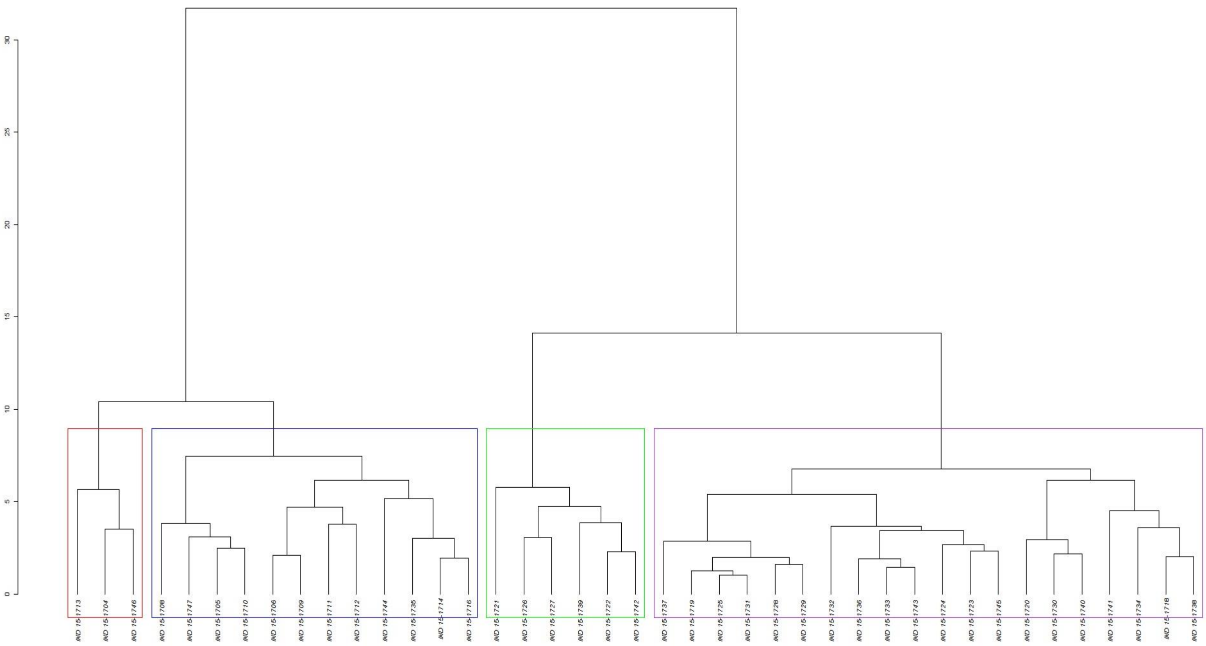

Fig. 3. Dendrogram based on quantitative traits

Table 6. Mean intra-clusters values for quantitative traits

Variables	Cluster 1	Cluster 2	Cluster 3	Cluster 4
PHT	175.4	159.49	102.66	112.45
STD	4.13	3.88	2.83	2.93
STL	130.17	126.48	74	71.72
INN	10	13.03	10.9	8.83
INL	12.17	9.75	6.72	6.65
RZW	0.6	0.44	0.43	0.42
LLL	77.23	60.38	65.05	94.78
LLW	0.67	0.64	0.46	0.47
MDW	0.13	0.15	0.1	0.18
SHL	20.53	13.51	11.49	18.65
DEW	0.33	0.21	0.2	0.23
BR	9.43	9.51	9.21	8.72
Number of accessions	3	12	20	6

Plant height (PHT cm), Stalk diameter (STD cm), Stalk length (STL cm), Inter node numbers (INN), Internode length (INL cm), Root-zone width (RZW cm), Leaf lamina length (LLL cm), Leaf lamina width (LLW cm), Midrib width (MDW cm), Leaf sheath length (SHL cm), Dewlap width (DEW cm) and Brix% (BR)

Studnicki *et al.,*2013). According to Akhtar *et al.* (2001), sugarcane varietals exhibit different morphological traits. The aerial organs of sugarcane varieties are the most important morphological features, according to Piscitelli *et al.*(1994). Because qualitative traits are unaffected by environmental influences, they can be used to correctly identify accessions and to help eliminate duplicates and closely related materials. The Shannon-Weaver diversity index (H') was used to determine the degree of phenotypic diversity in this study as shown in **Table 7**.The root eye alignment was found to be irregular in all accession and hence it has not taken for analysis. The estimates of phenotypic diversity indices (H') ranged from 0.17 for the presence or absence of a prominent nodal wax band to 0.96 for apical and sub apical germpore for 24 qualitative traits. In this study, leaf dewlap colour, bud shape and internode colour exposed to sun showed major diversity index among the 41 accessions.

Twelve quantitative and 24 qualitative traits were utilised to characterise the 41 *Saccharum spontaneum* accessions that showed the most diversity and might be used to improve sugarcane breeding programmes. In terms of quantitative and qualitative parameters, the fifteen *Saccharum spontaneum* accessions *viz.,* IND 15-1704, IND 15-1713, IND 15-1746, IND 15-1705, IND 15-1706, IND 15-1708, IND 15-1709, IND 15-1710, IND 15-1711, IND 15-1712, IND 15-1714, IND 15-1716, IND 15-1735, IND 15-1744 and IND 15-1747 could be considered viable genetic stocks for sugarcane improvement. Furthermore, the results of this study have crucial implications for

Table 7. Shannon-weaver diversity index

S.No.	Features	H'	S.No.	Features	H'
1	Stalk Colour (exposed)	0.90	13	Bud Germpore	0.96
2	Internode Waxiness	0.83	14	Bud Groove	0.76
3	Internode Shape	0.77	15	Bud Hairs	0.76
4	Internode Alignment	0.81	16	Bud Tip Extension	0.84
5	Internode Cross section	0.77	17	Bud Growth ring colour	0.77
6	Node - Wax Band	0.17	18	Bud Tip Extension	0.84
7	Node Swelling	0.85	19	Bud Growth ring Swelling	0.76
8	Node Root zone Colour	0.91	20	Leaf – attitude	0.78
9	Node Root zone swelling	0.89	21	Leaf Sheath Colour	0.86
10	Bud - Shape	0.93	22	Leaf Dewlap colour	0.94
11	Bud Size	0.53	23	Ligule shape	0.72
12	Bud Cushion	0.60	24	Ligule Hairs	0.28

making the greatest use of sugarcane germplasm, expanding our knowledge of essential accessions with favourable features, and, ultimately, improving sugarcane and energy cane.

ACKNOWLEDGEMENT

The authors express their gratitude to the Director, ICAR-SBI, Coimbatore, for the constant support to conduct research on germplasm characterisation

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