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Exploration of diversity and distribution of cytotypes of *Saccharum spontaneum*, a wild species of sugarcane, in India

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Abstract. The present investigation was undertaken to examine the geographic distributions of cytotypes of *Saccharum spontaneum* L., wild species of sugarcane, in India. New chromosome determinations has been done for 524 accessions collected mainly from four ecological zones, West, East, North and North-east, of the country. A detailed evaluation of cytogeographic pattern of *S. spontaneum* has been done with these clones along with the clones in which chromosome data is already available. Twenty six cytotypes ranging from 2n=40 (8x) to 2n=112 (14X) has been identified in *S. spontaneum* from India. Gangetic valley of Sub Himalayan region and deltaic region of South-east zone can be considered as regions of cytogeographic interest with largest concentration of different chromosome numbers. North-east region of the country appears to have had a higher evolutionary activity in this species which is evidenced by the occurrence of multiple cytotypes and sympatric growth with other species and genera. The overall cytogeographic pattern of *S. spontaneum* includes the incidence of mixed polyploidy, aneuploidy, sympatric with different ploidy levels and disjunct distribution of some cytotypes indicate that this species likely to have had multiple independent origin in different parts of India.

Keywords: *Saccharum spontaneum*, Chromosome number, Mitosis, India, Cytogeography, Polyploidy, Cytotype diversity.

INTRODUCTION

The genus *Saccharum* comprises of six species Viz., *Saccharum officinarum* L., *Saccharum barberi* Jesweit, *Saccharum sinense* Roxb. Amend. Jeswiet and *Saccharum edule* that are cultivated and *Saccharum robustum* Brandes and Jesweit ex Grassl and *Saccharum spontaneum* L. that are wild. Among these species *S. spontaneum* was subjected to detailed studies in India due to its wide distribution and high variability in morphology and chromosome number and most importantly its contribution towards the genetic improvement of cultivated sugarcane by conferring resistance to major diseases, providing vigor and hardiness for increased abiotic stress tolerance (cold and drought), increased tillering and improved ratoonability.

The commercial success of the early interspecific hybrids involving *S. spontaneum* generated interest in the collection and utilization of wild sugarcane germplasm. India is one of the major centers of diversity for *S. spontaneum*. It has a wider distribution throughout the country, from sub – Himalayan region to Peninsular India. The sugarcane germplasm collection from India dates back to 1912. During that time Dr. C.A. Barber collected *S. spontaneum* clone Coimbatore which later become the male parent of the first sugarcane variety, Co 205. Further collections of *S. spontaneum* were made by Sir Venkataraman and Dr. Janakiammal. Later the 'Spontaneum Expedition Scheme (SES)' sponsored by the Indian Central Sugarcane Committee was operated during 1948-1957, with the objective of collecting the wild *Saccharum* germplasm from the distributional areas in the country and outside. Collection efforts were revived during 1980s and six more explorations were conducted to North Eastern states of India during 1981-1990. Further exploration for collection of *Saccharum* germplasm was conducted under the National Agricultural Technology Project on Plant Biodiversity (NATP-PB) during 1999-2004. Under this programme explorations were conducted in Arunachal Pradesh, Mizoram, Orissa, Andra Pradesh, Karnataka, Tamil Nadu, Kerala and Andaman and Nicobar Islands (Nair, 2013). Explorations are being continued thereafter under the Institute programmes and the states of India like Tripura (2005), Meghalaya (2006), Gujarat (2007), Rajasthan (2008), Himachal Pradesh (2009), Uttarakhand (2009), West Bengal (2010), Nagaland and Manipur (2011), Maharashtra (2015), Punjab and Haryana (2016), Jharkhand (2017), West Bengal and Sikkim (2018 and Assam (2019) were explored during the subsequent years.). The explorations covered all states of the country and the cytogenetic studies revealed that the *S. spontaneum* germplasm collected represent the whole range of cytotype diversity present in the respective state.

Extensive cytological studies have been conducted in *S. spontaneum* accessions available in germplasm collection at ICAR-Sugarcane Breeding Institute, Coimbatore, India. Natural occurrence of around 31 cytotypes in *S. spontaneum* ranging from 2n=40-128 was established from these studies of accessions from in and out of the country (Janakiammal 1939; Panje and Babu 1960; Mehra and Sood 1974; Sreenivasan 1975; Kandaswamy et al. 1983; Sreenivan and Sreenicasan 1984, 1994; Praneetha and Nair, 2005; Sobhakumari and Mallika 2007; Sobhakumari 2009; Sobhakumari 2013 and Sobhakumari and Stanly 2017

Survey on the geographical distribution of different cytotypes of *S. spontaneum*, which were identified till 1960, has been conducted by Panje and Babu (1960) and they could substantiate the proposal of Parthasarathy and Rao (1946) that the Indian sub-continent (including Nepal, Bangladesh, Pakistan and Sri Lanka) has mostly low numbers ranging 2n=40-80. After this survey the geographic distribution of the cytotypes of *S. spontaneum* has not been analyzed critically. A detailed knowledge of the geographic distribution of ploidy variation within the species comprising of polyploid complexes is critical to our understanding of the history and evolution of such complexes. In this study an elaborative cytological analysis of accessions of *S. spontaneum* from different states of India is used to resolve its cytogeographic pattern in the country. In particular, the study will address the following questions. (1) What is the ploidy variation of *S. spontaneum* across its distribution range (based on the representative samples collected from different states of India), (2) Is this variation geographically/ecologically structured (3) Where is the center of ploidy level diversity (4) How many cytotypes are available for this species in India and its role in the evolution of euploids and aneuploids in the same species.

MATERIALS AND METHODS

The materials included in the study are collections about 524 in number, from different states of the country which were made during 2001-2017. The details of the number of clones used in the study from different states of the country are given in Table 1. Most of these clones were documented in S. spontaneum catalogues (Sreenivasan et al. 2001, Nair et al. 2013). The clones were numbered based on the following method. IND represents the country of origin (INDIA), next number (01-17) represents the year of collection and the last number represents the accession number. From the place of origin the materials were collected as clumps/ suckers and were established at germplasm fields of ICAR-Sugarcane Breeding Institute, Coimbatore after proper quarantine. For cytological studies small parts of clumps were planted in pots to collect root tips. For mitotic studies the root tips were pretreated with saturated solution of alpha bromo naphthalene at 4°C for 2h. Then the washed materials were fixed in 3:1 (alcohol: acetic acid) fixative overnight. Washed root tips were hydrolyzed in 1N HCl and stained in 1% acetocarmine. A minimum of 10 well spread metaphase plates were used for chromosome count and photographed in Carton 402T microsystem.

The cytologically analyzed 524 accessions of *S. spontaneum* include 478 new determinations of chromosome number from the clones collected under Institute project

Table 1. Details of *S. spontaneum* clones cytologically analyzed.

Clone	Year of collection	State	No. of clones studied
IND 01	2001	Orissa	3
IND 02	2002	Andhra Pradesh	6
IND 03	2003	Andaman	24
IND 04	2004	Mizoram	39
IND 05	2005	Tripura	13
IND 06	2006	Meghalaya	16
IND 07	2007	Gujarat	25
IND 08	2008	Rajasthan	11
IND 09	2009	Himachal Pradesh & Uttaranchal	45
IND 10	2010	West Bengal	26
IND 11	2011	Nagaland & Manipur	91
IND 15	2015	Maharashtra	39
IND 16	2016	Punjab & Haryana	88
IND 17	2017	Jharkhand	52
IND 90	1990	Arunachal Pradesh	19
SES,IND81 & R collections	1954, 1981, 1937	Madhya Pradesh, Bihar and AP	27

(2001-2017), 19 clones collected from Arunachal Pradesh during 1990 (IND 90 clones) and 27 clones which were collected under SES programme and R collections done by Dr Janakiammal. The clones recently analyzed (478) were mostly covered the West zone, East zone, North zone and North-East zone of India. In order to cover the whole country the earlier cytological reports of *S. spontaneum* were also considered for cytogeographic survey. For this purpose the clones studied by Panje and babu (1960), Sreenivasan (1975), Sreevivasan and Sreevivasan (1984 and 1994), Praneetha and Nair (2005), Sobhakumari and Mallika (2007), Sobhakumari (2009), Sobhakumari (2013) and Sobhakumari and Stanly (2017) were included. All the chromosome number data have been pooled together and a superimposed map of on the cytogenetic distribution of *S. spontaneum* in India has been resolved. To avoid confusion the chromosome numbers of all the clones are referred by their 2n numbers, although most of the earlier determinations have been made on pollen mother cells (meiosis). To avoid hinder during discussion the authorship references are not given each time when a cytotype is mentioned.

RESULTS AND DISCUSSION

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. Its somatic chromosome number as on now known extends from 2n=40-128 with basic chromosome number x=8. In the present study we examined the geographic distributions of cytotypes of *S. spontaneum* in different parts of India. For the present study India has divided into 6 geographic zones viz., (1) South zone, (2) West zone, (3) East zone, (4) North zone, (5) North-East Zone and (6) Central zone. The cytotype of 524 accessions were determined based on somatic chromosome count which were mainly distributed in four zones viz. (1) West zone, (2) East zone, (3) North zone and (4) North-East zone. Few clones were studied from Peninsular India and also from Andaman islands.

West zone

This zone includes Rajasthan, Gujarat and Maharashtra. Three cytotypes, 2n=64, 72 and 80 were common in these states. Of 34 *S. spontaneum* clones collected from Gujarat, 25 were studied cytologically. Majority of the clones were 2n=80 (80%) and many of them were morphologically dwarf. Surprisingly only one clone, IND 07-1486, showed 2n=64 chromosomes. With two clones of 2n=72 two aneuploids of 2n=74 and 76, one each, were also present in Gujarat. Though Rajasthan is a nearby state, its collection was having only one clone, IND 08-1502, with 2n=80. Other than this 2n=64 and 2n=72 cytotypes were also present in low frequencies. This state reports only cytotypes with multiples of eight, i.e., 8x, 9x and 10x. In Maharashtra out of 41 clones collected, 39 clones were cytologically analyzed. Six cytotypes, 2n=60, 62, 64, 66, 72 and 80 were identified from this collection. Majority of the clones were with 2n=64 (77%). Other two euploids present in the collection were 2n=72 (9x) and 2n=80 (10x). Few aneuploids like 2n=60, 62 and 66 were also present with polyploids that of multiples of eight.

As the range of somatic chromosome number reported in *S. spontaneum* is 2n= 40-128, from west zone of India intermediate numbers of this range were reported. Majority of the clones were 2n=62, 72 and 80. As far as the distribution of the cytotypes concerned a specific segregation could observe in the states of this zone. In Gujarat majority of the clones were with 2n=80 (80%). This was an unusual occurrence while compare to other

states of the country where mixed ploidy was observed. This finding contrast with the report of Panje and Babu (1960) where they have identified 2n=80 chromosome forms restrictedly in Nepal, Assam and the Western Ghats of India. The nearest state Rajasthan was having only one clone with 2n=80 cytotype and also absent in aneuploids. This indicates that 2n=64, 72 and 80 cytotypes are cytologically stable with normal meiosis and normal chromosome segregation.

East zone

This zone covers the states Bihar, Jharkhand, West Bengal and Odisha. The Clones that studied from Bihar were collected during 1950 (SES) and 1981 (IND 81). Most of them were 2n=64 cytotypes and one clone each of 2n=56 and 2n= 90 cytotypes were also there. Jharkhand collections were done recently in 2017. From this collection 52 clones were subjected to cytological analysis. Cytotype range identified in this collection was 2n=40-72. Among this, lower cytotypes i.e., 2n=54 and 56 were in higher frequency. The reported lowest chromosome type of *S. spontaneum* has identified in Jharkhand collection, i.e., 2n=40 (IND 17-1852). A total of 8 cytotypes were identified from this state in which aneuploids were lesser in number while compared to euploids. In west Bengal collection (IND 10) only four cytotypes were identified, i.e., 2n=60, 64, 70, 72. Out of 26 clones analyzed cytologically it was found that majority of them were with 2n=60 (73%). The euploids (multiples of 8) like 2n=64 and 72 were present only in low frequency. From Odisha three cytotypes were identified as 2n=52, 64, 112. IND 01-1157 (2n=112) from Odisha is the *S. spontaneum* clone with highest number reported from the present study.

From the four states of East zone of India, the Lowest chromosome number, 2n=40, and the highest chromosome number, 2n=112 were reported. These extreme types were only in low frequency, i.e., one in each. West Bengal was showing a peculiar cytotype, i.e., 2n= 60 in high frequency. This cytotype was identified from other states also, but in low frequency. In Bihar the lowest number was 2n=56 and highest number was 2n=90. As very few clones were studied from this state at present it is not sensible to conclude the position of *S. spontaneum* cytotypes existing in this state. There are chances for having the intermediate chromosome numbers from natural hybridization. To substantiate this view earlier reports revealed that from Bihar 14 cytotypes were identified by analyzing more number of accessions of *S. spontaneum* (Sreenivasan and Sreenivasan, 1994). Jharkhand was having low cytotypes like 2n= 40, 56, 64, 72 (euploids) and this may be the cause of the existence of other aneuploids in this region due to intraspecific natural hybridization. Panje and Babu (1960) reported the possibility of existence of cytotypes with different range of chromosome numbers in the region where low cytotypes are abundant. Odisha was having the highest chromosome number 2n=112 and lowest chromosome number 2n=52. In this study only few clones from Odisha has been included and the picture of chromosome survey in this state is not adequate to come to the conclusion about its cytogeographic pattern. This will be clearer in the later part of the study where all previous reports on cytological analysis of *S. spontaneum* from the same area were considered.

North zone

In this zone *S. spontaneum* has been collected from four states namely Himachal Pradesh, Uttaranchal, Punjab and Haryana. During 2009 a combined collection has been done from Himachal Pradesh and Uttaranchal. All the collected clones of *S. spontaneum*, 45 cloones, were subjected to cytological analysis. Majority (71%) of them comes under the cytotype category 2n=54 and 2n=56. More number of clones (6 clones) were identified from here with 2n=40. Other cytotypes present in Himachal Pradesh and Uttaranchal were 2n=60, 64 and 72. Surprisingly only one clone was with 2n=64 though it is considered as the most prevailing cytotype in India. During 2016 a combined collection has been made from Punjab and Haryana. High chromosomal diversity has been revealed by studying 88 accessions collected from these states. In this region twelve cytotypes were identified such as 2n=40, 48, 50, 52, 54, 56, 60, 64, 70, 72, 74 and 76. As in Himachal Pradesh and Uttaranchal the lower cytotypes 2n=54 and 56 were more in Punjab and Haryana also. It covered around 60% of the whole collection. All the other cytotypes were present only in less than 8%.

Very clear demarcation in the chromosome number of *S. spontaneum* has been shown by North zone of India from other zones because of the high frequency of low chromosome number types like 2n=40, 54 and 56. 2n=64 cytotype was in less frequency in this zone. In Punjab and Haryana among 12 cytotypes 2n=40, 48, 56, 64, and 72 were euploids (multiples of basic chromosome number 8) with chromosome constitution 5x, 6x, 7x, 8x and 9x respectively. Others were aneuploids and may be originated from intraspecific hybridization among the different ploidy cytotypes at the place of origin itself. Though in Punjab and Haryana six clones of 2n=64 were available, only one clone was with 2n=64 in Himachal

Pradesh and Uttaranchal collection. It has been reported that inter and intraspecific natural hybridization are responsible for the existence of extensive euploidy and aneuploidy in *S. spontaneum* (Janaki Ammal, 1936; Janaki Ammal and Singh, 1936; Raghavan, 1953; Kandasami. 1961a; Bremer, 1961a; Kandaswamy and rao, 1963; Sreenivasan and Jagthesan, 1973). Analysis on the evolutionary origin of different cytotypes of Punjab and Haryana collection revealed its independent as well as multiple origins (data not published).

North-East zone

In this zone the cytological analysis has been done for *S. spontaneum* clones collected from the states Sikkim, Meghalaya, Tripura, Mizoram, Manipur, Nagaland and Arunachal Pradesh. In Sikkim only one cytotype, i.e., 2n=64 was identified. From Meghalaya 16 clones were studied and four cytotypes, 2n=60, 64, 70 and 80 were identified. Of this 2n=64 (8x) was showing majority (59%) and next to it was 2n=80 (10x). The other two aneuploids would have been derived as a result of intraspecific hybridization of euploids. These aneuploids, 2n=60 and 2n=70, were in 12% and 16% respectively. In 2005 collections of were made from Tripura. Thirteen clones were cytologically analyzed and chromosome number has been determined by root tip mitosis. It was found that majority of the clones were with 2n=64 (31%). The other cytotypes available in Tripura were 2n=80 (23%), 2n=72 (23%). These clones were with chromosome numbers that were the multiples of 8. Two types of aneuploids identified from Tripura were 2n=60 and 52. They were less in percentage, 15% and 8% respectively. Mizoram collections were made during 2004. Thirty nine clones were cytologically analyzed from this collection and 12 cytotypes were identified. They were 2n= 56, 58, 60, 62, 64, 70, 72, 76, 78, 80, 88 and 90. While considering eight as the basic chromosome number of *S. spontaneum*, 2n=56, 64, 72, 80 and 88 were polyploids with chromosome constitution 7x, 8x, 9x, 10x and 11x respectively. 2n=64 (26%) and 2n=80 (28%) cytotypes were in majority in this state and next to this was 2n=56 (13%). All the other cytotypes were in less frequency and it was found that all together the nine cytotypes covered 33% of the total clones studied. Combined collection was made from Manipur and Nagaland during 2011 and cytological analysis has been done in 91 clones of *S. spontaneum*. Nine cytotypes, 2n=54, 56, 58, 60, 64, 70, 72, 74, and 80 were identified from these states. Majority of the collection (62%) was with 2n=64 and next to it was 2n=80 (22%). All other cytotypes were in low frequency. Nineteen clones were cytologically analyzed from Arunachal Pradesh and six cytotypes were identified as 2n=54, 56, 58, 62, 64 and 90. Majority of the collections were with 2n=64. All other cytotypes present in this collection were in less number. From Arunachal Pradesh one clone, IND 90-755, was with 2n=90 which is a rare cytotype with high chromosome number occurred in India.

From the result of cytological studies conducted in *S. spontaneum* clones from the states of North East region of India make us to recall the statement of Dr. C.A. Barber that "one of the keys which can unlock the question of ancestral sugarcane forms is concealed in North India". In the present study 179 clones of *S. spontaneum* from different states of North East Zone were cytologically analyzed. These clones have been collected from diverse habitats and different altitudes. Fourteen cytotypes including 2n=52, 54, 56, 58, 60, 62, 64, 70, 72, 74, 76, 80, 86 and 90 were identified from this study. This revealed that this region is showing high ploidy diversity for its cytotypes. This high genetic variability is due to its high compatibility between the groups and even with other related genera and species. Though we could see variable numbers with euploids of 7x, 8x, 9x, 10x and many intermediate aneuploids it was interesting to note that the lowest chromosome numbers in this species, 2n=40 and 48, were absent in this region. In earlier report the cytotype 2n=40 has been reported from Sikkim and Arunachal Pradesh (Sreenivasan and Sreenivasan, 1994). They observed that irrespective of the climatic condition prevailing in the distributional area, all clones with 2n=40 were short, saturated, less cane forming with very narrow leaves due to reduction of lamina to midrib. Contradictory to this 2n=80 cytotypes in most of its morphological characteristics it resembles *S. barberi*. In the present study determination of chromosome numbers from the recent collections have revealed new cytotypes. Earlier reports of the occurrence of low chromosome types in Sikkim and Arunachal Pradesh, occurrence of other related species and genera and existence of natural hybrids with different chromosome numbers in the North east region provides further evidence for the evolutionary significance of this zone. Due to the existence of all members of "*Saccharum* complex", overlapping of flowering time, and high compatibility between the species makes this area much evolutionary significant as far as sugarcane is concerned.

During 2003 collection of *S. spontaneum* has been done in Andaman Islands and the cytological analysis showed that only 2n=64 (8X) and 72 (9x) were available here.

The essential first step when gaining insight into

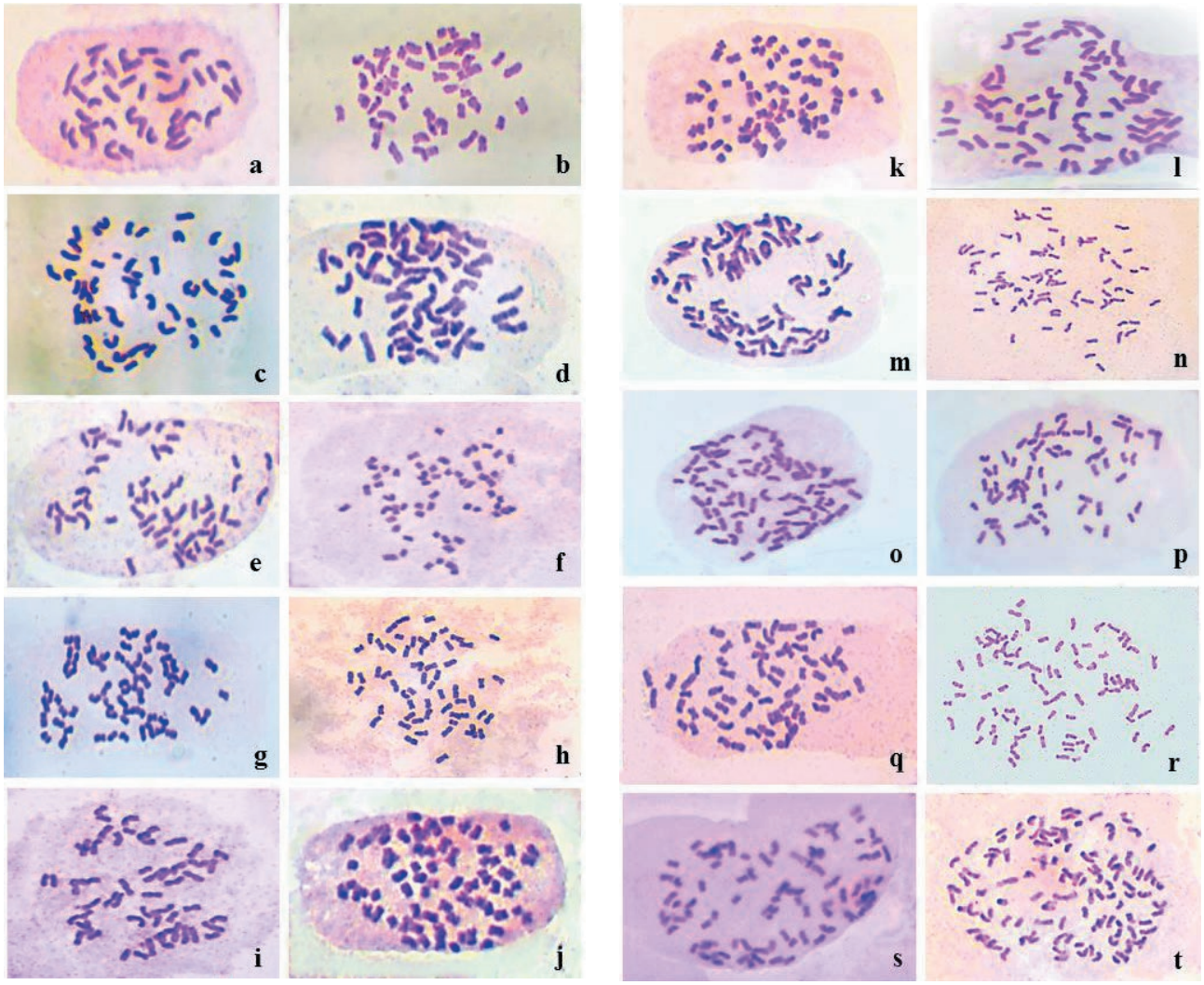

Figure 1. (a) – (t) Somatic chromosomes in different cytotypes of *S. spontaneum* a) IND 16-1812 (2n=40), b) IND 16-1792 (2n=48), c) IND 11-1606 (2n=54), d) IND 11-1604 (2n=56), e) IND 11-1614 (2n=58), f) IND 10-1574 (2n=60), g) IND 89-754 (2n=62), h) IND 11-1610 (2n=64), i) IND 01-1156 (2n=52), j) IND 17-1862 (2n=66), k) IND 17-1866 (2n=70), l) IND 15-1741 (2n=72), m) IND 10-1585 (2n=74), n) IND 09-1552 (2n=76), o) IND 07-1457 (2n=80), p) IND 08-1494 (2n=64), q) IND 03-1312 (2n=64), r) IND 04-7353 (2n=86), s) IND 90-775 (2n=90), t) IND 01-1157 (2n=112).

the evolution of polyploid is cytogeography, the study of cytotype diversity and its past and predicted future distribution patterns. Knowledge of cytotype distribution pattern usually reveals phenomena such as environmental segregation or productive isolation of cytotypes (Rejlova et al., 2019). While superimposed the map of Figure 1 which explained the state wise chromosome numbers of recently collected *S. spontaneum* clones from different zones of India with the earlier reports on the same aspect, we are getting a comprehensible picture of the geographic distribution of different cytotypes of *S. spontaneum* throughout Indian sub-continent. In Figure 2 the merged map with distribution details of so far reported cytotypes of *S. spontaneum* has been given. In Figure 3 the distribution pattern of 26 cytotypes of *S. spontaneum* in six geographical zones of India has been specified.

The earlier report says that in the case of *S. spontaneum* wherever the low cytotypes occur there is a certain concentration of other chromosome numbers also (Panje and Babu, 1960). The cytotype distribution pattern of North and North-East zones substantiate this statement by having maximum number of cytotypes that in the country reported. Although there are many incidents of polyploid coexistence in nature, the minority cytotype exclusion hypothesis predicts that mixed

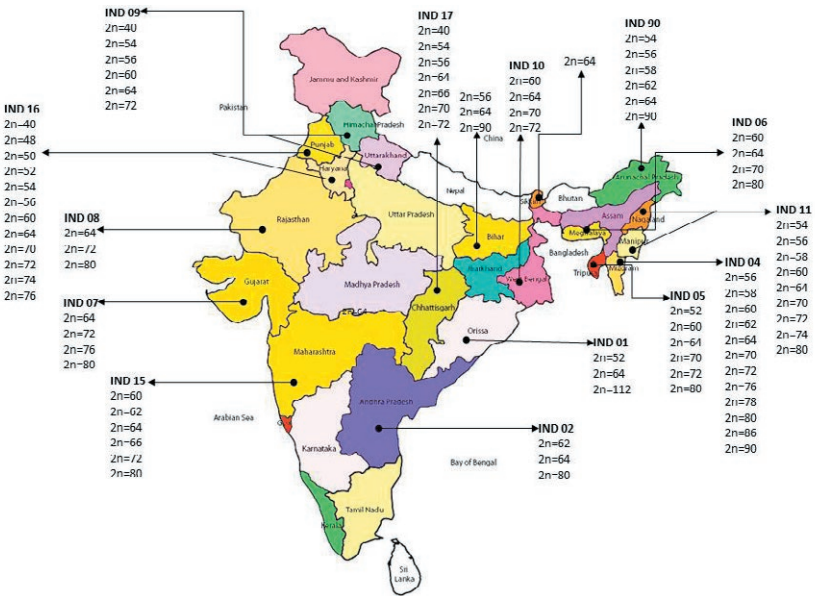

Figure 2. Distribution of *S. spontaneum* cytotypes (from 2001-2017 collection) in different states of India.

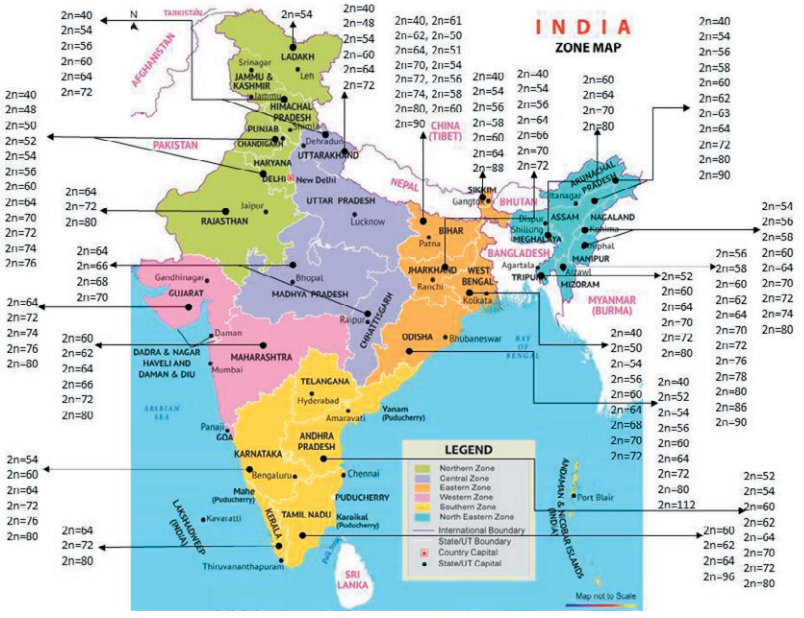

Figure 3. Superimposed map showing distribution of *S. spontaneum* cytotypes (so far reported) in different states of India.

cytotype population will eventually lose one cytotype (Levin, 1975). This may be the reason for absence of 2n=40 and 2n=48 in majority of the states of North east zone even though around 17 cytotypes were available in this zone.

A wide ranging track could be marked to incorporate all lowest chromosome number cytotypes (2n=40, 48, 54 and 56). These cytotypes could be identified from two areas which covers the entire northern sub Himalayan plain of Ganga and Yamuna river and this extended to the south east coast which encircled the deltas of Mahanadhi, the Godavari and Krishna. This identified area is coincides with the geographic pattern explained by Panje and Babu (1960). While considering the geographic pattern of different cytotypes of *S. spontaneum* in India the above mentioned track is of special interest

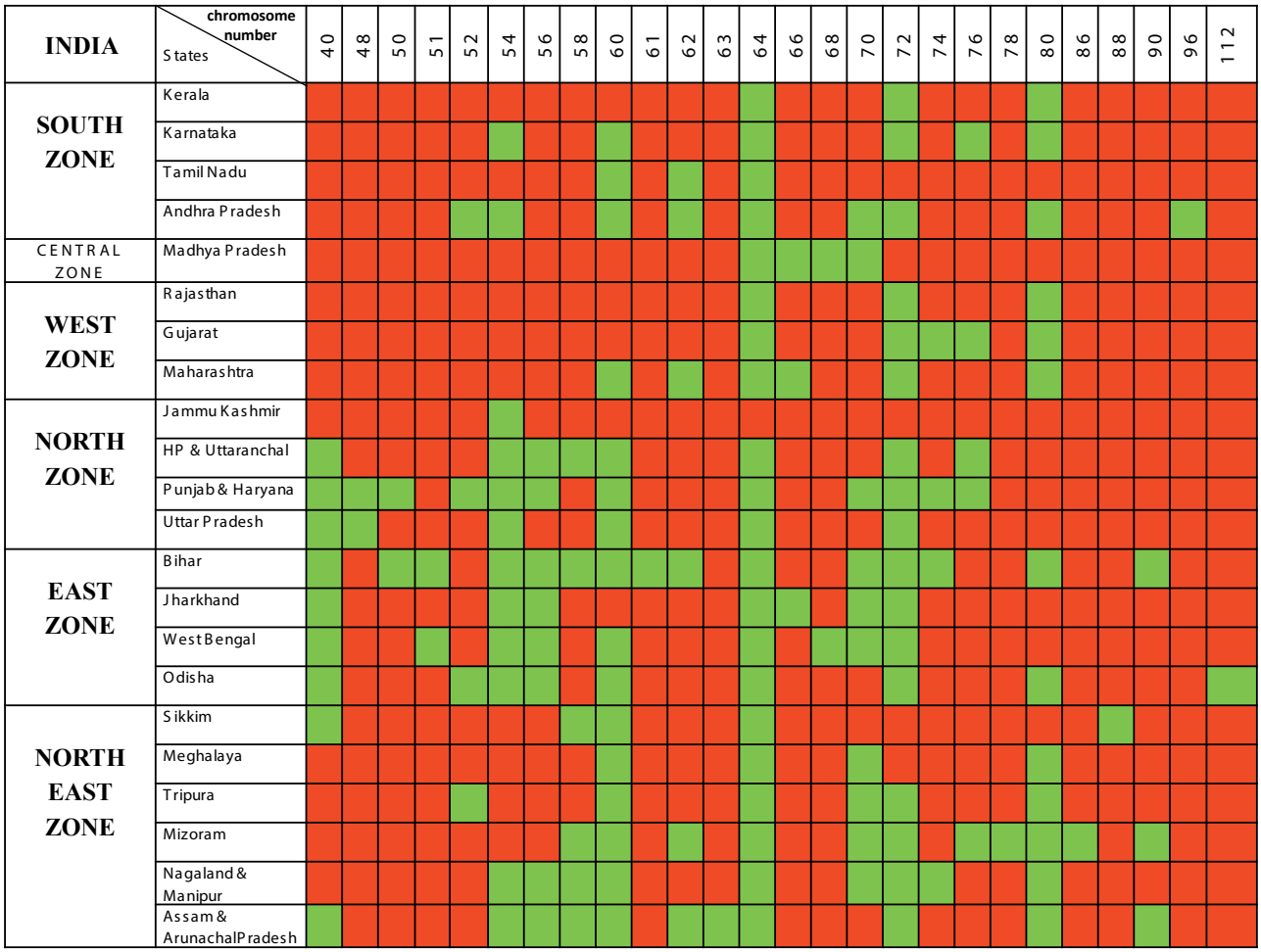

Figure 4. Distribution pattern of *S. spontaneum* cytotypes in different geographic zones of India.

because it is found to have as many as 18 out of 26 cytotypes reported from India.

The cytotype of 2n=64 has the widest distribution in India (Figure 3). This may be due to its better competitive ability compare to other cytotypes. The lower part of the peninsular India which consists of Tamil Nadu and Kerala was having more number of 2n=64 types than in North India. In Kerala only euploids like 8x, 9x and 10x were present and no intermediate chromosome numbers. Given the geographical separation and habitat similarity among cytotypes, mixed-ploidy populations may be transitional and subject to the forces of minority cytotype exclusion which lead to pure-ploidy populations (Castro et al, 2018). In most of the states with 2n=64 types, its aneuploids, 2n=60, 62, and 66, were also observed in low concentration. Mating between intraspecific cytotypes which are at the levels of ploidy often produce offsprings with odd number of genomes or imbalanced ploidy having lower fitness than those produced by individuals of same cytotype. Generally, the odd polyploids show meiotic abnormalities and consequent decreased viability of gametes leading to little success in existence (Rani et al., 2015). In contrast to this in West Bengal relatively higher concentration of (73%) 2n=60 cytotype was observed which seems to be distinct from other states.

2n=54 cytotype groups are more confined to the North zone of the country. Its relative concentration is more in Himachal Pradesh, Uttaranchal, Punjab and Haryana. This is the only chromosome number so far reported from Jammu and Kashmir (SES 352, 2n=54). Though many cytotypes inhabit the neighboring zones around Gangetic plain, the central zone of India was having only few cytotypes of *S. spontaneum* which consist of 2n=64 and its aneuploids. Chromosome number less than 64 was not reported from here. 2n=80 cytotype has been identified from all parts of the country except in North zone states. In many states it was in

low frequency whereas in Gujarat 80% of the *S. spontaneum* clones were with 2n=80. This disproves the prior assumption that this cytotype was restricted Assam and Western Ghats.

The world collection of sugarcane germplasm maintained by ICAR-Sugarcane Breeding Institute, Coimbatore, India is the largest germplasm collection at present. A large assembly of *S. spontaneum* representing the entire range of availability collected from its distributional areas of the country is currently available in the Institute. These accessions were used for the present study and it revealed that *S. spontaneum* is rich in genetic variability and are compatible group for inter and intraspecific hybridization. Polyploid series from lowest chromosome number 2n=5x=40 to the highest of 2n=14x=112 in this species has been revealed from this study. Natural hybridization between the cytotypes with multiples of 8 (x=8) resulted in other cytotypes and also aneuploids in its distributional areas. A total of 26 cytotypes were identified from India. In North and North-East India the evolutionary mechanism are highly active in this species and it is found that the cyto-morphological variability favor the accumulation of adaptability characters, especially to biotic and abiotic stresses. In sugarcane, compared to most of the other commercial crops, the information on genetic variability and geographic distribution pattern of the wild species, *S. spontaneum*, is available and it can be contributed to the development of superior clones with desirable characters.

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