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Research Article

Characterization of *Saccharum spontaneum* accessions from North-Eastern India

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Abstract
Saccharum spontaneum, an important wild species of Saccharum complex, has been instrumental in improvement of sugarcane for yield, sucrose content, biotic and abiotic resistance. A total of 304 collections of *Saccharum spontaneum* from four North-Eastern states of India, conserved in the field gene bank at SBI-RC, Kannur, were characterized for 21 qualitative and 12 quantitative descriptors to assess the extent of variability and also to have reference database for maintaining their identity. The accessions showed high variability for both quantitative and qualitative traits. While the Arunachal Pradesh collections predominantly recorded erect and semi-erect plant habit type, the Bushy type was dominant in the collections of Bihar, Sikkim and Manipur. Discriminant analysis of quantitative characters classified the majority of the clones to their respective geographical origin. Shannon Diversity index was found to be more than 0.50 for most of the characters. These collections provide gene pool for broadening the genetic base and to introduce genes for cold and waterlogging tolerance, high vigour and yield.

Key words
Saccharum spontaneum, Sugarcane, North-Eastern states, India, qualitative and quantitative descriptors, Discriminant analysis

Introduction

Saccharum spontaneum, a wild relative of sugarcane is an important, highly variable and diverse among the *Saccharum* species. It is characterized by thin stalks with little or no sugar and has a wide range of agromorphological and cytological variation with an extensive distribution from Africa to Asia and across the Pacific islands (Artschwager and Brandes 1958). Plant height ranged from less than 1 m (Dwarf types) to over 7 m (tall types) (Nair *et al*. 1993) and different cytotypes of *S. spontaneum* with the varied chromosome numbers (2n = 40 to 128) were also reported (Sreenivasan *et al*. 1987). *S. spontaneum* has most diverse germplasm due to its very wide geographic distribution from tropics to subtropics (Ming *et al*., 2006). Morphologically and cytologically diverse germplasm of *S. spontaneum* can effectively contribute for genetic base broadening in sugarcane (Legendre and Breaux 1983; Burner and Legendre 1993). The first hybrids derived from interspecific crosses, made between *S. officinarum* L. and *S. spontaneum* in India and Java during the beginning of the 20th century, resulted in improved production, adaptability, ratoonabilty and resistance to biotic and abiotic stress. Diversity of *Saccharum spontaneum* had also been used in the development of bioenergy cultivars (Tew and Cobill, 2010). *Saccharum spontaneum* is also considered as valuable medicinal herb and folk medicine in traditional systems of medicine in India. Fresh juice from the stem of the plant has been used for the treatment of mental illness and mental disturbances by the vaidhiyars (Suresh kumar *et al*. 2010)

Sugarcane varieties around the world have narrow genetic base and sugarcane crop improvement at present is slow due to use of a few accessions of ancestral species and intensive selection pressures in the breeding programmes (Berding and Roach, 1987; Nair *et al*., 2002; Tew 2003). Understanding and documentation of the pattern and extent of genetic variation in the germplasm of *Saccharum* species has been limited due to its complex genome of heterozygosity, polyploidy, aneuploidy and environmental influence on it. Hence it is emphasized to collect, characterize, conserve, and utilize the new variability of *Saccharum* species, particularly *Saccharum spontaneum*, for sustaining the production and productivity of sugarcane and energy canes. Thus the research institutes (private and public sector) from across the world are increasingly demanding the sugarcane germplasm for crop improvement. In this context, germplasm characterization will be helpful in addressing the concerns of breeder's rights and benefit sharing in the context of exchange and utilization of sugarcane germplasm. In this study, a total of 304 *Saccharum spontaneum* clones collected from North Eastern states of India were characterized for various quantitative and qualitative parameters

Materials and Methods

Several expeditions, focused mainly on collections from the North Eastern states, were organized by the ICAR-Sugarcane Breeding Institute, Coimbatore since 1947, to collect and conserve and utilize the wild *Saccharum* germplasm from the distributional areas in the country (Naidu and Sreenivasan 1987; Nair *et al.* 1991, 1993). North Eastern India has a rich diversity of *Saccharum* and related grasses. The present study involves characterization of 304 *S. spontaneum* clones maintained in the field gene bank at the ICAR-Sugarcane Breeding Institute Research Centre (ICAR-SBI-RC), Kannur, Kerala (India). The mean sea level was 11 m with latitude of 11.87N and longitude of 75.37E. The monthly average rainfall, minimum and maximum temperatures during the crop period (2011-12) was given in Figure 2 (ICAR-SBI-RC meteorological data, 2011-12).

These clones represented four states of North Eastern India and majority of the accessions were representing the states of Arunachal Pradesh (143) and Bihar (129). The states of Sikkim and Manipur were represented by 25 and 7 accessions respectively. Table 1 and Figure 1 shows the collection areas in the four states of North Eastern India.

Arunachal Pradesh is primarily a hilly tract nestled in the foothills of Himalayas with rainfall ranging from 1000 mm in higher altitudes to 5750 mm in the foot-hill areas. Its climate is tundra in the higher altitude, temperate at lesser altitude, and sub-tropical climate at sea-level areas. 143 accessions represented Arunachal Pradesh and the collection areas were mainly in the southern border of the state experiencing temperate and subtropical climate. Bihar located in the gangetic plains of India has a humid warm tropical to subtropical climate with average annual rainfall of 1200 mm. It has mainly alluvial soils in addition to swamp soil and terai soil in northern parts of the state. 129 clones were represented from North-western districts of Bihar, which have subtropical climatic conditions. Sikkim basically has temperate climate with an altitude of 280 m to 8,585 m. Nestled below the eastern Himalayas, it experiences tropical to subtropical, temperate and frigid weather in different regions. Temperature ranges from zero to 28^0C with the average annual temperature of 18^0C. Twenty five collections from Sikkim represent the South Central and South Eastern areas of the state. The climate of Manipur is mostly tropical and in elevated areas alpine in nature. The temperature ranges from 0 to 32^0C depending on the elevation and season. The average rainfall is 1467.5 mm. Seven accessions included in this studies were from North Central areas of Manipur.

These accessions were planted in single replication on raised beds, with 180 x 90 cm spacing. Planting was in a zig-zag manner to prevent spread of vegetative propagules like runners between them. The crop was irrigated till the onset of monsoon upto first week of June. Earthing-up was carried out for each accession to ensure proper support and restrict spreading of runners. The accessions were completely randomized irrespective of their states (regions) of collection and growth habit. There was no apparent variation in the soil fertility in the experimental layout though no soil test was carried out during the experiment and 166:88:90(Kg/ha) NPK was applied in addition to application of lime @ 750 Kg/ha. All the package of practices as recommended for sugarcane cultivation were followed. These collections were characterized for 12 quantitative (Table.2) and 21 qualitative (Table.3) descriptors to assess the extent of variability among these clones and also to have reference database for maintaining the identity of clones in the field gene bank. Descriptors as elaborated in the publication by Artschwager and Brandes (1958) and Sreenivasan *et al* (2001a) were used.

Data were recorded on all collections during the post monsoon period (December 15 –January 30). Individual traits were recorded one trait at a time so that the observations on all 304 clones were completed for a specific trait within one or two days. The matured canes, which were present in the center of the clump were used for data recording, as they represent the maximum growth potential of a particular accession. Data on quantitative characters were recorded as average of five samples from each accession. Descriptors of bud were recorded from the matured central region of the cane/stem. Generally descriptors of the bud vary within a clone depending on the nature and extent of growth, duration of the clones, and portion of the cane observed for recording the data. Hence the specific descriptor which has maximum frequency at the central portion of the matured cane was recorded. Plant height was measured from collar region to tip of the whorl and stalk height was from collar region to last internode with cane formation.

Data analysis

Discriminant Function Analysis

Accuracy of grouping was determined by Predictive Discriminant function analysis using SPSS. A discriminant score was calculated based on the weighted combination of the independent variables using the formula

$$Di = a + b_1x_1 + b_2x_2 + \ldots + b_n x_{n,}$$

Di is predicted score (discriminant score or dependent variable) used for classifying accessions

into groups, x is predictor (Independent variable) and b is discriminant coefficient.

Discriminant function was based on linear combinations of predictor variables. Maximum likelihood technique was used to assign an accession to a group (state) from a specified cut-off score. Since group size (number of accessions in each state) was not equal, the cut-off was calculated from weighted means. Accuracy of the discriminant function is given by the classification matrix, which tells what percentages of the existing accessions are correctly classified by the discriminant function.

Statistical significance of the model was explained by the Wilk's lambda value and the eigen value. When Eigen value > 1, groups were considered distinct, and hence the model had good discriminating power. Wilk's lambda is a measure of the extent of misfit of the discriminant solution. Values of lambda range from 0 to 1. Values close to 0 indicate that groups were distinctly different. Values close to 1 indicate that groups were overlapping. When lambda < 0.5, solution was statistically significant and acceptable. Standardized Canonical Discriminant Function Coefficient was used to identify which one (best discriminator) of the independent variables is more important in discriminating between groups. It also overcomes the problem of different measurement units. The higher the standardized discriminant coefficient of a variable, the higher is its discriminating power. Once a model was finalized and the discriminant functions were derived, we predicted to which state (region) a particular accession belonged by using Classification functions (S). Each function allowed us to compute classification scores for each accession for each group, by applying the formula:

$$Si = c_i + w_{i1}*x_1 + w_{i2}*x_2 + ... + w_{im}*x_m$$

Where, the subscript i denotes the respective group; the subscripts 1, 2… m, denote the m variables; c_i is a constant for the i^{th} group, w_{ij} is the weight for the j^{th} variable in the computation of the classification score for the i^{th} group; x_j is the observed value for the respective case for the j^{th} variable. Si is the resultant classification score.

Shannon-weaver diversity index

Variation in qualitative traits was quantified using Shannon-weaver diversity index (H') using the formula

$$H' = - \sum pi \, (\log_2 pi)/\log_2 n$$

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 maximum diversity.

Results and Discussion

Quantitative and qualitative traits

A total of 304 accessions were characterized for twelve quantitative traits and all the traits were found to be highly variable among the accessions from both within and between the states (regions). The minimum, maximum, mean, standard deviation and coefficient of variation for the quantitative traits are given in the table 2 (Appendix).

The data on some qualitative traits and flowering in the accessions of different states is given in the table 3 and 4 respectively. Accessions from Arunachal Pradesh were predominantly tall growing types with erect growth habit and among them twelve, eighty six and forty five accessions had bushy, erect and semi growth habit respectively (table 3). The accessions had moderate to high internode wax. Majority of the accessions from Arunachal Pradesh were nonflowering in the field gene bank at SBI-RC, Kannur (Table 4) and out of 143 accessions, only 28(19.58%) were flowering irregularly. Flowering and its intensity was mainly from September to January and reduces later, though, it was observed in some accessions with less intensity in the remaining months of the year also. Only < 20% of the accessions from different growth habit were flowering at ICAR-SBIRC, Kannur, indicating environmental influence on flowering. Pollen fertility ranged from 70 to 90%.

The erect and semi erect accessions had vigorous and tall growth habit with good cane formation. The bushy accessions had slow growth with thin and short cane. The plant height ranged from 1 to 4 m with the mean plant height of 2.96 m. While the node length ranged from 4.67 to 30.50 cm, the stalk thickness ranged from 2.90 to 16 mm.

Bushy growth habit was the dominant type in the accessions from Bihar, Sikkim and Manipur. Out of 129 accessions from Bihar, 67(51.94%) accessions were flowering irregularly. Thirty-six (42%) accessions out of 86 bushy and 28(73%) out of 38 semi erect growth types were flowering. Flowering and its intensity was mainly from July to August. The plant height ranges from 0.90 to 3.30 m with the mean plant height of 2.01 m. While the internode length ranged from 2.84 to 15 cm, the stalk thickness ranged from 2.20 to 9.60 mm. Pollen fertility was in the range of 60-98%. Thirteen (52%) out of 25 were flowering in accessions of Sikkim. The Coefficient of variation (CV) (table 2) was high and above 20 % for many

economically important quantitative traits like plant height, stalk height and thickness, internode number and internode length except for leaf length (<20%).

S. spontaneum has been reported to be cytologically and morphologically diverse (Tai *et al.* 1995), and generally show greater genetic variability than *S. robustum*, *S. officinarum* (Lu *et al*. 1994; Besse *et al*. 1997), and elite germplasm (*Saccharum* spp hybrids) (Arceneaux 1967; Harvey and Botha 1996; Harvey *et al*. 1994). High variability was also reported for morphological and physiological traits in various studies in sugarcane germplasm collections (Chu *et al*. 1962; Kandasami *et al*. 1983; Rao and Vijayalakshmi 1963; Tai *et al*. 1995; Lu *et al*. 1994). Morphological characterization is an efficient method of classification for the management of sugarcane germplasm (Brown *et al.* 2002). Govindaraj *et al*. (2014) had reported that 30 accessions of *S. spontaneum*, from arid and semi-arid regions of North-Western India, varied greatly for 19 qualitative traits except for sheath hair, wax band, node swelling, ring swelling, bud hair, bud extension and root eye alignment. In the same study, five quantitative traits recorded CV more than 20%.

Shannon diversity index (SDI)

In the present study, Shannon Diversity Index (SDI) (Table 5) was calculated for twenty qualitative traits. SDI was found to be high for all the traits except for spines and ligule shape in the accessions of different states. SDI was high for the descriptors of bud, growth habit, internode wax and node swelling. Some qualitative descriptors like growth habit, bud shape, spines on the leaf sheath were more reliable for characterization of *Saccharum spontaneum* germplasm.

Discriminant analysis

Discriminant analysis using 11 quantitative descriptors correctly classified the state (region) membership of accessions from Arunachal Pradesh and Bihar by 79.72% and 62.8% respectively and that of Sikkim and Manipur by 71.40% and 40.0% respectively. Overall 68.85% of the accessions were correctly classified and 66.23% of the cases were classified by cross validation of the accessions involved in discriminant analysis. *Saccharum spontaneum* accessions from Arunachal Pradesh were more unique as nearly 80% of the accessions were correctly classified. Forty-one (13.44 %) accessions were not assigned to any state by the discriminant analysis and majority of them (27 or 20.98%) being from Bihar indicates that there was more variation in accessions from Bihar. The results of Discriminant analysis also indicates that even though the 11 quantitative descriptors classified majority of the accessions, some more quantitative descriptors may be required for assigning the accessions to their respective states/geographical location of collection. Original and cross validated group membership predicted through Discriminant analysis is given in the Table 6. Statistical significance of the model was explained by Eigen values and Canonical correlation (Table. 7) and Wilk's lambda (Table. 8). Eigen value = 1.95 for Canonical Discriminant Function 1, hence accessions of states are distinct, and the model had good discriminating power. Wilks' lambda was 0.27 for test of functions 1 through 3, and thereby it was statistically significant and acceptable.

Standardized Canonical Discriminant Function Co-efficients (Table 9) explains that the sheath length was the best discriminator in Function 1 and 2. Sheji Mary *et al*. (2006) reported grouping of *S. spontaneum* clones based on their location of collection in RAPD study. Govindaraj *et al*. (2014) have reported that the collections were grouped into separate clusters based on morphological traits, geographical distribution and location's rainfall pattern. Grouping was reported to be random with respect to location of collection or morphological traits with a different set of *S. spontaneum* clones (Tai *et al.* 1995; Pan *et al*. 2004).

The bushy and semi erect types of *Saccharum spontaneum* generally colonised plains near stagnating or slow moving water bodies or marshy areas. North-western districts of Bihar had gangetic river systems, alluvial plains and altitudinal range of 53-188m which provided congenial ecogeographical conditions for the establishment and evolution of mainly bushy and to some extent semi erect growth habit types. The accessions from these districts generally reported to have somatic chromosomes of 2n= 50, 54, 60, 62 and 64 types (Panje and Babu. 1960)

Accessions from Arunachal Pradesh represented southern and south-western districts of the state. These districts had hilly tracts, high rainfall and seasonal variation in temperature. Tall and vigorous accessions from Arunachal Pradesh indicated that the ecogeographical conditions of Arunachal Pradesh favoured better colonisation and evolution of erect and semierect types than bushy types of *Saccharum spontaneum*. Erect and semi-erect growth habit types with thick stems might have been ecologically selected and evolved in hilly and high rainfall tracts as they can better withstand and sustain competition with other plant species. These accessions are expected to provide genetic tolerance to cold and waterlogging due to high rainfall and seasonal variation in their natural

ecosystem. Though the extent of internode wax appears not to be correlated with the growth habit type but generally majority of the high internode wax accessions had erect or semierect growth habit type.

All the accessions of Bihar and Sikkim had deltoid shaped ligule, though they had different growth habit and bud traits. Majority of the accessions from Arunachal Pradesh had erect or semi erect growth habit with crescent shaped ligule and conical shaped internode though some accessions had cylindrical and few accessions had obconoidal internodes. Majority of the bushy growth types had small, ovate buds and bobbin shaped internode. It was reported that the Arunachal collections were distinct genetically, representing different morphotypes *viz*., the tall, medium and short/dwarf forms (Nair *et al*. 1993; Mary *et al.* 2006) and the most variable group among the Indian *S. spontaneum* in terms of morphological variation and number of cytotypes (Sreenivasan *et al*. 1986, 2001)

The results also indicated that the deltoid shaped ligule was predominantly present in the accessions of bushy growth habit though the semierect types from Bihar and Sikkim also had deltoid shaped ligule. Hence the results indicated that the extent of internode wax, ligule shape and bud traits were not exclusive with a particular growth habit or geographic location or habitat. This indicates that extensive natural crossing and recombination might have occurred between *Saccharum spontaneum* accessions having different cyto-morphology.

Accessions from Sikkim generally reported to be having 2n=54, 56, 58, 60 and 64 cytotypes (Sreenivasan *et al*. 2001; Kandasami *et al*. 1983). Akira Moriya (1965) had reported 2n=56 chromosomes in Sikkim forms of *Saccharum spontaneum*. Sikkim forms that were characterised in this study were very similar to the Japanese forms of *Saccharum spontaneum* with respect to very slender stalks, narrow leaves and also deltoid pattern of ligule as reported by Akira Moriya (1965). Accessions from Arunachal Pradesh reported to be of mainly 2n=80 cytotypes though few accessions had been reported to have 2n= 60, 64, 72. 2n=80 was reported in the accessions of Nepal and Assam (Panje and Babu 1960). Bremer (1934), Santos (1937) and Akira Moriya (1965) have also reported 2n=80 in Manila forms of *Saccharum Spontaneum*. The possibility of different ancestry and evolution may have contributed for different cyto-morphological types in accessions of Arunachal Pradesh as compared to the accessions from Bihar and Sikkim. Colder climate in Himalayan Mountains and high altitude may have had an impact on genetic differentiation and evolution of *Saccharum spontaneum* in the states of Arunachal Pradesh, Sikkim and Manipur. The sub Himalayan foot hills, rivers banks and indo-gangetic plains from west to east have distribution of diversity in the wild species of *Saccharum* and related wild genera like *Erianthus fulvus*, *Erianthus arundinaceus*, *Erianthus bengalensis* and other grasses like *phragmitis*, *Imperata* spp etc. Nair *et al.*(1993) reported that varied climatic and ecogeographical conditions prevailing in north-eastern states together with profuse flowering and congenial factors for dispersal and germination of seeds has lead to extensive colonisation in the riverbanks and evolution of different cyto-morphological types in *Saccharum spontaneum*. The river slopes and sandy nature of river banks helps in easy spread of runners.

Extensive Phenotypic diversity analysis has not been carried out in *S. spontaneum* germplasm and is difficult due to its rhizomatous growth, large plant size and environmental influence. (Aitken and McNeil 2010). This study also had the limitations of one replication, due to the requirement of larger plot, for statistical analysis and interpretation of the results of quantitative traits. But the errors were reduced by taking the average of five samples from the centre of clump for each accession and the interdependence between growth habit and quantitative traits; as erect and semi-erect growth habit types had taller and thicker stems and larger leaves in comparison to bushy types irrespective of their geographical region.

The results emphasises that the tall, robust and cold-tolerant germplasm collection from diverse eco-geographical niche in the north-eastern Himalayan foot hills and river systems of India provides gene pool for broadening the genetic base and to introduce genes for cold and waterlogging tolerance, high vigour, yield and non-flowering nature. The *Saccharum spontaneum* clones from Arunachal Pradesh have huge potential for utilization in breeding programmes for high biomass or energy canes due to their tall and high vigour potential. The important clones *viz.*, IND 85-529 IND 85-531, IND 85-532, IND 85-537, IND 85-539, IND 85-545 (Table 10) identified in the this study can be utilized in sugarcane breeding programmes for improving yield and resistance to cold and waterlogging or developing energy canes. Though these clones were non-flowering at the climatic conditions of SBI-RC, Kannur, Kerala, if they were induced to flowering and used in breeding programmes, it would be of significant economic importance as the non-flowering nature influences sucrose content and duration of sugarcane hybrids and bioenergy canes. Further exploration in the above states may be helpful in

collection and conservation of diverse germplasm as these states represent diverse eco-geographical conditions.

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Table 1. The states, the collection areas/districts and number of accessions

S No.	State	Districts/collection areas	Number of accessions
1	Arunachal Pradesh	West Kameng, East Kameng, Papum Pare, Lower Subansiri, West Siang, Anjaw, Lohit , Changlang, Tirap	143
2	Bihar	West Champaran, Motihari, Sitamahri and Muzaffarpur	129
3	Sikkim	Gangtok	25
4	Manipur	Imphal, Ukhrul and Thoubal	7

Table 2: Variation in quantitative traits in the accessions of four states*

	State	**Arunachal Pradesh**					**Bihar**				
SN	**Descriptor**	Min.	Max.	Mean	Std. dev.	CV	Min.	Max.	Mean	Std. dev.	CV
1	Number of internodes	7.00	23.00	14.57	3.35	23.02	3.00	21.00	9.70	2.22	22.85
2	Number of root eyes	1.00	3.00	1.93	0.28	14.61	1.00	2.00	1.40	0.49	35.14
3	Leaf length(cm)	72.00	174.00	127.06	19.35	15.23	53.60	174.60	115.89	21.80	18.81
4	Leaf width(mm)	1.00	32.00	16.53	7.45	45.06	1.50	12.00	3.80	2.01	53.00
5	Midrib width(mm)	1.00	6.00	2.91	1.01	34.57	1.00	3.00	1.70	0.42	24.88
6	Sheath length(cm)	9.70	33.60	18.65	3.81	20.41	9.50	31.80	19.86	4.66	23.44
7	Plant height(cm)	100.00	400.00	296.52	62.14	20.96	90.00	330.00	201.53	41.42	20.55
8	Stalk thickness(mm)	2.90	16.00	10.45	2.77	26.52	2.20	9.60	5.22	1.68	32.18
9	Stalk height(cm)	37.33	268.50	148.77	47.05	31.63	12.40	175.00	65.67	24.58	37.44
10	Internode length(cm)	4.67	30.50	14.28	4.62	32.39	2.84	15.00	8.48	2.54	30.00
	State	**Manipur**					**Sikkim**				
SN	**Descriptor**	Min.	Max.	Mean	Std. dev.	CV	Min.	Max.	Mean	Std. dev.	CV
1	Number of internodes	10.00	19.00	14.00	3.54	25.31	5.00	14.00	9.56	2.62	27.35
2	Number of root eyes	2.00	2.00	2.00	0.00	0.00	1.00	2.00	1.50	0.51	34.13
3	Leaf length(cm)	94.80	136.80	109.83	15.11	13.76	57.60	156.40	117.16	22.13	18.89
4	Leaf width(mm)	4.00	28.00	10.43	8.34	80.01	1.50	17.00	6.11	5.02	82.09
5	Midrib width(mm)	2.00	3.00	2.04	0.36	17.65	1.00	4.00	1.79	0.76	42.18
6	Sheath length(cm)	16.20	27.40	21.40	3.62	16.93	9.80	29.30	20.30	4.60	22.68
7	Plant height(cm)	145.00	332.00	229.29	78.59	34.28	76.00	315.00	214.09	60.84	28.42
8	Stalk thickness(mm)	3.70	10.10	6.29	2.66	42.36	4.10	9.10	5.98	1.62	27.15
9	Stalk height(cm)	73.00	243.40	116.03	60.45	52.10	42.75	155.00	85.80	27.91	32.53
10	Internode length(cm)	4.70	15.10	9.76	3.45	35.34	4.50	13.50	8.82	2.52	28.63

* The descriptors Root band width and dewlap width are not shown in the table

Table 3. Frequency of qualitative traits within and among the states*

Trait	State	AP	BH	MNP	SKM	Total
1.Growth habit	Bushy	12	86	5	14	117
	Erect	86	1	1	3	91
	Procumbent	0	4	0	0	4
	Semi Erect	45	38	1	8	93
2. Ligule shape	Deltoid	29	129	2	25	185
	Crescent	109	0	5	0	114
	Arcuate	4	0	0	0	4
	Subarcuate	1	0	0	0	1
3. Bud shape	Narrow Ovate	19	21	3	6	49
	Obovate	13	13	0	3	29
	Ovate	22	65	2	4	93
	Rhomboid	30	5	1	6	42
	Tall Deltoid	19	18	1	2	40
	Pentoid	40	7	0	4	51
4. Node shape	Conical	117	41	7	20	185
	Cylindrical	15	19	0	4	38
	Bobbin	11	59	0	1	71
	Obconoidal	0	10	0	0	10
5. Spines on leaf sheath	Present	20	1	0	1	22
	Absent	123	128	7	24	282

*The data on the remaining 16 qualitative traits has not been shown the table and these descriptor states includes: Internode wax, Node alignment, Node cross section, Wax band, Node swelling, Root band swelling, Root eye alignment, Bud size, Bud cushion, Bud germpore, Bud groove, Bud hairiness, Bud extension, Growth ring swelling, Sheath hair and Ligule hair.

Table 4: Number and percentage of different growth habit types and flowering clones in the field gene bank

State/Growth habit	No. of clones	Percentage of each growth habit type	No. of clones flowering	Percentage of flowering clones
Arunachal Pradesh	143	-	28	19.58
Bushy	12	08.39	2	16.67
Semi erect	45	31.47	9	20.00
Erect	86	60.14	17	19.77
Bihar	129	-	67	51.94
Bushy	86	66.67	36	41.86
Semi erect	38	29.46	28	73.68
Erect	1	0.78	1	*
Procumbent	4	03.10	2	*
Sikkim	25	-	13	52.00
Bushy	14	56.00	7	50.00
Semi erect	8	32.00	3	37.50
Erect	3	12.00	3	*
Manipur	7	-	4	57.14
Bushy	5	71.43	3	60.00
Semi erect	1	14.29	1	*
Erect	1	14.29	0	*

* Percentage of flowering was not calculated where ever sample size was less than four for each growth habit type

Table 5. Shannon Diversity Index (SDI) for qualitative traits

Trait/State	Arunachal Pradesh	Bihar	Sikkim	Manipur
Growth habit	0.80	0.56	0.88	0.86
Internode waxiness	0.78	0.64	0.72	0.92
Inter node shape	0.54	0.87	0.68	0.00
Node alignment	0.65	0.07	0.27	0.99
Node cross section	0.62	0.45	0.77	0.99
Wax band	0.11	0.07	0.00	0.59
Node swelling	0.79	0.40	0.57	0.59
Root band swelling	0.62	0.82	0.98	0.99
Root eye alignment	0.62	0.95	0.57	0.59
Bud shape	0.96	0.80	0.97	0.92
Bud size	0.81	0.93	0.69	0.59
Bud cushion	0.91	1.00	0.57	0.59
Bud germpore	0.71	0.91	0.96	0.59
Bud groove	0.87	1.00	0.99	0.99
Bud hairiness	1.00	0.99	0.98	0.59
Bud extension	0.76	0.66	0.65	0.99
Growth ring swelling	0.11	0.20	0.27	0.00
Sheath hair	0.37	0.20	0.68	0.59
Spines	0.58	0.07	0.27	0.00
Ligule shape	0.48	0.00	0.00	0.35

*Ligule hair was not considered for SDI as all the accessions show its presence.

Table 6. Discriminant analysis Classification Results (b,c)

Count and respective %	State	Predicted Group Membership					
		Arunachal	Bihar	Manipur	Sikkim	Others	Total
Original Count	Arunachal	114	4	8	7	10	143
	Bihar	0	81	3	18	27	129
	Manipur	1	0	5	1	0	7
	Sikkim	2	8	1	10	4	25
%	Arunachal	79.72	2.8	5.6	4.9	7	100
	Bihar	0	62.8	2.3	14	20.9	100
	Manipur	14.3	0	71.4	14.3	0	100
	Sikkim	8	32	4	40	16	100
Cross-validated(a) Count	Arunachal	112	4	10	7	10	143
	Bihar	0	77	4	22	26	129
	Manipur	1	0	5	1	0	7
	Sikkim	2	10	1	8	4	25
%	Arunachal	78.32	2.8	7	4.9	7	100
	Bihar	0	59.7	3.1	17.1	20.2	100
	Manipur	14.3	0	71.4	14.3	0	100
	Sikkim	8	40	4	32	16	100

a-Cross validation is done only for those cases in the analysis. In cross validation, each case is classified by the functions derived from all cases other than that case.
b-68.85% of original grouped cases correctly classified.
c-66.23% of cross-validated grouped cases correctly classified.

Table 7. Wilks' Lambda

Test of Function(s)	Wilks' Lambda	Chi-square	df	Sig.
1 through 3	.274	382.124	44	.000
2 through 3	.809	62.652	30	.000
3	.934	20.287	18	.317

Wilk's Lambda was significant for the first three functions

Table 8. Eigen values and Canonical correlation

Function	Eigenvalue	% of Variance	Cumulative %	Canonical Correlation
1	1.953	89.7	89.7	.813
2	.154	7.1	96.8	.366
3	.050	3.2	100	.218

First 3 canonical discriminant functions were used in the analysis.

Table 9. Standardized Canonical Discriminant Function Coefficients*

	Function		
	1	2	3
Number of internodes	.120	.347	.890
Root band width	.256	.263	.568
number of root eyes	.249	.508	.001
Leaf length	-.077	-.395	.057
Leaf width	.230	.399	-.343
Midrib width	-.031	-.128	.130
Sheath length	-.436	.656	.023
Plant height	.008	-.298	.128
Stalk thickness	.420	-1.123	-.095
Stalk height	.173	.784	-1.414
Node length	-.020	-.423	.613

*Dewlap width was not included for Discriminant function analysis

Table 10. Traits of prominent accessions

Accession	State (region)	Chromosome No. reported	Growth habit	Plant Ht. (cm)	Stalk height (cm)	Stalk thickness (mm)	Leaf length (cm)	Leaf width (mm)	Flowering
IND 85-529	ARP	64	Erect	358	237.50	12.20	141.00	28.00	NF
IND 85-531	ARP	NR	Erect	380	259.00	14.50	162.80	28.00	NF
IND 85-532	ARP	NR	Erect	350	212.00	14.50	160.40	26.00	NF
IND 85-537	ARP	NR	Erect	380	268.50	14.00	152.00	28.00	NF
IND 85-539	ARP	NR	Erect	342	227.50	15.25	135.80	32.00	NF
IND 85-545	ARP	80	Erect	335	249.00	16.00	132.00	27.00	NF

ARP: Arunachal Pradesh, NR: Not Reported, NF: Non flowering at SBIRC, Kannur, Kerala

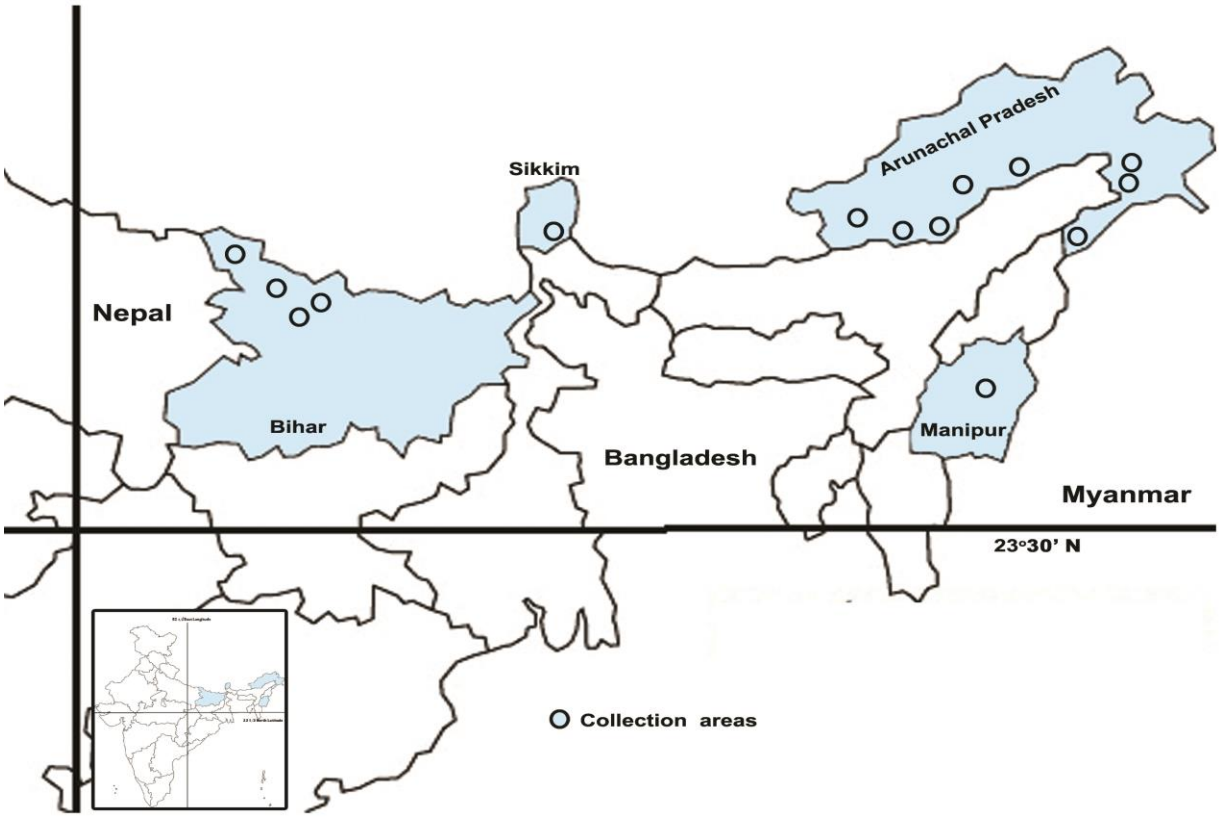

Fig.1 Collection areas of *Saccharum spontaneum* in North Eastern states of India