RESEARCH ARTICLE

HIGH PLOIDY DIVERSITY IN *SACCHARUM SPONTANEUM* POPULATION OF NORTH-EAST REGION OF INDIA

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Abstract

Saccharum spontaneum L., a wild relative of sugarcane is the most variable and diverse among the *Saccharum* species. This species had been successfully exploited in sugarcane improvement programmes and most of the present day commercial varieties are derivatives of interspecific hybrids involving *S. spontaneum*. Cytological characterization has been done in *S. spontaneum* accessions collected from five North-East states of India. The cytological characterization of 39 accessions of Mizoram collected during 2004 revealed the presence of 12 cytotypes with 2n= 56, 58, 60, 62, 64, 70, 72, 76, 78, 80, 88, 90. From Tripura and Meghalaya 13 and 17 clones were studied respectively and were showing 4-5 cytotypes. Somatic chromosome number of 91 clones collected from Nagaland and Manipur was determined. It showed that nine different cytotypes were present in this collection. It was found that the diversity of this species is very pronounced in North- East region of India. On the whole it appears that among the chromosome number categories multiples of eight are more common. Among the studied population of *S. spontaneum* 2n=64 was in majority in all five states. While considering 8 as the basic chromosome number for *S. spontaneum* the cytotypes of 2n=52, 54, and 58 can be the aneuploids of 2n=56 (7x) with elimination or addition of 2-4 chromosomes. This type of aneuploidy may be the reason for the presence of 2n=70, 74 and 76 cytotypes in the same locality. 2n=80 can be considered as a typical cytotype of *S. spontaneum* for North-East region of the country because in all the North-East states it showed majority next to 2n=64. In general, the pattern of the ploidy level distribution shows that higher ploidy diversity is exist in these collections with more than 12 cytotypes and among them higher ploidy levels like 7X, 8X, 9X, 10X and 11X were present.

Keywords : *Saccharum spontaneum*, chromosome, cytology, germplasm, polyploids

Introduction

India is one of the major centers of diversity for *Saccharum* species and related genera. *Saccharum spontaneum* has a wider distribution throughout the country, from the sub-Himalayan regions to the peninsular India. The species show extensive variation in terms of morphology and cytotypes. Short bushy types to tall thick types growing to over 7 m in height had been reported from India. Nearly 30 cytotypes of *S. spontaneum* are present in the subcontinent. A major breakthrough in sugarcane improvement was achieved through the use of wild species viz., *S. spontaneum* in breeding programmes. The first sugarcane variety, Co 205, produced at Sugarcane Breeding Institute, Coimbatore, India was an interspecific hybrid involving *S. officinarum* clone Vellai and wild species *S. spontaneum* clone Coimbatore. This initial success in transferring desirable traits from *S. spontaneum* to cultivated sugarcane led to the systematic collection and maintenance of these species from their distributional areas. Although, exploration and collection of sugarcane germplasm was initiated as early as 1912-1915 by Dr. C. A. Barber at Imperial Sugarcane Breeding Station, Coimbatore, but well organized and systematic

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collection of *S. spontaneum* and related genera was taken-up in 1947 under a programme named *Spontaneum* Expedition Scheme (SES) sponsored by Indian Central Sugarcane Committee. A large collection of widely variable forms of this species as a result of the efforts made by Sugarcane Breeding Institute during the last 100 years is being clonally maintained at Coimbatore.

The first comprehensive study of cytogenetics of *S. spontaneum* was made by Janaki Ammal in a series of papers (Janaki Ammal 1936, 1939; Janaki Ammal and Singh 1936). She postulated the origin of certain chromosome types and found that a polyploidy series exist in this species with *x*=8 as the basic number. Panje and Babu (1960) summarized 443 clones of *S. spontaneum* with their location and chromosome number from their own work and from the literature up to 1960. Natural occurrence of 31 cytotypes in *S. spontaneum* ranging from 2*n*=40 to 2*n*=128, ie. 2*n*=40, 48, 50, 52, 54, 56, 58, 60, 61, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 90, 93, 96, 100, 104, 112, 116, 120, 124, 126 and 128 was established from this study. Time to time the *S. spontaneum* clones available at world sugarcane germplasm has been subjected to cytological characterization (Sreenivasan 1969, Sreenivasan 1975, Sreenivasan and Sreenivasan 1984, Sreenivasan and Sreenivasan 1994, Sobhakumari and Mallika 2007, Sobhakumari 2009, Sobhakumari 2013). *S. spontaneum* has been subjected to extensive cytological studies resulting in postulations of several hypotheses regarding its origin, basic number and interrelationship with other species. Cytologically, the species has both euploid and aneuploid chromosome numbers.

S. spontaneum has been extensively used in interspecific hybridization with cultivated sugarcane for producing improved varieties of sugarcane. During the sugarcane improvement programme this wild species have contributed to resistance to disease and pests, improvement in cane yield through increase in number of millable canes and better ratoonability and expanded the area of sugarcane cultivation by imparting wider adaptability. In view of its importance in sugarcane breeding as source for high productivity and adaptability, efforts made to collect, conserve and characterize *S. spontaneum* germplasm since 1933. Recently many explorations were conducted at and North-East regions of India. Among these explorations were conducted during 2004 in Mizoram, 2005 in Tripura, 2006 in Meghalaya and 2011 in Nagaland and Manipur. After quarantine these collections are clonally being maintained at ICAR-Sugarcane Breeding Institute, Coimbatore and systematic characterization has been carried out.

This paper describes the cytological characterization of 160 clones of *S. spontaneum* collected from five states of North-East region of India i. e., Mizoram, Tripura, Meghalaya, Nagaland and Manipur. This information will be useful for the effective utilization of these clones in sugarcane improvement programme.

Materials and methods:

The present cytological survey has included 160 clones of *S. spontaneum* collected from Mizoram (39 Nos.), Tripura (13 Nos.), Meghalaya (17 Nos.) and Nagaland and Manipur (91 Nos.) during 2004, 2005, 2006 and 2011 respectively. These clones have been collected from diverse habitats and different altitudes. After quarantine these clones are clonally maintained at the germplasm fields of ICAR-Sugarcane Breeding Institute, Coimbatore.

For cytological studies small clumps of the clones

were planted in pots with soil mixture to collect root tips for mitotic analysis. The root tips were pretreated with saturated solution of α-Bromo naphthalene at 4°C for 2 h, after washing, the roots were fixed in alcohol : acetic acid (3:1) solution and kept at 4 ℃ overnight. Hydrolyzed the roots in 1N HCl at 60° C for 13 minutes and stained in leuco-basic fuchsin for 30 minutes. The squashes were prepared in 1% acetocarmine. In each clone at least 10 well spread metaphase plates were counted for determining the chromosome number. Cells with well spread chromosomes were photographed in Carton CM 402T microsystem.

Results and discussion

The cytological investigations are made in 160 *S. spontaneum* clones collected from North–East region of the country (Table 1 and Fig. 1). The chromosome number has been determined from well spreaded mitotic preparations (Fig.2). Thirty nine clones were collected from Mizoram during 2004. This is the collection which shows more diversity for the cytotypes. Twelve cytotypes were identified in this collection like 2n=56, 58, 60, 62, 64, 70, 72, 76, 78, 80, 88 and 90. Among this 2n=56, 64, 72, 80 and 88 were polyploids with chromosome constitution of 7x, 8x, 9x, 10x and

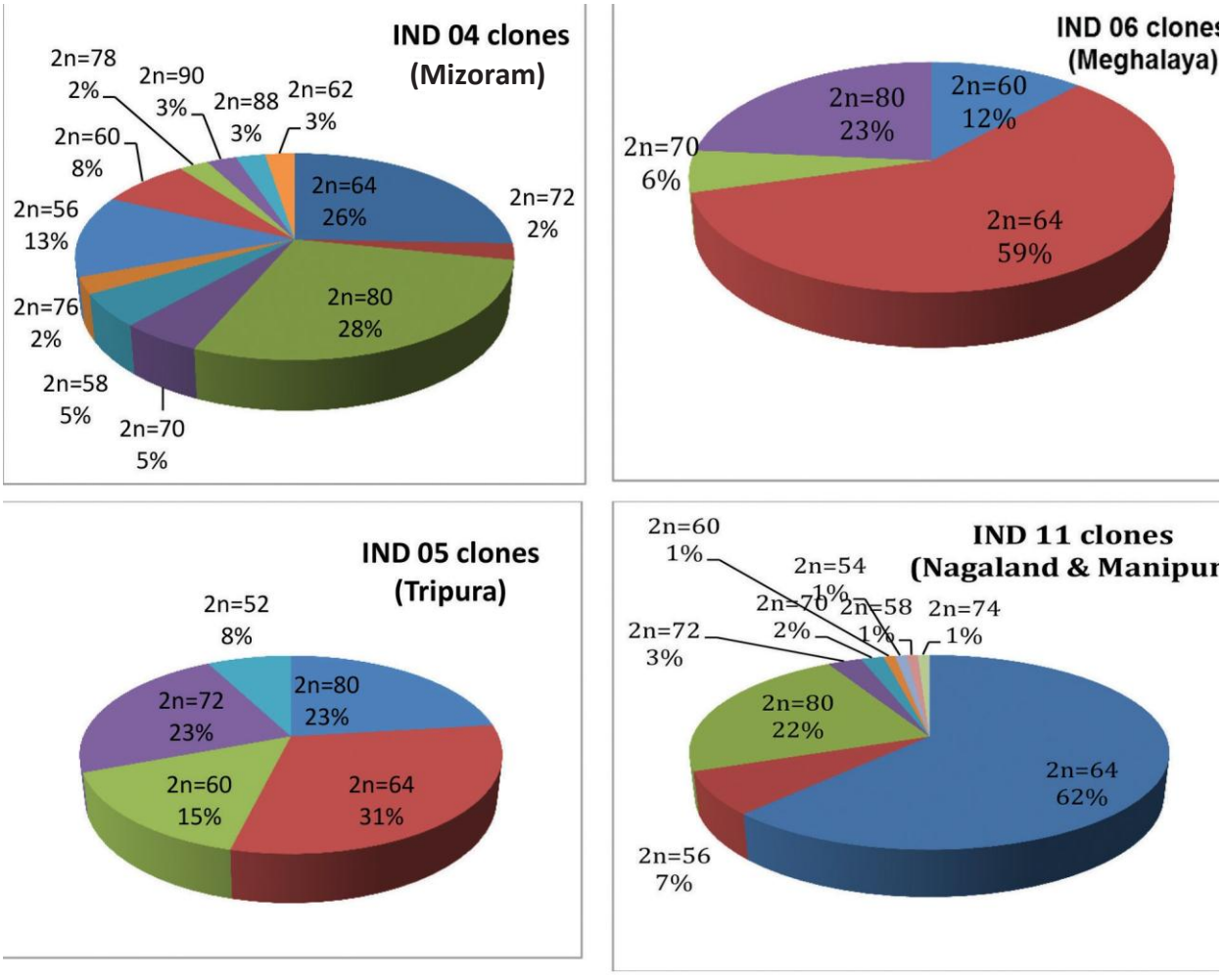


Fig.1. Distribution of different cytotypes of *S. spontaneum* from North-East states of India

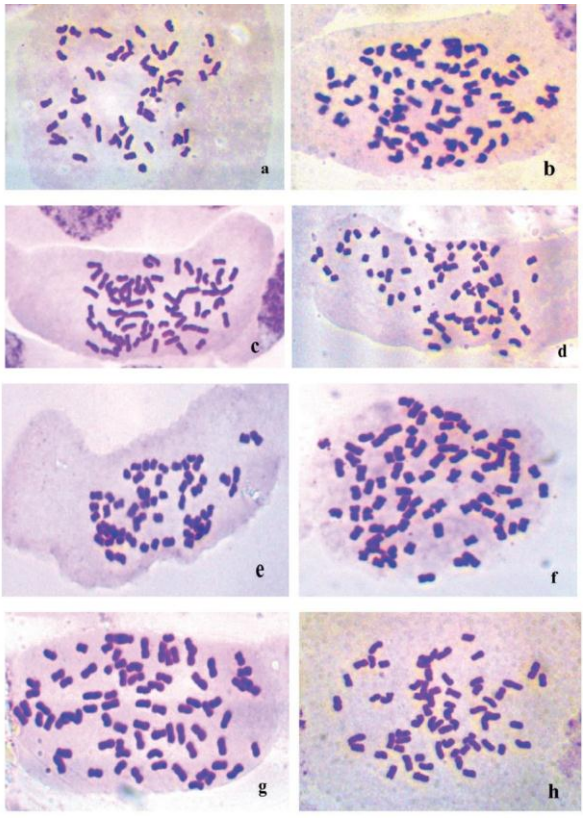


Fig.2. Mitotic metaphase chromosomes in *S. spontaneum* clones (a) IND 04-1374 (2n=56), (b) IND 04-1353 (2n=82), (c) IND 05-1405 (2n=64), (d) IND 05-1410 (2n=72), (e) IND 06-1453 (2n=60), (f) IND 06-1456 (2n=80), (g) IND 11-1685 (2n=80), (h) IND 11-1690 (2n=64)

these may be aneuploids of 8x and 9x polyploids.

Ninety one clones of *S. spontaneum* from Nagaland and Manipur was collected during 2011 were cytologically characterized and nine different cytotypes were identified in this collection. In this collection majority of the clones were with 2n=64 (62%). Clones with 2n=80 were in the next position (22%). 7x and 9x ploidy clones were also present in this area but only in low percentage (3-7%). 2n=54 and 58 may be derived from 2n=56 by addition or deletion of two chromosomes.

Cytological study of clones synthesized by hybridization of different cytotypes suggested that

Table 1. Somatic chromosome number (2n) of different clones of S. *spontaneum* from North - East states of India

Misoram		
Sl.No.	**Clone**	**2n No**
1	IND 04-	70
2	IND 04-	64
3	IND 04-	72
4	IND 04-	80
5	IND 04-	64
6	IND 04-	80
7	IND 04-	64
8	IND 04-	80
9	IND 04-	60
10	IND 04-	58
11	IND 04-	80
12	IND 04-	64
13	IND 04-	56
14	IND 04-	80
15	IND 04-	86
16	IND 04-	60
17	IND 04-	60
18	IND 04-	64
19	IND 04-	64
20	IND 04-	62
21	IND 04-	64
22	IND 04-	76
23	IND 04-	80
24	IND 04-	64
25	IND 04-	56
26	IND 04-	56
27	IND 04-	58
28	IND 04-	56
29	IND 04-	64
30	IND 04-	80
31	IND 04-	78
32	IND 04-	80
33	IND 04-	80
34	IND 04-	80
35	IND 04-	90

36	IND 04- 1390	80
37	IND 04- 1393	70
38	IND 04-1329	80
39	IND 04-1369	56

Tripura

Sl.	Clone	2n
1	IND 05- 1399	60
2	IND 05- 1400	52
3	IND 05- 1403	64
4	IND 05- 1404	60
5	IND 05- 1405	64
6	IND 05- 1407	72
7	IND 05- 1410	72
8	IND 05- 1411	80
9	IND 05- 1413	80
10	IND 05- 1416	64
11	IND 05-1417	64
12	IND 05-1419	80
13	IND 05- 1421	72

Meghalaya

Sl.No.	Clone	2n No
1	IND 06-1428	64
2	IND 06-1430	64
3	IND 06-1431	70
4	IND 06-1432	80
5	IND 06-1433	64
6	IND 06-1434	80
7	IND 06-1437	64
8	IND 06-1439	64
9	IND 06-1441	80
10	IND 06-1443	64
11	IND 06-1445	64
12	IND 06-1447	64
13	IND 06-1451	64
14	IND 06-1452	60
15	IND 06-1453	60
16	IND 06-1455	64
17	IND 06-1456	80

Nagaland & Manipur

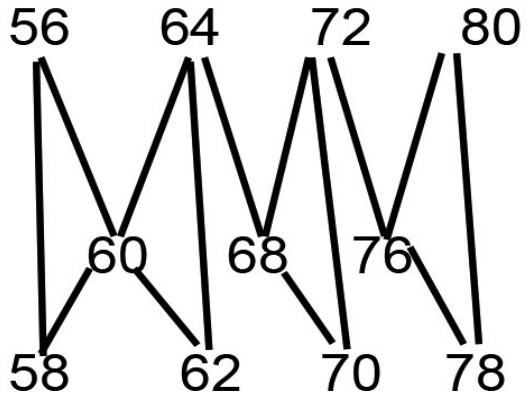
Sl.No.	Clone	2n
1	IND 11-1600	64
2	IND 11-1601	64
3	IND 11-1604	56
4	IND 11-1606	54
5	IND 11-1608	64
6	IND 11-1609	64
7	IND 11-1610	64
8	IND 11-1611	64
9	IND 11-1614	58
10	IND 11-1619	64
11	IND 11-1620	64
12	IND 11-1622	64
13	IND 11-1629	64
14	IND 11-1636	72
15	IND 11-1637	80
16	IND 11-1640	64
17	IND 11-1642	64
18	IND 11-1643	80
19	IND 11-1644	80
20	IND 11-1645	64
21	IND 11-1646	80
22	IND 11-1647	80
23	IND 11-1648	64
24	IND 11-1649	80
25	IND 11-1651	64
26	IND 11-1653	80
27	IND 11-1656	64
28	IND 11-1659	80
29	IND 11-1660	80
30	IND 11-1661	80
31	IND 11-1665	64
32	IND 11-1666	64
33	IND 11-1668	80
34	IND 11-1674	80
35	IND 11-1675	80
36	IND 11-1676	64

37	IND 11-1677	64
38	IND 11-1678	80
39	IND 11-1655	70
40	IND 11- 1615	64
41	IND 11- 1618	64
42	IND 11- 1633	72
43	IND 11- 1634	56
44	IND 11- 1664	64
45	IND 11- 1667	64
46	IND 11- 1683	56
47	IND 11-1597	56
48	IND 11-1603	74
49	IND 11-1616	64
50	IND 11-1624	64
51	IND 11-1625	56
52	IND 11-1626	64
53	IND 11-1627	64
54	IND 11-1630	64
55	IND 11-1632	72
56	IND 11-1638	64
57	IND 11-1641	80
58	IND 11-1652	64
59	IND 11-1654	64
60	IND 11-1669	60
61	IND 11-1673	80
62	IND 11-1679	80
63	IND 11-1681	56
64	IND 11-1682	80
65	IND 11-1683	56
66	IND 11-1686	56
67	IND 11-1687	64
68	IND 11-1689	64
69	IND 11-1690	64
70	IND 11-1692	80
71	IND 11-1693	80
72	IND 11-1694	80
73	IND 11-1697	80
74	IND 11-1699	64
75	IND 11-1700	64
76	IND 11-1701	64
77	IND 11-1702	64
78	IND 11-1969	80
79	IND 11- 1628	64
80	IND 11- 1669	64
81	IND 11-1598	64
82	IND 11-1599	70
83	IND 11-1600	64
84	IND 11-1613	64
85	IND 11-1619	64
86	IND 11-1620	64
87	IND 11-1642	64
88	IND 11-1663	64
89	IND 11-1685	80
90	IND 11-1691	64
91	IND 11-1695	80

natural interspecifichybridizationwereresponsible for the extensive euploidy and aneuploidy in *S. spontaneum* (Janaki Ammal 1936, Janaki Ammal and Singh, 1936 a; Raghavan, 1953; Kandasami, 1960; Bremer, 1961a, Kandasami and Rao, 1963, Sreenivasan and Jagathesan, 1973). Earlier reports postulated that the 56 chromosome form arose as a hybrid between 2n= 48 and 2n=64 forms (Janaki Ammal, 1936). But in the studied collections 2n=48 cytotype was absent. The 2n=56 cytotype may arose from selfing or intraspecific hybridization of the clones. The reports on selfing of synthetic aneuploids with 2n= 63 chromosomes produced plants with chromosome number ranging from 2n=50 to 2n=110 (Jalaja, 1983), suggested that there was no need to have sympatric distribution of cytotypes for the origin of new forms.

Asia-Africa region including Burma has mostly medium and medium high number 2*n*=80 to 2*n*=112. Polyploidy series from the lowest chromosome number of 2*n*=5*x*=40 to the highest of 2*n*=16*x*=128 in wild *S. spontaneum* occurring

within the country or nearby areas was established (Janaki Ammal, 1939; Panje and Babu, 1960). The extensive studies on chromosome number in *S. spontaneum* widely distributed in South-east Asia and adjoining areas revealed that 8 is the basic number (x=8) in this species. In Mizoram collection twelve cytotypes were identified in this collection like 2n=56, 58, 60, 62, 64, 70, 72, 76, 78, 80, 88 and 90. While considering this we found that natural hybridization between the cytotypes with multiples of 8 (x=8) may be resulted in other cytotypes which are present in less frequency, and a few aneuploids also.



These are some of the possibilities of natural hybridization for getting different cytotypes in Mizoram collection.

Panje and Babu (1960) could substantiate the proposal by Parthasarathy and Subba Rao (1946) that there is an increasing trend in chromosome number from North-West to South–East. The distribution area of *S. spontaneum* is in three principal geographical sectors such as African-Mediterranean area, Indian subcontinent and South-east Asia-cum- Africa region. The first region is characterized by high and medium high chromosome numbers ranging from 2*n*=104 to 2*n*=128. The second area which includes India, Nepal, Bangladesh, Pakistan and Sri Lanka has mostly lowest numbers ranging from 2*n*=40 to 2*n*=80. In our studies we found that 2n=80 can be considered as a typical cytotype of *S. spontaneum* for North-East region of the country because in all the North-East states it showed majority next to 2n=64. In our earlier reports on cytological characterization of *S. spontaneum* from other parts of the continent clones with 2n=80 was rare not available so that 2n=80 cytotype of *S. spontaneum* may be originated and adapted in the North-East region of the country. The cytotypes with 2n=76, 78, 88 and 90 might been originated from 2n=80 (10x) by natural hybridization. It is also observed that the population percentage of anueploids are less in all the states while compared to polyploids or clones with somatic number of exact multiples of eight.

Among the *Saccharum* species, the wild *S. spontaneum* was subjected to detailed studies in India due to its wide distribution, extensive variability in morphology and chromosome number, and most importantly its use in genetic improvement of cultivated sugarcane. The *S. spontaneum* germplasm collected from different states of the country represent the whole range of variability present in the respective states. The cytological characterization of 160 *S. spontaneum* clones of five North- East states of India revealed that this region is showing high ploidy diversity for its cytotypes. This high genetic variability is due to its high compatibility between groups and even with other related species and genera. Since this species has the capacity for modification through natural selection and having varying degree of genome constitution with different polyploid groups with high degree of adaptability

this identified sources can be utilized in sugarcane improvement programme especially to improve biotic and abiotic stress tolerance.

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