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RESEARCH ARTICLE

Expedition Collection, Characterization and Diversity Analysis of the New Wild Sugarcane Germplasm from Manipur

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Saccharum spontaneum, a wild relative of sugarcane had contributed significantly for introgressing wider adaptability, high tillering, resistance to biotic and abiotic stresses in the commercial sugarcane varieties. Manipur state representing tropical to sub-alpine climatic condition was explored and 61 *S. spontaneum*, 4 *S. officinarum*, 1 *Erianthus fulvus* and 1 *Narenga fallax* accessions were collected. *S. spontaneum* had wider distribution and were found in river bank to filed bunds, isolated clumps to large population. High variability was observed for many quantitative characters including plant height, tillering, stalk diameter and internode length. Clustering analysis separated the collection into 6 major clusters. While *Erianthus fulvus* and *S. officinarum* formed individual clusters, the *S. spontaneum* accessions were separated into 4 clusters mostly based on biomass potential, plant height and morphotypes. Genetic diversity and utilization of these clones in improving low temperature tolerance, biomass improvement and widening genetic base of sugarcane varieties are discussed.

Key Words: Clusters, *Erianthus*, Germplasm collection, *Narenga*, North East India, *Saccharum spontaneum*

Introduction

Plant genetic resources play a major role in crop improvement programmes not only for improving yield and quality but also for imparting resistance to pests and diseases and tolerance to abiotic stresses. Sugarcane is one of the classical examples wherein wild species have significantly contributed for the development of new varieties with high cane yield and wider adaptability (Panje, 1972; Giamalva *et al.,* 1984; Berding and Roach, 1987, Govindaraj *et al.,* 2005). *Saccharum officinarum* (L.) was the cultivated cane in tropical countries including India and *Saccharum barberi* (Jeswiet) was the main sugarcane species under cultivation in northern India before the introduction of man-made sugarcane hybrids. Area under *S. officinarum* varieties could not be expanded in India because they essentially required well managed condition due to lesser adaptability to varied agro-climatic conditions and susceptibility to biotic and abiotic stresses although they had thick and tall canes and high juice sucrose content. However major breakthrough in sugarcane varietal development was achieved in 1918 when the first interspecific hybrid (ISH) between *S. officinarum* (Vellai: 2n=80) and *S. spontaneum* (Coimbatore local: 2n=64) was made and first-generation interspecific hybrid Co 205 with wider adaptability was released for cultivation in subtropical India. This was the first interspecific hybrid involving a wild species which became commercially successful without resorting to back crosses for eliminating the undesirable genes from wild species which was mainly attributed to the 2n+n transmission of gametes ensuring the full complement of *S. officinarum* in the F_1 hybrid.

After the successful introduction of Co 205 for commercial cultivation, several ISH hybrids and their backcrosses with either *S. officinarum* or near commercial 'Co' canes were developed for further improving cane yield, juice quality and wider adaptability, a process called nobilization. Introduction of array of varieties developed through nobilization process occupied sizable areas in both tropical and subtropical India thus totally replacing the traditional varieties. The success of these varieties was primarily due to high tillering, wider adaptability to various agro-climatic conditions and resistance to biotic and abiotic stresses contributed by *S. spontaneum* (D'Hont *et al.,* 1996; Terajima *et al.,* 2007; Wang *et al.,* 2008; da-Silva 2017; Govindaraj and Mahadevaswamy 2021). Other species /genera of *Saccharum* complex viz., *S. robustum*, *S. barberi* and *Erianthus* were also

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introduced later in breeding programmes but their contribution to varietal development was relatively very less (Govindaraj *et al.,* 2012). Genome analysis of the modern sugarcane varieties also indicated around 15% of the sugarcane genome was contributed by *S. spontaneum* genome (D'Hont *et al.,* 1995). Red rot, a major disease is practically managed with the introgression of *S. spontaneum* as vertical resistance was positively and significantly correlated with the number of *S. spontaneum* chromosomes present in their genome (Natarajan *et al.,* 2001). At present it is also used for transferring cold tolerance (Hale *et al*., 2014), high biomass energycane development (Govindaraj 2017; Govindaraj, 2020), winter hardiness and winter ratoonability and improving vegetation and restoration programmes (Pandey *et al.,* 2015). As the ISH or *S. spontaneum* introgressed clones showed higher fibre, lignocellulose or higher biomass (Govindaraj *et al*., 2014; Cosentino *et al.,* 2015; Terajima *et al.,* 2007; Govindaraj and Nair, 2014), there was renewed interest among sugarcane breeders in utilizing *S. spontaneum* and *Erianthus arundinaceus* in the development of energycanes and multipurpose sugarcane varieties (Govindaraj *et al.,* 2012).

For the successful introgression programme, new germplasm carrying new genes /allelic variation for agronomic and stress tolerance traits should be included. Hence, several explorations were conducted by the ICAR-Sugarcane Breeding Institute, Coimbatore for the collection of new *S. spontaneum* and other species of *Saccharum* complex in India and other countries (Nair *et al.,* 1991; Nair *et al.,* 1993; Nair *et al.,* 2006; Abraham *et al.,* 2008; Nair and Sekaran, 2009; Govindaraj *et al.,* 2014; Govindaraj *et al.,* 2016; Karthigeyan *et al.,* 2020; Govindaraj and Mahadevaswamy, 2021; Govindaraj and Mahadevaswamy, 2021a). However, a part of North Eastern Region of India especially the Manipur State which still conserves rich source of biodiversity due to less human interference was not explored so far, hence an expedition was carried out in the state to collect new variability found in *Saccharum* complex.

Materials and Methods

Manipur state of India was explored for the collection of wild sugarcane germplasm during 2011 by a team of scientists consisting of Dr. VA Amalaraj and Dr. P Govindaraj, Principal Scientists, ICAR-Sugarcane Breeding Institute, Coimbatore. The state had two distinct physiography – the hills and valley and flat plain and representative locations of these geography were covered during the expedition. The expedition route was prepared and 10 major districts viz., West Imphal, Ukhrul, Thoubal, Tamenglong, Senapati, Kangpoki, East Imphal, Churachandpur, Chandal and Bishnupur were explored (Fig. 1). A minimum of 5km distance was maintained between the adjacent collections to avoid duplications. Visits were made to home gardens where cultivated *S. officinarum* were grown either for chewing or pooja purposes. While *S. spontaneum* and *E. fulvus* accessions were collected as clumps with germinating underground stolon, *S. officinarum* and *Narenga fallax* were collected as stem cuttings with active buds. Data on the latitude, longitude and altitude of the collection site were recorded. Quantitative traits such as plant height (cm), leaf length (cm), leaf breadth (cm), stalk diameter (cm), arrow length (cm), peduncle length and intermodal length were recorded *in situ* (Table 1) in three replicates for further analysis. Variability statistics such as range, mean and SD were worked out to understand the variation among the collections (Table 2). Genetic diversity was estimated through hierarchical clustering with the morphological data recorded on new accessions. Jaccard's coefficient of similarity was calculated (Rohlf, 2002) by the following equation:

$$GSij = Nij /[Ni + Nj+ Nij]$$

where

Nij is the total number of attributes common to both accessions i and j, and Ni and Nj are the number of attributes only present in accession i and j, respectively.

Fig. 1. Exploration route for the collection of wild sugarcane germplasm covering different districts of Manipur

Table 1. Location, species status and quantitative traits recorded at the collection site of the new germplasm collected from Manipur

S. No	Accession No	Place of collection	Species status	Plant height (cm)	Leaf length (cm)	Leaf width (cm)	Arrow length (cm)	Peduncle length (cm)	Stalk diameter (cm)	Internode length (cm)
1	IND 11-1657	Ukhrul	EF	140	100	1.7	34	42	–	–
2	IND 11-1680	Chandal	NF	154	101	0.7	88	77	0.5	7.1
3	IND 11-1671	Thoubal	SO	400	145	5.0	–	–	3.7	18.0
4	IND 11-1672	Thoubal	SO	450	166	9.0	–	–	5.4	19.0
5	IND 11-1684	Thoubal	SO	300	140	6.0	–	–	4.1	14.0
6	IND 11-1703	West Imphal	SO	320	160	5.0	–	–	3.8	13.0
7	IND 11-1637	Bisnupur	SS	130	79	0.5	30	40	0.5	10.5
8	IND 11-1638	Bisnupur	SS	400	102	0.7	50	43	0.8	14.0
9	IND 11-1639	Churachandpur	SS	400	132	0.5	55	32	0.4	13.0
10	IND 11-1640	Churachandpur	SS	275	112	0.4	45	61	0.6	16.0
11	IND 11-1641	Churachandpur	SS	310	93	0.6	60	72	1.8	14.0
12	IND 11-1642	Churachandpur	SS	310	124	0.4	52	55	0.8	13.0
13	IND 11-1643	Bisnupur	SS	600	119	3.8	60	60	2.3	25.0
14	IND 11-1644	Bisnupur	SS	320	114	3.8	55	53	2.2	16.0
15	IND 11-1645	Bisnupur	SS	100	57	0.6	27	42	0.5	13.0
16	IND 11-1646	Bisnupur	SS	200	93	0.6	34	31	0.5	8.0
17	IND 11-1647	West Imphal	SS	190	112	1.0	32	30	0.5	14.0
18	IND 11-1648	Senapati	SS	185	103	0.7	50	64	0.5	21.0
19	IND 11-1649	Senapati	SS	200	120	1.5	49	61	0.7	11.0
20	IND 11-1650	Senapati	SS	215	129	2.6	57	58	1.1	12.0
21	IND 11-1651	Tamenglong	SS	260	110	0.8	50	55	0.5	17.0
22	IND 11-1652	Tamenglong	SS	250	120	0.3	40	44	0.5	9.0
23	IND 11-1653	Tamenglong	SS	210	125	0.7	63	53	0.9	8.0
24	IND 11-1654	Tamenglong	SS	160	65	0.3	36	60	0.4	7.5
25	IND 11-1655	Tamenglong	SS	200	118	0.5	25	28	0.7	9.5
26	IND 11-1656	West Imphal	SS	180	93	0.4	22	34	0.9	24.0
27	IND 11-1658	Ukhrul	SS	180	95	1.3	65	59	0.8	13.0
28	IND 11-1659	Ukhrul	SS	170	82	0.6	40	55	0.7	11.0
29	IND 11-1660	Ukhrul	SS	175	71	0.8	27	42	0.7	14.0
30	IND 11-1661	Ukhrul	SS	210	81	0.4	42	54	0.6	13.0
31	IND 11-1662	Ukhrul	SS	130	73	0.7	38	39	0.9	14.0
32	IND 11-1663	Ukhrul	SS	120	62	0.4	30	36	0.5	12.0
33	IND 11-1664	Ukhrul	SS	122	104	0.2	45	59	0.5	10.0
34	IND 11-1665	Ukhrul	SS	215	101	0.2	37	55	0.5	5.6
35	IND 11-1666	Ukhrul	SS	120	79	0.2	33	53	0.4	8.0
36	IND 11-1667	Senapati	SS	124	77	0.2	79	74	0.6	14.0
37	IND 11-1668	Senapati	SS	380	95	1.2	25	43	0.6	8.0
38	IND 11-1669	Thoubal	SS	140	120	1.1	30	48	0.9	25.0
39	IND 11-1670	Thoubal	SS	180	76	0.3	40	53	0.7	11.0
40	IND 11-1673	Thoubal	SS	260	94	0.6	59	77	0.6	7.5
41	IND 11-1674	Thoubal	SS	130	131	1.0	50	62	1.1	7.5
42	IND 11-1675	Thoubal	SS	210	145	2.0	40	65	0.8	8.5
43	IND 11-1676	Thoubal	SS	480	115	3.7	42	39	2.1	30.0
44	IND 11-1677	Thoubal	SS	190	105	0.5	57	65	0.5	6.5
45	IND 11-1678	Chandal	SS	200	106	0.3	40	52	0.6	7.5
46	IND 11-1679	Chandal	SS	167	126	0.3	41	40	0.5	13.0
47	IND 11-1681	Chandal	SS	200	101	0.6	57	62	0.6	8.0
48	IND 11-1682	Chandal	SS	198	48	0.2	29	48	0.6	9.5
49	IND 11-1683	Chandal	SS	380	109	1.0	59	72	1.1	28.0
50	IND 11-1685	Thoubal	SS	350	93	1.9	56	64	1.4	12.5
51	IND 11-1686	Thoubal	SS	270	144	2.0	46	44	0.8	11.9
52	IND 11-1687	Thoubal	SS	209	116	0.9	46	54	0.7	7.0

S. No	Accession No	Place of collection	Species status	Plant height (cm)	Leaf length (cm)	Leaf width (cm)	Arrow length (cm)	Peduncle length (cm)	Stalk diameter (cm)	Internode length (cm)
53	IND 11-1688	East Imphal	SS	105	130	0.6	55	50	0.8	13.0
54	IND 11-1689	East Imphal	SS	200	124	0.2	55	67	0.5	10.0
55	IND 11-1690	East Imphal	SS	160	114	0.5	30	42	0.5	8.5
56	IND 11-1691	East Imphal	SS	290	132	1.3	48	61	1.9	32.0
57	IND 11-1692	East Imphal	SS	80	24	0.1	27	33	0.4	4.5
58	IND 11-1693	East Imphal	SS	170	96	0.2	39	55	0.5	12.5
59	IND 11-1694	West Imphal	SS	380	126	0.3	54	60	0.9	21.5
60	IND 11-1695	West Imphal	SS	220	95	0.3	50	46	0.6	14.0
61	IND 11-1696	West Imphal	SS	200	121	0.4	49	52	0.5	20.5
62	IND 11-1697	West Imphal	SS	120	59	0.4	39	51	0.2	8.0
63	IND 11-1698	West Imphal	SS	150	68	0.3	21	35	0.1	14.0
64	IND 11-1699	West Imphal	SS	260	89	0.2	48	57	0.5	10.0
65	IND 11-1700	Kangpokpi	SS	210	126	0.9	20	24	0.9	13.5
66	IND 11-1701	Kangpokpi	SS	230	121	2.0	47	39	0.8	12.0
67	IND 11-1702	West Imphal	SS	130	103	0.2	31	39	0.5	5.5

EF: *Erianthus fulvus* NF: *Narenga fallax* SO: *S. officinarum* SS: *S. spontaneum*

Table 2. Descriptive statistics on the seven quantitative traits among the new *S. spontaneum* accessions

	Plant height (cm)	Leaf length (cm)	Leaf width (cm)	Arrow length (cm)	Peduncle length (cm)	Stalk diameter (cm)	Internode length (cm)
S. officinarum							
Mean	367.50	152.75	6.25	–	–	4.25	16.00
Maximum	450.00	166.00	9.00	–	–	5.40	19.00
Minimum	300.00	140.00	5.00	–	–	3.70	13.00
SD	69.94	12.26	1.89	–	–	0.79	2.94
S. spontaneum							
Mean	221.48	101.59	0.84	43.57	50.84	0.76	12.97
Maximum	600.00	145.00	3.80	79.00	77.00	2.30	32.00
Minimum	80.00	24.00	0.10	20.00	24.00	0.10	4.50
SD	98.43	24.82	0.86	12.63	12.30	0.45	6.03
Erianthus fulvus							
Mean	140	100	1.7	34	42	–	–
Narenga fallax							
Mean	154	101	0.7	88	77	0.5	7.1

Dendrogram based on similarity coefficients were generated through NTSYS-PC by using unweighted pair group method with arithmetic averages (UPGMA).

Results and Discussion

Distribution of Saccharum species complex in Manipur

Manipur is blessed with an amazing variety of flora and fauna and 67% of the geographical area is hill tract covered forests. Depending on the altitude of hill ranges, the climatic condition varies from tropical to sub-alpine. The wet forests and the pine forests occur between 900-2700 m above mean sea level (AMSL) and they together sustain a host of rare and endemic plants. Exploration was conducted in ten districts of Manipur namely West Imphal, Ukhrul, Thoubal, Tamenglong, Senapati, Kangpoki, East Imphal, Churachandpur, Chandal and Bishnupur and 67 new accessions were collected which included 61 *S. spontaneum*, 4 *S. officinarum* (Thoubal and West Imphal districts), 1 *Erianthus fulvus* (Ukhrul district) and 1 *Narenga fallax* (Chandal district) accessions. Distribution of species was not even across the state while *S. spontaneum* was found abundant and could be collected from all the districts, *E. fulvus*, *N. fallax* and *S officinarum* were restricted to certain areas.

Earlier expeditions in different states of India also indicated the wider adaptability of *S. spontaneum* under

drought (Govindaraj *et al.,* 2014), water logging and salinity (Govindaraj and Mahadevaswamy, 2021), low temperature (Govindaraj and Mahadevaswamy, 2021a) and hilly slopes (Govindaraj *et al.,* 2016). During the exploration various forms of *S. spontaneum* could be collected from river banks (IND 11-1639, IND 11-1651, IND 11-1653, IND 11-1654, IND 11-1665, IND 11-1682, IND 11-1702), irrigation channels (IND 11-1670, IND 11-1681, IND 11-1686), bunds of paddy fields (IND 11-1646, IND 11-1659, IND 11-1692), small water ponds (IND 11-1663, IND 11-1656, IND 11-1677), hilly slopes (IND 11-1648, IND 11-1655, IND 11-1664, IND 11-1688), rocky crevices (IND 11-1640) and forest areas (IND 11-1666). Distribution of the species in different geo-agro-climatic conditions indicated its wider adaptability as reported earlier by Govindaraj and Mahadevaswamy (2021) and Govindaraj *et al*. (2014). A possible reason attributed for the wider distribution of *S. spontaneum* could be its mode of reproduction. The underground rhizomes are carried away by rivers, river fed canals, irrigation channels, ponds and the true seed called, fluff dispersed through wind and scattered over dry land, waste lands, hilly slopes, rocky crevices and forest areas can germinate under favourable conditions and establishes in different ecological niches (Govindaraj and Mahadevaswamy, 2021a).

During the exploration we could found establishment of *S. spontaneum* as individual clump (IND 11-1652, IND 11-1659, IND 11-1670, IND 11-1692), small groups (IND 11-1650, IND 11-1693, IND 11-1694) and very large populations (IND 11-1651, IND 11-1653, IND 11-1681, IND 11-1682, IND 11-1695) (Fig. 2). All the accessions were in flowering stage during our exploration (September and October 2011) and three accessions namely IND 11-1646, IND 11-1658 and IND 11-1686 were early in flowering and the fluff started shedding at the time of collection.

Four *S. officinarum* (IND 11-1671, IND 11-1672, IND 11-1684, IND 11-1703) were collected from the home gardens in Thoubal (3) and West Imphal (1) districts. A splendid accession (IND 11-1672) was collected from Thoubal district with 4.5 m cane height and 5.4 cm cane diameter (Fig. 4). The new accessions were very tall with high biomass and more juice volume hence can be used in the breeding programme after induction of flowering for not only incorporating these useful traits but also broadening the genetic base of the new sugarcane varieties (Govindaraj *et al.,* 2021).

Fig. 2. Large population of *S. spontaneum* (IND 11-1682) spotted in the river bank at Chandel district

Variability in the new collections

High variation was found among *S. spontaneum* collections for tillering ability. A high proportion of accessions collected were high tillering (IND 11-1639, IND 11-1651, IND 11-1673) while very few were low in tillering (IND 11-1640, IND 11-1649, IND 11-1699). The high tillering accessions could be utilised in the breeding programmes after characterization to improve the genetic base of the modern sugarcane varieties (Sindhu *et al.,* 2011; Govindaraj *et al.,* 2011).

The accession, IND 11-1637 was collected from fallow land in West Imphal district. This was a short plant with short leaf blade (79 cm), leaf width (0.5 cm) and thin stalks (0.5 cm) (Table 1). The tallest accession (IND 11-1643 – 600 cm) was collected from a small pond fed with perennial water source (Fig. 3). It had broader leaves (3.8 cm), thicker stalk (2.3 cm diameter) and longer internodes (25 cm). IND 11-1676 (480 cm), IND 11-1638 (400 cm), IND 11-1639 (400 cm), IND 11-1668 (380 cm) and IND 11-1683 (380 cm) were the next best accessions for plant height and all these accessions could be potential parents for breeding for biomass improvement. Very short accession IND 11-1692 (80 cm) was spotted in a paddy field bund. It had very narrow leaf (0.1 cm) with very short arrow (27 cm) and peduncle (33 cm). Very large population (IND 11-1638) was spotted on the river bank of Khuga river while another large population (IND 11-1639) was found along irrigation channels in Bhisnapur and Chrurachandpur, respectively. IND 11-1665 and IND 11-1682 were the two representative samples collected from the large population of *S. spontaneum* found in

Fig. 3. IND 11-1643 – the tallest collection (600 cm) of *S. spontaneum* from a small pond with perennial water source in Bisnapur district

Fig. 4. Thick (5.4 cm) and tall (450 cm) *S. officinarum* (IND 11-1672) collected from a home garden in Thoubal district

Ukhrul and Chandal districts, respectively and both were medium in height and with internode length of 9.5 cm each. A heavy tillering type IND 11-1651 with more than 100 tillers per clump was collected along the Barak river bank in Tamenglong district which receives an average of 3330 mm annual rainfall. The longest internodes were observed in IND 11-1691 (32 cm) and IND 11-1676 (30 cm) and the shortest internode was noticed in IND 11-1692 (4.5 cm). Long internodes are the indication of faster growth which is one of the important selection criteria in sugarcane varietal development programme (Lingle and Tew, 2008). While IND 11-1667 (79 cm) and IND 11-1673 (77 cm) recorded the longest arrow and peduncle length respectively, IND 11-1700 showed the shortest arrow (20 cm) and peduncle (24 cm) length. High variability for morphological traits (Govindaraj *et al.,* 2014; Govindaraj *et al.,* 2016; Karthigeyan *et al.,* 2020) and molecular markers (Alwala *et al.,* 2006; Govindaraj *et al.,* 2011; Sindhu *et al.,* 2011; Govindaraj *et al.,* 2021) among the *S. spontaneum* accessions were reported earlier. The new collections have unique germplasm with tall growing and high biomass *S. officinarum* and high tillering and long internodes *S. spontaneum* and will add more variability and diversity to the world collection of sugarcane germplasm maintained at ICAR - Sugarcane Breeding Institute, India.

Among the ten districts, two districts namely Kangpokpi and part of Ukhrul were situated in the high attitude hilly areas and other districts were in the plains. Ten accessions were collected from Tamenglong followed by 9 each in West Imphal and Ukhrul districts. IND 11-1700 and IND 11-1701 were collected from high altitude Kangpokpi district (Table 3). Both the accessions were robust in stature with high mean values for leaf length (123.5 cm), leaf width (1.45 cm) and stalk diameter (0.85 cm) but reduced arrow (33.5 cm) and peduncle (31.5 cm) lengths (Table 4). Six accessions namely IND 11-1658 (1876 m AMSL), IND 11-1659 (1210 m AMSL), IND 11-1660 (1202 m AMSL), IND 11-1661 (1300 m AMSL), IND 11-1662 (1457 m AMSL) and IND 11-1663 (1053 m AMSL) were located in hilly areas experiencing low temperature during winter and the other collections were from the plain areas. All the accessions were medium statured with average stalk diameter (0.62 cm). All these high-altitude accessions can be screened for cold tolerance for using in breeding programme for transferring winter ratoonability in sugarcane. Churachandpur had the lowest collection of

4 accessions and all were tall with mean plant height of 323.75 cm and high mean leaf length (115.25 cm) and stalk diameter (0.9 cm). Six collections were made from Bishanpur district and all the collections were tall with the mean plant height of 291.67 cm, leaves width of 1.67 cm, cane diameter of 1.13 cm and internode length of 14.42 cm. From East Imphal, six more accessions were collected which were short in nature with reduced leaf width (0.48 cm) and 5 accessions each were collected from Tamenglong, Senapati and Chandal.

One *E. fulvus* (IND 11-1657) was collected in the hilly slopes in the high altitude Ukhrul district. IND 11-1657 occurred as small population in an abandoned field and was in flowering stage. It was a medium tall plant (140 cm) with short leaf and medium leaf width (1.7 cm). Among the species of *Erianthus*, *E. arundinaceus* is the only cane forming species while other species produce stalks only during flowering to bear the inflorescence called arrow. Hence, in the sugarcane improvement programmes mostly *E. arundinaceu*s was used as parent for introgressing high biomass, more tillering and pests and disease resistance (Govindaraj, 2020). Recently non-cane forming species like *E. procerus* was also used for incorporation of biomass, drought tolerance and red rot resistance (Nair *et al.,* 2017, Mohanraj *et al.,* 2019). The new accession of *E. fulvus* is a high altitude (1879 m AMSL) collection and supposed to possess low temperature tolerance hence can be crossed with commercial and near commercial canes for improving winter ratoonability after screening under cold condition.

Narenga fallax is another genera in the *Saccharum* complex which can be hybridized with the *Saccharum* species including commercial canes. IND 11-1680 was the *N. fallax* accession collected from the fallow land in Chandal district. This was found as an isolated clump with heavy tillering with the height of 154 cm, leaf length of 101 cm, leaf width of 0.7 cm and stalk diameter of 0.5 cm. Flowering was almost completed with the

Table 3. Distribution of *S. spontaneum* and other species in different districts of Manipur

District	*S. spontaneum*	*S. officinarum*	*E. fulvus*	*Narenga fallax*
West Imphal	9	1	–	–
Ukhrul	9	–	1	–
Thoubal	10	3	–	–
Tamenglong	5	–	–	–
Senapati	5	–	–	–
Kangpokpi	2	–	–	–
East Imphal	6	–	–	–
Churachandpur	4	–	–	–
Chandal	5	–	–	1
Bishnupur	6	–	–	–
Total	61	4	1	1

Table 4. Variation in the mean performance of the *S. spontaneum* accession collected from different districts

Districts	Plant height (cm)	Leaf length (cm)	Leaf width (cm)	Arrow length (cm)	Peduncle length (cm)	Stalk diameter (cm)	Internode length (cm)
West Imphal	203.33	96.22	0.39	38.44	44.89	0.52	14.61
Ukhrul	160.22	83.11	0.53	39.67	50.22	0.62	11.18
Thoubal	241.90	113.90	1.40	46.60	57.10	0.96	12.74
Tamenglong	216.00	107.60	0.52	42.80	48.00	0.60	10.20
Senapati	220.8	104.8	1.24	52.00	60.00	0.70	13.2
Kangpokpi	220.00	123.50	1.45	33.50	31.50	0.85	12.75
East Imphal	167.50	103.33	0.48	42.33	51.33	0.77	13.42
Churachandpur	323.75	115.25	0.48	53.00	55.00	0.90	14.00
Chandal	229.00	98.00	0.48	45.20	54.80	0.68	13.20
Bishnupur	291.67	94.00	1.67	42.67	44.83	1.13	14.42

arrow and peduncle of 88 cm and 77 cm respectively. This accession can be used in hybridisation for the broadening the genetic base as intergeneric hybrids involving *Narenga* were reported earlier (Chang *et al.,* 2020).

Genetic diversity among 67 accessions

Sugarcane is a cross pollinated crop and high genetic diversity was reported earlier due to complex genome structure, polyaneuploidy, di or tri species origin and high heterozygosity (D'Hont *et al.,* 1996; Grivet and Arruda 2002; Hemaprabha *et al.,* 2006; Govindaraj *et al.,* 2012; Saravanakumar *et al.,* 2014; Senthilkumar *et al.,* 2014). New accessions collected from Manipur were also analysed for variability and clustering pattern. Hierarchal clustering established six major groups with 1, 4, 1, 6, 1 and 54 accessions in different groups. Cluster I had the single *E. fulvus* accession IND 11-1657. Cluster II grouped all four *S. officinarum* collected from Thoubal and West Imphal districts. This species had long and thick canes and presence of 3 rows of root eyes in the nodal region. Mean stalk diameter (4.25 cm) was the highest among the clusters and it also showed the longest (152.75 cm) and the widest (6.25 cm) leaves. Cluster III had only IND 11-1643, a *S. spontaneum* accession which was the tallest plant (600 cm). This was unique among the accessions with the highest arrow length (60 cm), peduncle length (60 cm) and internode length (25 cm). Among the 61 *S. spontaneum* accessions collected, IND 11-1643 recorded the highest leaf width (3.80 cm) and stalk diameter (2.3 cm) also. It clearly indicated that morphological traits have greater power in grouping of genotypes based on variability present (Govindaraj *et al.*, 2016). Cluster IV had 6 accessions (IND 11-1638, IND 11-1639, IND 11-1668, IND 11-1676, IND 11-1683, IND 11-1694) which were distributed in six different districts and all showed robust growth and high biomass with more than 380 cm plant height. The cluster IV had average plant height of 403.33 cm, leaf width of 1.23 cm and internode length of 19.08 cm. Accessions from this group can be used in the breeding programme for incorporating early growth and long internodes. Another small cluster V had only one accession of *S. spontaneum* (IND 11-1692) which was the shortest plant collected during the entire exploration. It recorded only 80 cm plant height with short (24 cm), very narrow (0.1 cm width) leaves, thin canes (0.40 cm stalk diameter) and short internodes (4.5 cm). Earlier reports revealed shorter plants among 2n=40 cytotype (Sobhakumari *et al.,* 2013). Hence, this accession is expected to have the basic cytotype (2n=40) which should be confirmed through cytological analysis.

The biggest cluster VI had 53 *S. spontaneum* and 1 *Narenga fallax* accessions representing all the 10 districts. Most of the districts in Manipur have overlapping agro-climatic conditions which may be one of the reasons for non-separation of accessions according to their location of collections. The average plant height, stalk diameter, internode length, leaf length and leaf width were 195.63 cm, 0.71 cm, 13.09 cm, 101.41 cm and 0.75 cm, respectively. All the *S. spontaneum* accessions were grouped in this cluster except the extreme accessions for plant height (IND 11-1692, IND 11-1643) and vigorous and high biomass (IND 11-1638, IND 11-1639, IND 11-1668, IND 11-1676, IND 11-1683, IND 11-1694). This largest cluster was subgrouped into two clusters namely VIa and VIb with 12 and 42 accessions respectively. Critical analysis of the pattern of sub grouping indicated that accessions in subgroup VIa were tall (282.08 cm) with thick canes (1.03 cm), broad leaf (1.19) and long internodes (18.66 cm) compared to the mean of the cluster VIb. *S. spontaneum* collected from Maharashtra (Govindaraj *et al.,* 2012, Gujarat (Govindaraj *et al.,* 2014), Assam (Govindaraj and Mahadevaswamy 2021a), Punjab and Haryana (Karthigeyan *et al.,* 2020) and Western Ghats (Govindaraj *et al.,* 2021a) also showed high genetic variability. *Narenga fallax* is the member of *Saccharum* complex and is crossable with the *Saccharum* species and possesses useful genes for tolerance to drought and water logging and resistance to pest, diseases and root parasites. Grassl (1977) reported successful intergeneric hybrids involving *Narenga fallax* and development of few hybrids. However, more efforts are required in utilising this genus in the regular breeding programme by overcoming the barriers in crossing and development of progenies for introduding new useful genes.

In general, clusters were formed as per their morphotypes. The new collections with high diversity will add to the genetic diversity of the gene pool maintained at ICAR-Sugarcane Breeding Institute, Coimbatore. The accessions collected from the high altitude and low temperature regions can be screened for low temperature tolerance and exploited in breeding for better winter ratoonability in the subtropical regions. New *S. officinarum* collections with high biomass production can be also used in the genetic enhancement programmes for improving cane yield and diversifying the genetic base of the modern sugarcane varieties.

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