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:** mixoploid, *Strobilanthes hamiltoniana*, karyotype, symmetric, polysomaty

**MATERIAL AND METHODS**

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 advanced 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.

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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 taxa-

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 (s/l) | Centromeric index 1, I1 (l/c%) | Centromeric index 2, I2 (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±2.42                   |
| 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±2.28                   |
| 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±0.08           | 1.17±0.12                      | 46.29±2.52                    | 53.71±2.52                   |
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 (Hameed 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 hyploid and diploid complements respectively. When the centromere shifts from median/submedian to terminal/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, R1 (s/l) | Arm ratio 2, R2 (l/s) | Centromeric index 1, I1 (s/c%) | Centromeric index 2 I2 (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                  |

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
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 reported in the species S. virendrakumanana (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 polysomy 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).

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**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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