Summary

Cytological study was carried out in 5 *Pseudoxytenanthera* species (tribe Bambuseae, family Poaceae) occurring in the Western Ghats, India. The chromosome numbers observed are 2n = 84 (*P. bourdillonii*), n = 38, 2n = 76, 72, 74 (*P. monadelpha*), 2n = 72 (*P. stocksii*), 2n = 72 (*P. ritcheyi*) and n = 96 and 2n = 192 in *P. sp.* which is the highest chromosome number in the entire Bamboo family reported for the first time here. *P. monadelpha* occurs here in 3 ployploid/aneuploid cytotypic forms (2n = 72, 74, 76). The chromosome numbers existing in species of the present genus is x = 12 basic which is suggested to be a secondarily derived one from an ancestral n = 6 taxon. Species of the present genus constitute a polyploid series on x = 12 (6x, 7x, 16x). Meiosis in one of the polyploid species, *P. monadelpha* with n = 38 was normal suggestive of its allohexaploid nature and that of other 2 species abnormal suggesting possibly their autoallopolyploid nature. Very small size of the chromosomes of the species in the genus appears to be grossly associated with their high polyploidy. The genus is cytologically heterogeneous and dynamic with polyploidy/aneuploidy having widely operated in speciation and evolution.

Keywords: Cytology, bamboos, *Pseudoxytenanthera*, Western Ghats, India.

Introduction

Chromosome information has been greatly recognized and exploited as a dependable tool for tackling taxonomic problems and for elucidating systematic relationships, phylogeny and evolution of related plant groups. Chromosome data comprising their number, morphology and behaviour at meiosis have been extensively used for making comparisons and interpretations of relationship and evolutionary trends in higher plants of quite a large number of taxa. The chromosome reports are known earlier in a number of taxa of the tribe Bambuseae (Fedorov 1974, Chen et al. 2004). Notable Indian reports were compiled by Seethalakshmi & Muktesh Kumar (1998). The present
report refers to meiotic and/or somatic chromosomes of 5 species of *Pseudoxytenanthera* (tribe Bambuseae) from the Western Ghats. These are first reports and the data have been used for discussing the chromosome evolution in the genus.

**MATERIALS AND METHODS**

The genus *Pseudoxytenanthera* Soderstr. & R. P. Ellis of subtribe Bambusinae (subfamily Bambusoideae) of family Poaceae consists of woody bamboos distributed in India, Sri Lanka and Indo-China. There are 4 species under this genus of which 2 species, *P. bourdillonii* (Gamble) H. B. Naithani and *P. ritcheyi* (Munro) H. B. Naithani are endemic to the Western Ghats, *P. monadelpha* (Thwaites) Soderstr. & R. P. Ellis occurs in India, Sri Lanka and Indo-China, while *P. stocksii* (Munro) T. Q. Nguyen in India and Vietnam (Clayton et al. 2015). These 4 species and yet to be named *P. sp.* found in the Western Ghats, India are presently studied.

Offsets of the species were collected from different parts of the Western Ghats, and grown in the Bambusetum of the Jawaharlal Nehru Tropical Botanic Garden of Research Institute (JNTBGRI), Palode, Thiruvananthapuram (Koshy 2010). Young spikelets and root tips for meiotic and somatic chromosome studies were procured from clumps bearing accession numbers 23 (*P. bourdillonii*), 354, 417, 670, 1044 (*P. monadelpha*), 42 (*P. ritcheyi*), 517 (*P. stocksii*) and 520 (*P. sp.*), the collection details and passport data of these clumps are published (Koshy 2010). Young spikelets and root tips were collected in the forenoon hours, and fixed in 3:1 Carnoy's fluid. The root tip materials were pre-treated in 0.002 M solution of 8-hydroxyquinoline, and kept at 4°C for 3 h before fixation. The smear and squash preparations were stained in 2% acetocarmine, and well spread chromosome preparations photomicrographed using a Leica DM 100 digital camera attached with Leica DM 2500 trinocular microscope. Pollen fertility was assessed based on stainability of mature pollen grains from a sample of 500 grains, stained in a 1:1 mixture of 2% acetocarmine and glycerine, the stained ones were considered fertile.

**OBSERVATIONS**

*P. bourdillonii*

The somatic chromosome number was 84. The chromosomes were very small varying in length from 1.22 to 1.75 µm (Fig. 1).

*P. monadelpha*

In Acc. No. 1044, meiosis was normal showing 38 bivalents, regular anaphase separation and appreciable pollen fertility (70%) (Fig. 2). The somatic chromosome number was 76. The chromosomes were very small varying in length from 0.5 to 1.2 µm (Fig. 3). In Accs 354 and 670 the somatic chromosome number was 72. The chromosome length varies from 0.74 to 1.75 µm in Acc. 354 and in Acc. 670 it ranges from 0.57 to 1.5 µm (Figs 4, 5). In Acc. 417, the somatic chromosome number was 74. The chromosomes vary in length from 0.57 to 2.1 µm (Fig. 6).
In this species, root tip cells showed 72 small chromosomes varying in length from 0.64 to 1.00 µm (Fig. 7).

**P. stocksii**

Meiosis was not normal due to formation of 2 to 4 multivalents in each PMC and irregular anaphase separation and low pollen fertility (40%) (Fig. 8). The somatic chromosome number was 72 with small chromosomes measuring 1.02 to 1.05 µm (Fig. 9).

**P. sp.**

This was a highly polyploid taxon showing n = 96 (Fig. 10). Anaphase separation was irregular and pollen fertility was very low (20%). Root tip cells showed 192 small chromosomes varying in length from 0.58 to 0.88 µm (Fig. 11).

**DISCUSSION**

The chromosome numbers observed in species of *Pseudoxytenanthera* are 2n = 84 (*P. bourdillonii*), n = 38 and 2n = 76 (*P. monadelpha*), 2n = 72 (*P. ritcheyi*), n = 36 and 2n = 72 (*P. stocksii*) and n = 96 and 2n = 192 in an unnamed species (*P. sp.*), all existing at high polyploid levels. 2n = 192 reported here is the highest so far known in the entire Bambuseae. Authentic data of chromosome numbers, meiotic behaviour and chromosome morphology are powerful tools in tracing the pattern of chromosome evolution and also in the resolution of systematic relationships, phylogeny and evolution of related plant groups. Within discrete groups and sometimes in large groups of plants, the trends of chromosome evolution has been unambiguous, and in some cases, the chromosome evidence has been crucial in settling disputed intra- and interfamilial relationships as was fairly well documented in many angiosperm families like the Asteraceae (Mathew & Mathew 1988), Rubiaceae (Philip & Mathew 1988), Liliaceae (Vijayavalli & Mathew 1990) and Pteridophytes (Abraham et al. 1962). For purpose of evaluation of the patterns of chromosome evolution in related plant groups, it is necessary to make use of valid data of various cytological parameters like basic chromosome constitution, and the direction and magnitude of numerical and structural changes of chromosomes. The chromosome numbers of 2n = 72 (*P. monadelpha*, *P. ritcheyi*, *P. stocksii*), 2n = 84 (*P. bourdillonii*) and 2n = 192 in the unnamed species (*P. sp.*) reported, appear to be very much suggestive of x = 12 as the for basic chromosome number the genus. The other numbers like 2n = 74 and 76 in *P.*
monadelpha are aneuploid derivations from 2n = 72. The chromosome data of the present taxa and the other numbers reported earlier in Bambuseae show that x = 12 is the most predominant basic number in the entire family. A perusal of literature pertaining to Bambuseae shows that a myriad of chromosome numbers exist globally based mostly on x = 12 and its derivatives (x = 11 and 10) constituting an assemblage of unrelated chromosome numbers (Ghorai & Sharma 1980, Yeasmin et al. 2015). Species based on x = 12 reported in previous studies are predominantly tetraploids with 2n = 48. Other, less common basic chromosome numbers known are, x = 10 and 11 which are aneuploid derivatives of x = 12, occurring mostly in herbaceous taxa (Yeasmin et al. 2015). The present 16x species with 2n = 192 is the highest chromosome number so far known in the entire Bambuseae. The next higher number in this group is 2n = 108 reported in Bambusa schizostachyoides (Kurz) Gamble (Sobita Devi & Sharma 1993) followed by 2n = 104 in 6 Chinese species (Chen et al. 2004). Sobita Devi & Sharma (1993) have also listed chromosome data on quite a number of Indian species with somatic numbers of 72 and its aneuploid variants. Other major Indian reports are those by Mehra & Sharma (1975) who reported several polyploid species based on x = 12 and its derivatives (2n = 48, 54 and 72) and by Janaki Ammal (1959) and Parthasarathy (1946) who reported 2n = 48 and 2n = 70 respectively. Koshy & Jee (2001) reported 2n = 72 to 82, with still lower numbers (2n = 32 and 34) in B. vulgaris. Other prominent reports of polyploids are, certain Japanese taxa with 2n = 48, 54 and 72 (Uchikawa 1935) and Canadian bamboos with 2n = 52 (Hunter 1934).

The factor of basic chromosome number at the level of genera, tribes and families has played a prominent role in shaping the prevailing concept of chromosome evolution in higher plants (Stebbins 1971). This has formed one of the widely used parameters in formulating phylogenetic speculations, in addition to being a stable and reliable marker of the direction of evolution. According to Stebbins (1950), diminution rather than increase of basic number has often occurred and played significant role in the process of chromosome evolution. Studies in many angiosperm families from the South Indian region have yielded convincing evidence in favour of this concept. Jones (1970), while agreeing in general to this concept, has pointed out that increase of basic numbers also has been prevalent in many plant groups. It is generally held that polyploidy, especially secondary hybrid polyploidy has played a major role in the evolution of higher basic constitutions. Apparently unrelated basic numbers are produced by the polyploid “drop” and “lift” processes resulting in dysploids leading to a very high basic numbers as in some angiosperms and pteridophytes, where the high polyploids are reckoned as “ paleopolyploids” (Mathew et al. 1998). The available store of chromosome data on the entire Bambuseae distributed in a wide range of habitats cutting across geographical differences and ecological singularities show that great bulk of woody taxa of the family are polyploids on x = 12. This is evident in the present genus in which the species studied occur in a regular polyploid series on x = 12 (6x, 7x, 16x). The other less common basic numbers (x = 11, 10) among some Indian genera and alien could be products of
progressive dysploidy, both ascending and descending (Ghorai & Sharma 1980). Stebbins (1971) and Grant (1982) have held that relatively high basic numbers so dominant among most angiosperm families, particularly those above the rank of \( x = 9 \) or 10, are secondary or tertiary ones derived by various processes like polyploidy, secondary hybrid polyploidy and aneuploidy at different polyploidy levels. On such a consideration, the present \( x = 12 \) may be construed as a derivation from some low chromosome-numbered ancestral taxon with \( n = 6 \).

There are innumerable examples of occurrence of more than one euploid and aneuploid types of plants in a previously recognized taxonomic species. Such differences in chromosome number within species complexes are often correlated with noticeable difference in plant morphology (Vijayavalli & Mathew 1990), and when they do so, the phenomenon may lead to species differentiation. One of the species of the present genus, \( P. \ monadelpha \) exists in the Western Ghats in 3 different intraspecific cytotypic forms (\( 2n = 72, 74, 76 \)). The cytotypes with \( 2n = 74 \) and 76 could be ascending aneuploid derivations from \( 2n = 72 \). The phenomenon of polyploidy is known to be the most widespread and distinctive cytogenetic process which has greatly influenced evolution in higher plants. This could be mainly due to the ability of the polyploids with increased chance of fertilization by breaking the reproductive barriers and also to their higher tolerance to adverse environmental conditions. Several hypotheses have been known to explain the higher incidence of polyploids in a given flora or a group of plants which include, (a) polyploids more resistant to extreme climates than their diploid counterparts, (b) polyploids are endowed with higher capacity for drought resistance and (c) polyploids having higher invasive potential for adapting to new and adverse habitats. Instances of expanding habitats and distribution of polyploid types in varying climatic conditions are known in bambusoid taxa. Bamboos have a long life period with an efficient method of vegetative reproduction and therefore enjoying considerable survival value in nature. On account of the infrequent flowering, they have notably fewer generations and hence lesser opportunities in recombinational and structural chromosomal alterations (Ghorai & Sharma 1980). The fairly high chromosome numbers at high ploidy levels of \( 6x \) and higher as in the present genus may be the outcome of the attribute of vegetative propagation.

Of the 5 polyploid taxa studied here, meiotic behaviour could not be studied in 2 species due to unavailability of flower materials in them during the period of the present study. Meiosis in one of the polyploid species studied, with \( n = 38 \) (\( P. \ monadelpha \)) was fairly normal characterized by regular bivalent formation and normal anaphase separation leading to appreciable pollen fertility. This taxon could be an allohexaploid. In the other 2 polyploid species (\( P. \ stocksii \) and \( P. \ sp. \)) meiosis was abnormal resulting in gross decline of pollen fertility. These species could
be possibly autoallopolyploids, the former hexa- and the latter 16x, because a criterion commonly used to distinguish between auto- and allopolyploids is the frequency with which chromosomes associate into multivalents in the former, and regular bivalent formation and appreciable pollen fertility in the latter (Stebbins 1950). Incidence of high polyploidy in all the taxa in one of them up to 16x, and occurrence of aneuploid cytotypes in one species (2n = 72, 74, 76), is suggestive that polyploidy and aneuploidy have been fairly operative in speciation and evolution of the genus. A glaring feature of the high polyploid species of the genus is their very small size of chromosomes, which is suggestive of association of very small chromosome size and high polyploidy in the genus. This is in conformity with the concept of Miksche & Hotta (1973) that very small chromosomes and smaller quantity of less repetitive DNA bring about more dynamic genetic and evolutionary diversification.

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