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CHROMOSOME NUMBERS

A new record of chromosome count in mixoploid karyotype of *Strobilanthes hamiltoniana* (Steud.) Bosser & Heine (Acanthaceae)

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ABSTRACT

The mixoploid karyotype found in the garden plant, *Strobilanthes hamiltoniana,* is a new record of chromosome count to the contemporary knowledge. The detailed karyomorphometrical analysis of the plant revealed a symmetric karyotype which is primitive. A hypoploid variant along with the normal chromosome complement was recorded within a root meristem with karyotype formulae as 10 nm and 20 nm respectively. In the diploid cells two pair of chromosomes have secondary constriction with an average chromosome length of 1.81 μm. The high values of symmetric indices and low values of asymmetric indices proved the primitive nature of the karyotype.

Keywords: mixoploidy, *Strobilanthes hamiltoniana*, karyotype, symmetric, polysomaty

РЕЗЮМЕ

Чембраммал Р., Топпил Дж.Э. Новое число хромосом в миксоплоидном кариотипе *Strobilanthes hamiltoniana* (Steud.) Bosser & Heine (Acanthaceae). Миксоплоидный кариотип, обнаруженный у садового растения *Strobilanthes hamiltonian*, – это новое число хромосом для данного вида. Детальный кариоморфометрический анализ растения позволил выявить симметричный кариотип, что является примитивным признаком. Гипоплоидный вариант наряду с нормальным дополнением хромосом был зарегистрирован в корневой меристеме с формулами кариотипа 10 нм и 20 нм, соответственно. В диплоидных клетках две пары хромосом имеют вторичное сужение со средней длиной хромосомы 1,81 мкм. Высокие значения симметричных индексов и низкие значения асимметричных индексов подтверждают примитивный характер кариотипа.

Ключевые слова: миксоплоидия, *Strobilanthes hamiltoniana*, кариотип, симметричность, полисоматичность

Cytological indices have immense importance as a proved tool for supporting other scientific data in explaining the evolutionary scenario of an individual. Karyotype analysis is a reliable procedure for aneuploidy identification and chromosomal rearrangements (Gouas et al. 2008). Karyotyping is an adavanced genotyping technique for the visual examination of genetic changes. It can be achieved by the modern techniques in cytogenetics along with the conventional stained karyotypes (Montazerinezhad et al. 2020).

Strobilanthes hamiltoniana (Steud.) Bosser & Heine is a small shrub native to North East India and is grown in South India as a garden plant. The peculiarity of the species is its annual flowering and perennial nature with continuous seasonal flowering (Augustine 2008). Chromosome studies on South Indian *Strobilanthes* species are rare. The chromosome morphology of the *S. hamiltoniana* is not studied yet. Hence, the present study is to unveil the karyotype and symmetry of the plant by conventional staining technique and analysing various karyomorphometric parameters.

MATERIAL AND METHODS

Strobilanthes hamiltoniana was procured from Wayanad district of Kerala (Fig. 1). The voucher specimens were herbarized and deposited in Calicut University herbarium (CALI-123785). The adventitious roots from healthy twigs were collected in sunny days at peak mitotic activity time. Cleaned roots were subjected to pretreatment in chilled paradichlorobenzene. They are first kept at 4ºC for 5 min and later 3 h in 16–18ºC for better separation of chromosomes. The pretreated roots were washed in distilled water twice and fixed in modified Carnoys fluid overnight. Hydrolysis was done. It is followed by aceto-orcein staining for 3–4 h. Destaining was made in 45 % acetic acid. It is followed by slide preparation by squash techniques (Sharma & Sharma 1990). Slides were observed under 100X of light microscope (Olympus CX21FSI, binocular research microscope, Japan) and images were taken by using an AmScope MU Series digital camera fitted into it.

Photographs with clear visibility of chromosomes were analysed in triplicate to prepare the karyograms and idiograms. Computer based programs such as AutoCAD, Adobe photoshop, Microsoft Paint and data based analyzing system such as Microsoft Excel were used for this. Based on the centromeric positions, length of arms were measured and chromosomes were identified. Numbering is done in the decreasing order of chromosome lengths. On the basis of chromosome length, arm ratio and centromeric indices, homologous chromosome pairs were identified and were

Figure 1 Habit of *Strobilanthes hamiltoniana* (Steud.) Bosser & Heine. Inset: enlarged view of inflorescence

classified according to Abraham & Prasad (1983). With the help softwares paired chromosomes were subjected to karyogram preparation.

The data represented throughout the work were subjected to statistical analysis using IBM SPSS Statistics Version 20. The data obtained were analyzed in One-Way ANOVA followed by the Duncans Multiple Range Test (DMRT) to confirm the variability of data and validity of results. All the values were expressed in mean ± standard error. The statistical significance was determined with $p<0.05$, which is considered significant.

RESULTS AND DISCUSSION

The annually flowering garden plant *S. hamiltoniana* have shown two different karyotypes (Fig. 2). The two different chromosome counts such as 2n = 10, 20 with karyotype formulae as 10 nm and 20 nm respectively (Tables 1, 2). The species has nearly median chromosome pairs in both hypoploid and somatic cells. The chromosome length was 9.88 and 18.07 μm in cell with 2n = 10 and 20 respectively. In somatic chromosome, 2 pair of chromosomes were having secondary constrictions with an average chromosome length of 1.81 μm (Table 3). The karyomorphometric parameters were calculated and tabulated to analyse the symmetry of karyotype. The karyograms and idiograms were also drawn (Fig. 3).

The cytogenetical characterisation of *S. hamiltoniana* revealed a mixoploid karyotype. Mixoploidy or polysomaty is the presence of diploid and polyploid cells within a root meristem in which dominating cell fraction will be diploid (Chembrammal & Thoppil 2021). The somatic chromosome in this study was found to be 2n = 20 with a base number as x = 10. In diploid as well as variant cells all the chromosomes were nearly median with karyotype formulae as 20 nm and 10 nm respectively. The presence of median/nearly median chromosomes signifies the primitiveness and the terminal centromeres represent the advanced ones (Imai et al. 2001). So, the species under investigation point out the primitive nature of the karyotype. The previous reports of the genus also convey the centromere position in the genus as mainly median to sub-median with a few having subterminal ones (Govindarajan & Subramanian 1983, 1985).

According to Vimala et al. (2021) karyotype description includes chromosome number, genome size, the absolute and relative size of a chromosome, centromere position leading to the symmetry of chromosome, number and position of secondary constriction /satellite and heterochromatin distribution. Chromosome number is an important parameter as it gives substantial information about a species (Anil et al. 2013). The value of the range of chromosomes of this species stands within the limits of the *Strobilanthes* which was given as 4.5–0.8 μm (Chembrammal & Thoppil 2021). The total chromosome length in diploid and hypoploid cells of *S. hamiltoniana* does not accompany exact duplication. Hence, it cannot be called true polyploidy. The changes in the number and structure of chromosomes are being linked to the evolution and speciation of plants (Astuti et al. 2017). The number of chromosomes with secondary constriction was 2 and 4 in variant and normal cytotype. The position of the centromere is the landmark of the chromosome while considering the chromosome morphology.

Karyotypic analysis has been successfully employed to estimate the intraspecific and interspecific levels of taxo-

Table 1. Detailed karyomorphometric data of *S. hamiltoniana* somatic variant cytotype with 10 chromosomes, all nearly medium. ***** – chromosome with secondary constriction, values are expressed in Mean ± SE.

Number of chromosome pairs	Total length of chromosome (μm)	Short arm length (μm)	Long arm length (μm)	Arm ratio 1, R_1 (s/l)	Arm ratio 2, R_2 (l/s)	Centromeric index 1, I_1 (s/c%)	Centromeric index 2 I_2 (l/c%)
2*	2.80±0.44	1.15±0.16	1.55±0.09	0.77±0.04	1.40±0.23	42.63±4.41	57.37±4.42
2	2.20±0.30	1.05±0.16	1.16±0.14	0.90±0.01	1.12±0.06	47.19±1.28	57.81±1.28
2	1.83±0.44	0.89±0.23	0.76±0.09	0.94±0.09	1.07±0.02	48.34±0.28	51.66±0.28
2	1.63±0.25	0.75± 0.14	1.03±0.18	0.86±0.09	1.19±0.14	45.92±2.70	54.08±2.70
2	1.42±0.17	0.66±0.08	0.88±0.13	0.87±0.05	1.17±0.12	46.29±2.52	53.71±2.52

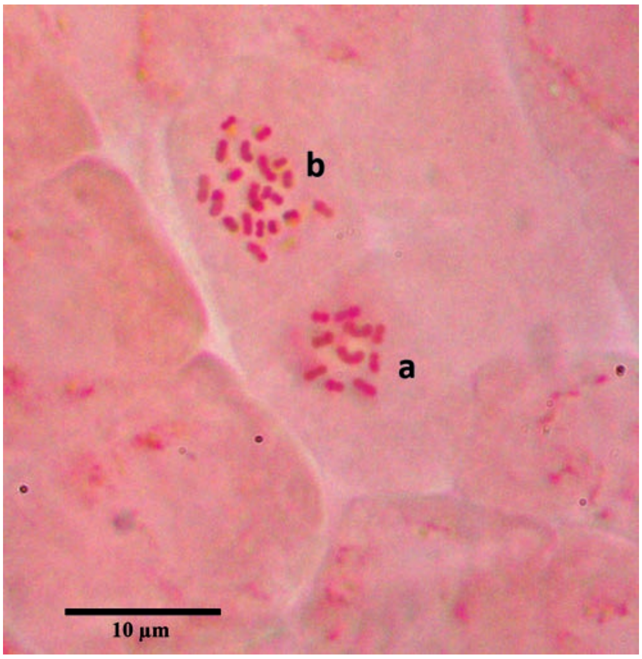

Figure 2. Somatic metaphase chromosome complements of *Strobilanthes hamiltoniana*; A – variant cytotype with 10 chromosomes; b – normal diploid with 2n = 20 chromosomes

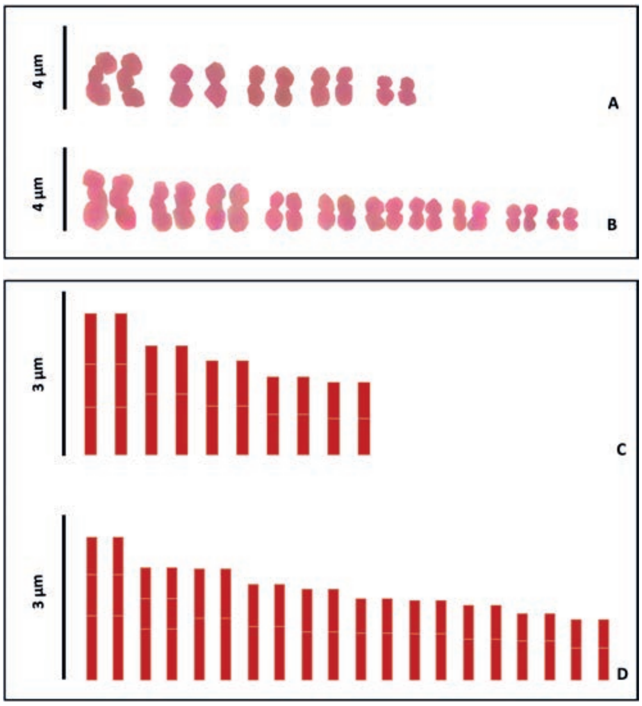

Figure 2. Mitotic chromosome complement images of *Strobilanthes hamiltoniana*. A – karyograms of variant cytotype with 10 chromosomes; B – karyograms of normal cytotype with 2n = 20, C–D – respective idiograms

nomic relationships between species (Beevy & Bai 2013). A combination of parameters was analysed to conclude the symmetry of the species. The dispersion index (DI) is used to differentiate closely related karyotype asymmetry in evolutionary order, easing the development of species (Vimala et al. 2021). This proportionate measure of the centromeric gradient to the coefficient of variation for chromosome length was very low in *S. hamiltoniana* indicating the poorly specialized karyotype (Lavania & Srivastava 1992). It has importance in arranging the species within the same class of karyotype asymmetry by permitting further gradations, as depicted by species rearrangements within sections (Hamideh et al. 2009). Total forma percentage (TF%) is widely used to evaluate the karyotype symmetry/asymmetry and the karyotypic relationship between species (Costa & Forni-Martins 2003). The highest TF% value in this plant is following with the previous reports suggesting the possible symmetric karyotype of the species (Biswas & Mukhopadhyay 2020).

The two indices developed by Greilhuber & Speta (1976) and later called by Venora were Syi and Rec indices. The higher value of Rec index, Syi index and lower values of Karyotype ksymmetry index (As K%) extent the symmetry of karyotype and decreases the probability for asymmetry (Saha et al. 2021, Prajitha & Thoppil 2018). In respect to centromere position karyotype, symmetry can be studied using As K%, intrachromosomal asymmetry index (A1), interchromosomal asymmetry index (A2) and degree of asymmetry of karyotypes (A) indices. The high symmetric and more primitive karyotype is depicted by the closer 50 % value of As K% (Ye et al. 2014). Accordingly in this investigation, the species has recorded 54.45 and 55.29 % for hypoploid and diploid complements respectively. When the centromere shifts from median/submedian to terminal/

Table 2. Detailed karyomorphometric data of *S. hamiltoniana* normal cytotype with 20 chromosomes, all nearly medium. * – chromosome with secondary constriction, values are expressed in Mean ± SE.

Number of chromosome pairs	Total length of chromosome (µm)	Short arm length (µm)	Long arm length (µm)	Arm ratio 1, R_1 (s/l)	Arm ratio 2, R_2 (l/s)	Centromeric index 1, I_1 (s/c%)	Centromeric index 2 I_2 (l/c%)
2*	2.78±0.42	1.25±0.22	1.53±0.20	0.81±0.05	1.25±0.07	44.60±1.45	55.39±1.45
2*	2.26±0.37	0.99±0.15	1.27±0.23	0.79±0.03	1.27±0.05	44.00±1.03	55.94±1.03
2	2.07±0.16	0.97±0.11	1.20±0.06	0.88±0.06	1.14±0.07	46.70±1.68	53.26±1.68
2	1.87±0.18	0.83±0.04	1.04±0.14	0.83±0.08	1.24±0.12	44.96±2.58	55.04±2.58
2	1.77±0.19	0.83±0.07	0.94±0.12	0.89±0.05	1.12±0.06	47.17±1.45	52.82±1.45
2	1.63±0.20	0.67±0.11	0.92±0.15	0.75±0.08	1.36±0.14	42.61±2.51	57.39±2.51
2	1.54±0.22	0.65±0.08	0.90±0.13	0.76±0.05	1.34±0.09	42.94±1.76	57.06±1.76
2	1.47±0.22	0.66±0.09	0.80±0.14	0.84±0.05	1.19±0.07	45.77±1.41	54.22±1.41
2	1.50±0.05	0.54±0.08	0.76±0.09	1.04±0.33	1.15±0.32	48.74±7.56	51.26±7.56
2	1.18±0.16	0.55±0.08	0.63±0.08	0.87±0.03	1.15±0.04	46.50± 0.91	53.50 ± 0.91

Table 3. Summary of karyomorphometrical data of different cytotypes of *S. hamiltoniana.*

Cromosome charactericties	Somatic chromosome number	
	10	**20**
Karyotype formula	10 nm	20 nm
Number of chromosomes with secondary constriction	2	4
Range of chromosome length (RCL)	2.8–1.42 μm	2.78–1.18 μm
Total chromosome length (TCL)	9.88 μm	18.07 μm
Average chromosome length (ACL)	1.98 μm	1.81 μm
Dispersion index (DI)	12.35	15.82
Total forma percentage (%)	45.54	43.94
Karyotype asymmetry index (As K%)	54.45	55.29
Syi index	84.29	79.07
Rec index	70.57	65
Intrachromosomal asymmetry index (A1)	0.14	0.21
Interchromosomal asymmetry index (A2)	0.26	0.27
Degree of asymmetry of karyotypes (A)	0.08	0.12

subterminal the intrachromosomal asymmetry increases. According to Romero-Zarco (1986) the intermediate value for A1 ranges between 0.18–0.22. The A1 and A values increase with an increase in asymmetry (Zuo & Yuan 2011). So, the minor values of A1 and A is due to the decreasing asymmetry leading to symmetric karyotype.

Polyploid mixoploidy is the most common mixoploidy seen in Angiosperms (Ranjbar et al. 2011). In other words, mixoploidy or the intra-individual variation in chromosome number is the presence of more than one chromosome number in a cellular population (Pierre et al. 2011). It is reported in *in vitro* propagation, in which the major report was in anther culture (Xin-Hua et al. 2010). Their presence is mostly reported in genera having small-sized chromosomes (Truta et al. 2011), in *Strobilanthes* it was first repoted in the species *S. virendrakumarana* (Chembrammal & Thoppil 2021). The average chromosome size in this species was found to be 1.98 and 1.81 μm in variant and normal cytotypes respectively reveals the small size of the chromosome. The adaptivity of a plant can be enhanced by a high level of mixoploidy which lead to genome plasticity (Kunakh et al. 2008).

By increasing the chromosome number by fragmentation, greater variability and adaptability will be acquired (Mola & Papeschi 2006). When plants are exposed to abiotic stresses karyotypes tend to evolve to polyploidy which is suitable for the environment (Mayrose et al. 2010). It is believed that polysomaty arises from diploid meristems on a particular stage of ontogenesis. It is most common in plants with vegetative and apomictic propagation (Kunakh 1995). The instability in cell genome within a meristem can induce ability for hybridisation and polyploidisation. These in turn can lead to epigenetic and epigenomic rearrangements (Kunakh et al. 2008). The interpretation of the evolution of a karyotype is based on chromosomal structural constraints and the degree to which persistent epigenetic chromatin alterations exist in a species (Vimala et al. 2021).

CONCLUSION

Chromosome studies of *S. hamiltoniana* revealed 2n = 20 with a base set of 10 chromosomes. The results point out the symmetric karyotype of the plant in the somatic variant as well as normal cytotype. While correlating all the assessed parameters the species seems to be more symmetric with equal-armed chromosomes. As the centromere shifts the asymmetry increases. The mixoploid condition might have arise to overcome the bottle neck effect of the environment. The findings are an addition to the chromosome data base for further tracking of the phylogenetic relationships of the genus.

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