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Variability and molecular diversity of wild sugarcane germplasm collected from low temperature regions Lohit and Changlang of Arunachal Pradesh

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Saccharum spontaneum L. is a perennial grass representing the most genetically diversified species in *Saccharum* genus. It has the potential to withstand severe biotic/abiotic stresses and frequently used as donor of stress tolerant genes in sugarcane improvement program through gene introgression. In this study, the phenotypic variation and molecular diversity of forty nine *S. spontaneum* accessions collected from Lohit and Changlang regions of Arunachal Pradesh, North Eastern India were investigated for morphometric traits and polymorphic STMS marker. The phenotypic coefficient of variation showed ample variability for the traits viz., plant height (27.19%), stalk diameter (28.21%), single cane weight (48.97%), internode number (22.60%) and internode length (29.15%). Further, twenty nine sequence-tagged microsatellite site (STMS) markers generated 495 bands with an average of 14.06 polymorphic bands. The accessions specific bands in respect to specific marker combinations were identified. The Jaccard's similarity coefficients among these accessions ranged from 0.42 to 0.78 with an average of 0.58 and clustering using unweighted pair group method of arithmetic-average (UPGMA) showed two major clusters with subclusters. Similarly polulation structure analysis based Bayesian approach grouped the individuals into two subpopulations, with alpha value of 0.112. The study shows that *S. spontaneum* accessions collected from Arunachal Pradesh is highly diverse, most of them will be harbouring the genes for cold tolerance and biomass. The set of markers which produced specific bands for the specific accessions identified in the study will help in identification of the particular accessions. The accessions studied are potential source for cold tolerance and high biomass, the results obtained in the present study will definitely help in planning and utilising them in sugarcane improvement programme.

Keywords: Molecular diversity; STMS marker; *Saccharum spontaneum*; germplasm; sugarcane

Introduction

Saccharum genus belongs to family Poaceae which has six species, *viz. S. officinarum, S. barberi, S. edule, S. robustum, S. spontaneum* and *S. sinense*. The *S. spontaneum* L. is a tall (1 to 7 m in height) perennial C_4 grass with deep roots and rhizomes and has diverse genetic composition[1]. Almost, thirty cytotypes of *S. spontaneum* with chromosome number ranging from 2n = 40 to 128 has been reported[2-3]. The species has wide adaptability and extensively distributed throughout India. It is usually found in banks of water bodies (river, lakes, and ponds), alongside the road and railway tracks, alluvial plains, damp depressions, swamps, sandy soils, etc[4]. It occurs at an altitude ranging from lowland eco-region or sea-level to more than 1,800 m[5]. The species has played a significant role in the development of improved sugarcane varieties, as the modern sugarcane cultivars are complex hybrids derived mainly from the interspecific crosses made between *S. officinarum* and *S. spontaneum*. The significant contribution of this species is imparting high productivity and resistance to diseases and pests in the modern sugarcane cultivars[6-7]. Realising the importance of the species in the sugarcane varietal improvement programmes, there had been concerted efforts in the past to collect and conserve the *S. spontaneum* diversity. Large *S. spontaneum* accessions representing different geographical groups are available in the Indian Sugarcane Germplasm Collection, which have been characterized for quantitative and morphological attributes[8-9]. Through regular explorations, collections were made and are conserved at the field gene bank of ICAR-Sugarcane Breeding Institute (SBI) Coimbatore, India. One such explorations was aimed to collect accssions which are likely to harbour the genes for cold tolerance from Lohit and Changlang regions of Arunachal Pradesh, a northeastern state which is most important region in terms of both abundance and diversity of *Saccharum* germplasm in

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India[5]. The tall, thick forms of *S. spontaneum* found exclusively in some part of Arunachal Pradesh are considered being a vital source of gene for vigour and high productivity in sugarcane breeding[5]. Molecular markers have been used for the genetic characterization of germplasm in a variety of crops including sugarcane. Various types of markers like RFLP, 5S rRNA ITS marker, RAPD, ISSR, AFLP, SRAP, TRAP, SNP, STMS, genomic simple sequence repeat (gSSR), EST-derived simple sequence repeats (EST-SSRs), SCoT have been used for the analysis of phylogeny, clone or cultivar identification, parent evaluation, genetic mapping, inter-species relationships and genetic diversity among the *Saccharum* species, related genera and their hybrids[10-11]. In the present study, we analyzed the phenotypic variation, genetic diversity and relationship of 49 accessions of *S. spontaneum* collected from Lohit and Changlang regions of Arunachal Pradesh, to provide a basis for utilization of the wild germplasm in sugarcane improvement.

Materials and Methods

Plant Material and Assessment of Trait Phenotypic Variation

Forty-nine accessions of *S. spontaneum* collected from Lohit and Changlang regions of Arunachal Pradesh, India were used in this study (Supplementary Table 1 and Supplementary Fig. 1). These accessions are being maintained at World Germplasm Collection, Indian Council of Agricultural Research (ICAR)-Sugarcane Breeding Institute (SBI), Coimbatore, Tamil Nadu, India. To assess the phenotypic variation, each accession was planted and replicated thrice in a randomized replicated blocks with plot size of 3 m length and 1.2 m spacing between rows. The regular field management was carried out as like for commercial sugarcane production[12]. Observations were recorded for five quantitative traits *viz.* plant height (cm), stalk diameter (cm), single cane weight (kg), internode number and internode length (cm).

Genomic DNA Isolation and PCR Amplification

Genomic DNA from a young leaf of each plant was isolated by following the procedure of Doyle and Doyle[13]. After RNAs treatment DNA quality was checked by 0.8% agarose and quantified using NanoDrop spectrophotometer (ND-1000, version 3.1.1, United States of America). Polymerase chain reaction (PCR) performed with a DNA concentration of 20 ng/µl.

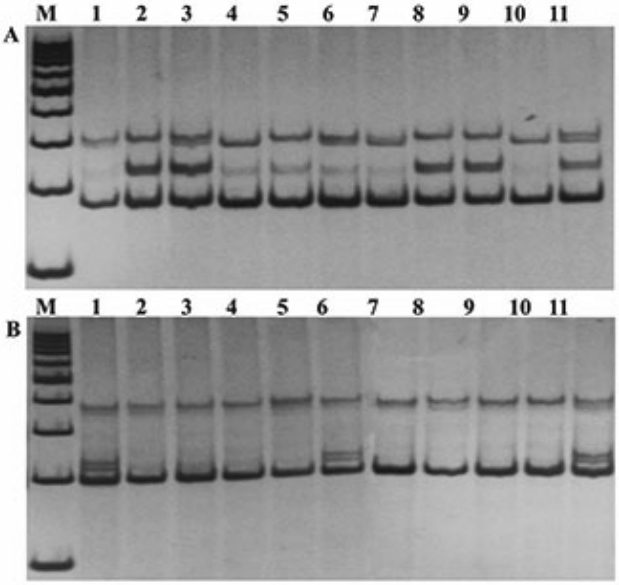

Fig. 1 — Gel electrophoresis of the amplified products obtained with STMS primers NKS52 (A) and NKS53 (B). Lanes; M, Marker; 1, *S. spontaneum* IND 00-997; 2, IND 00-998; 3, IND 00-1001; 4, IND 00-1002; 5,IND 00-1004; 6, IND 00-1011; 7, IND 00-1012; 8, IND 00-1015; 9, IND 00-1018; 10, IND 00-1019; 11, IND 00-1020.

Twenty nine STMS markers (Supplementary Table 2) were used for molecular diversity analysis of 49 *S. spontaneum* accessions. PCR reaction was performed by following the procedure mentioned by Saravanakumar *et al*[14]. A total of 10 µl reaction mixture contained 10X PCR buffer with 15 mM $MgCl_2$, 0.5 U of *Taq* DNA polymerase, 100 µM of each dNTPs, 20 pmol/µl of each primer and 20 ng/µl of genomic DNA. PCR reactions were performed in Thermal cycler (Eppendorf Master Cycler ProS, Germany) using a single primer pair in each reaction. The PCR cycle conditions were: initial denaturation at 94°C for 2 minute followed by 35 cycles consisting of denaturation at 94°C for 1 minute, primer annealing temperature varied depends on primers for 40 second, and extension at 72°C for 40 second followed by a final extension at 72°C for 7 minute. The PCR products obtained were resolved in 8% native PAGE and documented using Geldoc System (Alpha Imager version 4).

Data Analysis and Phylogeny Construction

Only clearly visible and reproducible amplicons from the sequence tagged microsatellite site (STMS) primers were considered for further analysis[15]. The markers were scored as absent (0) or present (1), genetic similarity relationship analysis between

the accessions was estimated by calculating Jaccard's similarity coefficients. Cluster analysis was performed with these coefficient values and a dendrogram was constructed by the unweighted pair group method of arithmetic-average (UPGMA) using NTSYS program (NTSYS-pc2.11)[16]. The quantitative traits *viz.* plant height, stalk diameter, single cane weight, internode number and internode length were used to estimate the phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV) and environmental coefficient of variation (ECV) using SAS (SAS version 9.2 software package; SAS Institute, Inc.; Cary, NC) software.

Population Structure

Analysis of the population structure of 49 *S. spontaneum* genotypes was carried out using STRUCTURE v.2.3.4, for which a Bayesian approach is used to infer the population structure[17]. Gene flow (Nm), or the number of migrants entering a population in each generation, was estimated using the formula Nm=0.25×(1−Fst)/Fst (five independent runs with K value ranging from 2 to 8 and five iterations for each value of K was set)[18]. To determine the actual K value, the following structure parameter was set with the possibility of admixture and allele frequency: the length of the burning period was 50,000 iterations followed by 200,000 Monte Carlo Markov Chain replicates. To obtain the optimal K value, it was plotted against the mean estimate of log probability of the data L(K). The actual number of sub-populations was identified using the maximum L(K) value. The final population structure was calculated with ΔK, based on the second-order rate of change of likelihood distribution mean L″(K) and with respect to K estimated using STRUCTURE HARVESTER and optimal K value was obtained[19-20]. Analysis of molecular variance (AMOVA) was done to detect the genetic variation within and among the population.

Result and Discussion

Phenotypic Variation Analysis

A total of 49 *S. spontaneum* accessions collected from Lohit and Changlang regions of Arunachal Pradesh, India was analyzed for phenotypic variation and their genetic diversity. These accessions were collected from twenty-five locations in two major districts of Arunachal Pradesh (Supplementary Table 1; Supplementary Fig. 1), of which 45 were from Lohit and four were from Changlang. The altitude of the areas explored were ranged from 140 m to 1650 m. Maximum number of accessions recorded the chromosome number of 64 (2n=64) while, one accession IND 00-1064 had chromosome number of 60 (2n=60). The moropological traits, like plant height, stalk diameter, single cane weight, internode number and internode length showed higly significant variation among the accessions (Table 1). The existence of high variation has been reported among *S. spontaneum* clones collected in arid and semi-arid zones of North Western India[21]. The plant height ranged from 89.6 to 293.3 cm with mean of 214.3 cm. Stalk diameter ranged between 0.36 and 1.86 cm with mean of 1.19 cm. The single cane weight ranged from 0.018 to 0.508 kg with mean of 0.277 kg. The number of internodes ranged from 8.7 to 25.3 with the average of 15.1 and the internode length ranged between 4.5 and 31.9 cm with mean of 18.2 cm. The phenotypic coefficient of variation (PCV), was highest for single cane weight (48.97%) followed by internode length (29.15%), stalk diameter (28.21%), plant height (27.19%) and number of internodes (22.60%; Table 1). The geneotypic coefficient of variation (GCV) and environmental coefficient of variation (ECV) were estimated (Table 1), GCV was highest for single cane weight (47%) and lowest for plant height (18.95%) while ECV was highest for plant height (19.51%) and lowest for stalk diameter (6.29%). PCV and GCV ranged between 0-10%, 10-20%, and over 20% respectively are considered as lower, medium and high variation levels[22-23]. The GCV and PCV provide a measure to compare the variability present among the traits. Values of PCV and GCV were close for most of the traits except for plant height, indicating that other traits are less influenced by environment and variation observed was due to their genetic constitution. These germplasm may harbour different combination of genes which may be highly helpful in genetic improvement of sugarcane for cold tolerance and biomass.

Polymorphism Detected Using STMS Markers

Twenty nine selected STMS markers which produced scorable, reproducible and well-resolved banding patterns were used for genetic diversity studies (Supplementary Table 2). The number of amplified DNA fragments by each primer ranged from 2 (NKS 53) to 31 (NKS 6, NKS 12, NKS 16 and NKS 21) with an average of 17.68 fragments per primer (Fig. 1, Table 2). Usually, high ploidy plants

Table 1 — Morphological diversity in agronomic traits of *S. spontaneum* L. clones from different areas of Arunachal Pradesh, India

Clone nos.	Plant height (cm)	Stalk diameter (cm)	Single cane weight (kg)	Internode number (no)	Internode length (cm)
IND00-997	241.7	1.33	0.233	17.9	19.1
IND00-998	233.3	1.26	0.262	15.7	30.3
IND00-1001	153.3	1.37	0.297	11.7	14.9
IND00-1002	196.7	1.29	0.305	11.7	21.8
IND00-1004	235.0	1.35	0.440	12.3	16.5
IND00-1011	246.7	0.85	0.072	15.0	20.7
IND00-1012	181.7	1.66	0.200	20.3	15.6
IND00-1015	215.0	0.81	0.196	19.3	14.2
IND00-1018	251.7	1.70	0.470	11.0	21.5
IND00-1019	216.7	1.28	0.402	12.3	16.8
IND00-1020	293.3	1.34	0.403	25.3	15.8
IND00-1022	201.7	1.72	0.538	12.7	15.3
IND00-1024	225.0	1.52	0.488	13.0	14.2
IND00-1025	228.3	1.52	0.460	14.3	16.1
IND00-1026	180.0	1.10	0.235	13.7	14.9
IND00-1027	153.3	1.44	0.292	10.0	20.3
IND00-1030	193.3	1.09	0.180	14.7	17.2
IND00-1032	125.0	1.07	0.140	8.7	11.8
IND00-1034	216.7	1.38	0.355	15.7	22.4
IND00-1035	206.7	0.96	0.115	15.7	15.2
IND00-1036	176.7	1.24	0.310	18.3	12.5
IND00-1037	218.3	0.95	0.148	18.0	9.4
IND00-1038	231.7	1.37	0.337	17.0	22.8
IND00-1039	218.3	1.26	0.293	14.3	24.2
IND00-1040	241.7	1.22	0.313	16.0	21.2
IND00-1041	235.0	1.09	0.300	14.7	22.1
IND00-1042	285.0	1.47	0.413	15.7	31.9
IND00-1043	256.7	1.35	0.365	19.0	18.1
IND00-1044	221.7	1.06	0.212	17.7	15.7
IND00-1045	251.7	0.67	0.128	13.3	25.3
IND00-1046	138.3	0.65	0.062	19.3	11.1
IND00-1047	90.0	0.36	0.018	13.7	4.5
IND00-1048	110.0	0.93	0.091	9.7	9.9
IND00-1051	205.0	1.96	0.378	13.0	21.6
IND00-1054	215.0	1.38	0.286	13.3	18.1
IND00-1056	220.0	1.37	0.300	13.7	24.2
IND00-1057	236.7	1.45	0.395	16.7	22.9
IND00-1058	236.7	1.05	0.292	16.7	19.6
IND00-1059	261.7	1.23	0.363	20.7	22.2
IND00-1060	236.7	0.99	0.170	17.0	17.1
IND00-1061	253.3	1.29	0.294	15.7	21.4
IND00-1062	288.3	1.47	0.508	16.7	23.2
IND00-1063	183.7	1.51	0.436	11.3	18.9
IND00-1064	105.0	0.54	0.035	11.3	12.1
IND00-1067	176.7	1.25	0.317	11.7	15.1
IND00-1068	288.3	0.74	0.111	15.7	16.3
IND00-1069	208.3	1.28	0.421	15.7	12.4
IND00-1070	246.7	0.63	0.102	13.7	18.1
IND00-1071	268.3	0.60	0.105	13.3	16.9
Mean	214.3	1.19	0.277	15.1	18.2
General Mean	214.29	1.19	0.28	15.05	18.16
MST	6693.51** (<.0001)	0.32** (<.0001)	0.055** (<.0001)	30.99** (<.0001)	80.77** (<.0001)
MSe	1747.36	0.0056	0.0008	1.86	1.64
General Mean	214.29	1.19	0.28	15.05	18.16
Minimum	89.6	0.36	0.018	8.7	4.5
Maximum	293.3	1.86	0.508	25.3	31.9

(Contd.)

Table 1 — Morphological diversity in agronomic traits of *S. spontaneum* L. clones from different areas of Arunachal Pradesh, India (Contd.)

Clone nos.	Plant height (cm)	Stalk diameter (cm)	Single cane weight (kg)	Internode number (no)	Internode length (cm)
LSD at 5%	67.75	0.12	0.046	2.21	2.08
CV(%)	19.51	6.27	10.23	9.06	7.06
PCV	27.19	28.21	48.97	22.60	29.15
GCV	18.95	27.51	47.92	20.70	28.28
ECV	19.51	6.29	10.10	9.06	7.06

** Highly significant, Values in the parenthesis indicates p-value *(0.05)*

Table 2 — Genetic diversity among 49 *S. spontaneum* accessions revealed by STMS primers

Marker name	Number of bands amplified	Number of polymorphic bands	Percentage of polymorphic bands (%)	Size range of bands		Unique fragment amplified
				Min	Max	
NKS1	22	18	81.82	216	1090	IND00-998
NKS2	24	19	79.17	180	915	-
NKS3	14	13	92.86	183	977	IND00-1024, IND00-1069
NKS6	26	24	92.31	147	1060	-
NKS7	23	19	82.61	174	490	-
NKS8	9	8	88.89	194	527	IND00-1059
NKS9	25	20	80.00	148	1613	IND00-1057
NKS12	26	21	80.77	147	2080	-
NKS16	26	19	73.08	157	944	IND00-1061
NKS21	26	22	84.62	165	693	IND00-1046
NKS24	11	11	100.00	136	993	-
NKS25	17	13	76.47	153	884	IND00-1068
NKS28	25	17	68.00	119	696	-
NKS29	12	9	75.00	145	425	IND00-1062
NKS30	16	11	68.75	129	696	IND00-1062
NKS31	13	8	61.54	101	582	-
NKS38	13	9	69.23	128	590	IND00-1036
NKS42	16	12	75.00	182	1375	-
NKS45	19	18	94.74	104	772	IND00-1039
NKS46	4	3	75.00	116	554	IND00-1047
NKS48	17	15	88.24	103	554	IND00-1047
NKS49	23	22	95.65	100	1035	-
NKS50	19	18	94.74	112	806	IND00-1041
NKS51	23	19	82.61	130	645	IND00-1041
NKS52	3	3	100.00	110	540	IND00-1043
NKS53	2	2	100.00	102	339	-
NKS56	14	13	92.86	129	407	-
NKS57	13	10	76.92	101	482	IND00-1036
NKS61	13	12	92.31	128	356	-

with high heterozygosity show multiple bands per marker per clone. The high number of DNA fragments obtained in *S. spontaneum* is possibly due to the ploidy level and as well as hetrozygosity. High number of amplicons were recorded in *Saccharum* interspecific and intergeneric hybrids using STMS markers[24] than in commercial cultivars, this could be possibly due to the high similarity between the commercial varieties. The chromosome number of *S. spontaneum* accessions used in this study were of 2n=60 (IND 00-1064) and 2n=64 (all other accessions)[25] indicating that their ploidy level is high. The size of the amplified fragment varied from 100 to 2080 bp, but most of the bands obtained in the range of 300-800 bp. The total number of bands scored was 495, of which 408 were polymorphic (82.40%) with an average of 14.06 per primer (Table 2). The polymorphism ranged from 61.54 (NKS 31) to 100% (NKS 24, NKS 52 and NKS 53), revealing a high degree of polymorphism among these clones. The different group of markers revealed a high degree of polymorphism in *S. spontaneum*[26-33].

Germplasm Accession Specific Markers

Out of 29 primers tested, the combined profile of polymorphic bands provided *S. spontaneum* accession specific markers that could distinguish from each other (Table 2). Unique markers were

identified either by their presence or absence in a particular accession. Unique DNA fragment amplified by some of the markers will be useful for identification/differentiation of clones and germplasm verification (Table 2). Different group of markers including STMS have been reported to be successfully used for identification or differentiation of individuals in *Saccharum* species clones and *Saccharum* hybrids[34-37]. These unique markers could also be used for identification of specific varieties in a mislabeled field and selfed progeny in conventional sugarcane breeding[38] and marker-assisted selection in varietal development programme[39]. STMS markers have been successfully used to identify the related species, landraces, new plant lines and varieties of many other crops of Poaceae family[40-41]. These clone specific markers identified in the study would also be helpful in monitoring the transmission of genomic regions introgressed from specific accession of *S. spontaneum* clones in the progeny generations.

Similarity Coefficient Analysis

Pairwise genetic similarity coefficient among the 49 *S. spontaneum* accessions ranged from 0.42 (IND 00-997 with IND 00-1048, IND 00-998 with IND 00-1048, IND 00-998 with IND 00-1051) to 0.78 (clones IND 00-1024 and IND 00-1025) with an average of 0.58. The overall diversity among the accessions was found to be high, which is much higher than the difference obtained with commercial sugarcane varieties based on STMS markers[42-43]. Lower variation in commercial varieties could be attributed to the utilization of only few germplasm accessions in varietal development process and also due to varital improvement is concentrated on few important traits. Lu *et al*[44] reported relatively higher genetic diversity of 69% in *S. spontaneum* from five different countries based on RFLP markers. However the present estimate of genetic diversity is higher than that has been reported for the Chinese *S. Spontaneum* (Hui *et al*. 2001, Fan *et al*. 2001). Arunachal Pradesh clones showed the maximum intragroup diversity consistent with its phenotypic diversity as represented by tall, medium and dwarf clones. The relatively high difference in the Arunachal Pradesh group is expected since this is the most variable group among the Indian *S. spontaneum* in terms of morphological variation[45]. Manechini *et al*[46] reported that based on microsatellite marker analysis highest putative exclusive alleles (39%) of the basic germplasm group with *S. spontaneum* were not found in the Brazilian cultivars.

Clustering Analysis

Clusters was constructed using UPGMA, based on the Jaccard's similarity coefficients to estimate the genetic relationships among the 49 *S. Spontaneum* accessions (Fig. 2). STMS markers were found to be

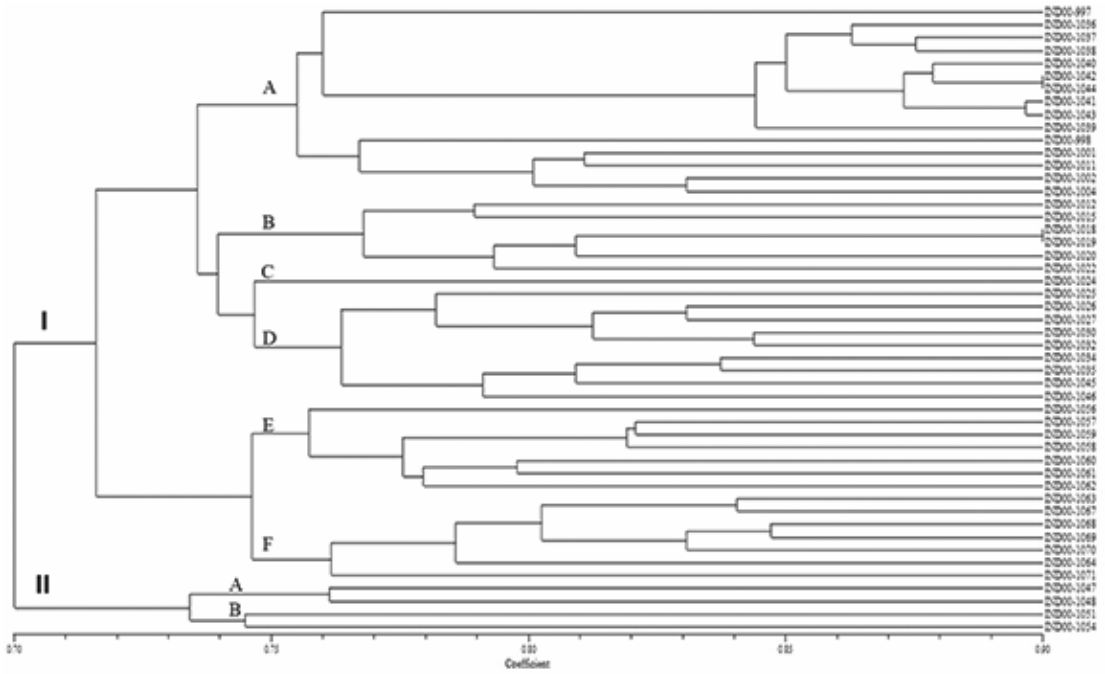

Fig. 2 — Dendrogram of 49 accessions of *S. spontaneum* L. collected from Arunachal Pradesh.

adequate for assessing genetic diversity. All these accessions distinctly clustered into two groups, which shared a common node at a similarity coefficient of 0.70. Cluster I and II consisted of 45 and 4 accessions, respectively. The cluster I was further divided into two sub-clusters. One sub-cluster consisted of four sub-clusters (A, B, C, D) and another consisted of two sub-clusters (E and F). Of *S. spontaneum* accessions, 15 were in sub-cluster A, six in B, one in C, nine in D, seven in E and F each, which shared a common node at similarity coefficient of 0.75. The result shows that most of the accessions collected from the same area/habitats or adjacent area gathered in the same cluster. Geographical factors play an important role in the pattern of accessions genetic structure[47]. The accessions IND 00-1036 - IND 00-1044 from high altitude regions (> 1000 m, supplementary Table 1) were formed a sub-cluster A. The IND 00-1012 - IND 00-1022 from neighboring regions classified into sub-cluster B and a clone IND 00-1024 classified into sub-cluster C. The clones IND 00-1025, IND 00-1026, IND 00-1027, IND 00-1030, IND 00-1032, IND 00-1034, IND 00-1035, IND 00-1045 and IND 00-1046 from Paya-Tiding and Tiding regions classified into sub-cluster D.

The accessions IND 00-1056, IND 00-1057, IND 00-1058, IND 00-1059, IND 00-1060, IND 00-1061 and 00-1062 from Kherum, Medo, Kamlang and Wakro regions were classified into sub-cluster E, and IND 00-1063, IND 00-1064, IND 00-1067, IND 00-1068, IND 00-1069, IND 00-1070 and IND 00-1071 from Pokri, Mahadevpur and Derakgade Namsi were classified into sub-cluster F. One accession each from Khamba (IND 00-1012), Hawa-Camp (IND 00-1045) Namsai (IND 00-1046) grouped in different cluster than clones from same region suggesting their distinct genetic identity. The clones collected from high altitude ranging from 1000-1640 m under severely cold and windy conditions. Evaluation of these clones under low-temperature conditions revealed tolerant to cold stress conditions[48] and thus these could be an important source of genes for improving cold tolerance[49-50]. The cluster II further subdivided into two subgroups, that is two accessions were in sub-cluster A and B each. The accessions from Namsai (IND 00-1047) and Piyong (IND 00-1048) grouped into sub-cluster A, and accessions IND 00-1051 (Innoa) and IND 00-1054 (Changlang) were classified into sub-cluster B. These areas closely located, and accessions belong to cluster II were from fertile plains. Geographical distribution is in agreement with earlier studies (Mary *et al.* 2006, Fan *et al.* 2013). The genetic relationship between the accessions of *S. spontaneum* was related to geographical distribution and ecological conditions. All of these studies indicated that geographic factors play an essential role in the pattern of the genetic structure within *S. spontaneum*.

Population Structure

The two optimum number of clusters were decided using the value of ΔK distribution (Fig. 3, Supplementary Table 3). From the total clones investigated 46 clones had more than 60% membership in the given cluster. Three clones IND 00-1001, IND 00-1012 and IND 00-997 share similar membership coefficients in both the clusters and considered as admixtures. The alpha value 0.112 indicating most of the individuals are pure and only few admixtures. The mean F_{ST} values with 0.58 (cluster 1) and 0.09 (cluster 2) confirmed the existence of differences among clusters. It is generally accepted that F_{ST} values under 0.05 indicate negligible genetic differentiation while those over 0.25 indicate a great deal of genetic differentiation[51]. The average distances (expected heterozygosity) among individuals in same cluster were 0.13 (cluster 1) and 0.27 (cluster 2). High heterozygosity means lots of genetic variability, we found moderate to low variability in the accessions studied. The present study of 49 *S. spontaneum* from Lohit and Changlang regions of Arunachal Pradesh, North Eastern India based on morphological traits (plant height, stalk diameter, single cane weight, internode number and internode length) and genetic analysis has revealed that there is obvious high variation. These highly diverse *S. spontaneum* accessions could be a potential source for exploitation in sugarcane improvement programme for high biomass and cold tolerance. The present study revealed variations existing among the accssions collected from Lohit and Changlang regions of Arunachal Pradesh, which would definitely helpful in planning and utilizing them in sugarcane improvement programme.

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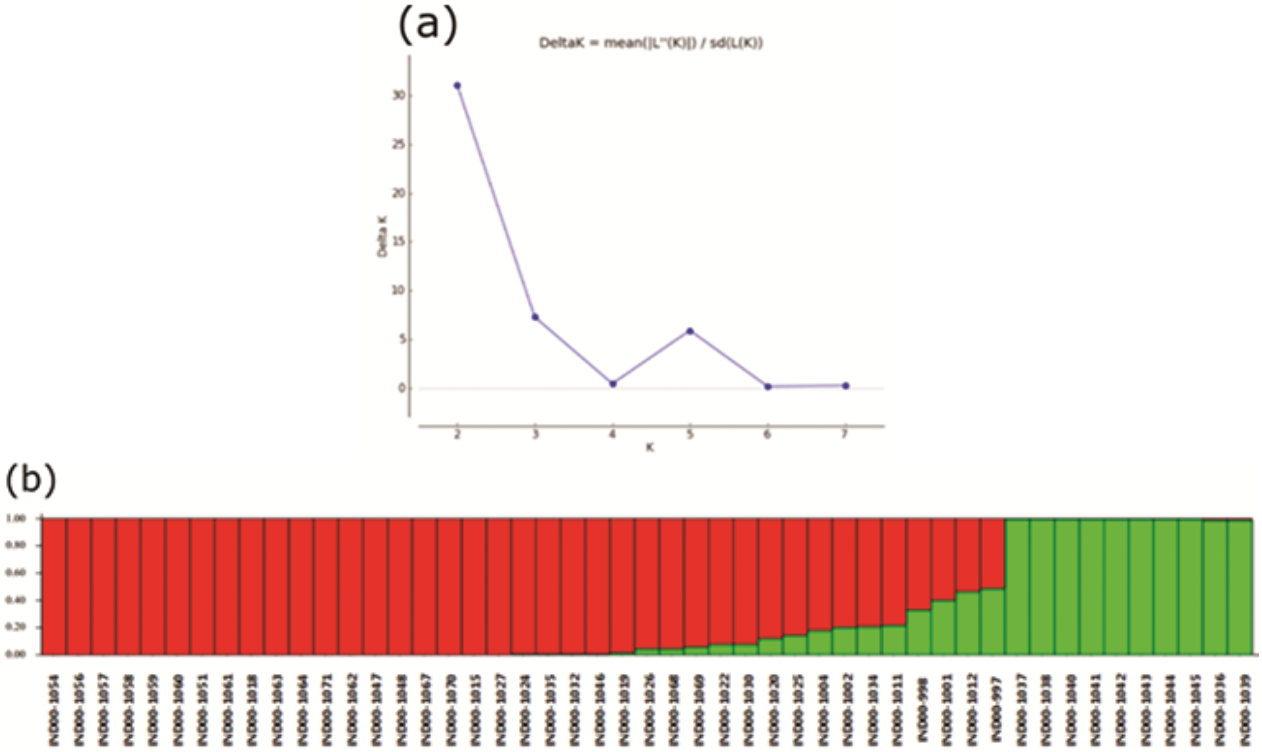

Fig. 3 — (a) Estimates of the rate of the slope of the log probability curve (ΔK) plotted against K. (b) Population structure of accessions based on Bayesian assignment probabilities.

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