Karyotic analysis among populations of *Senna spectabilis* (DC.) H.S. Irwin & Barneby (Leguminosae: Caesalapinioideae), an invasive alien species of South India

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Abstract

Senna spectabilis is an invasive alien species known for its extremely fast-growing habit, profusely flowering and fruit setting nature, and high coppicing ability. In India, its introduction is not well documented,which probably may have been introduced as an ornamental plant. Presently this species has aggressively spread across the moist deciduous forests of Wayanad wildlife sanctuary in the Western Ghats, peninsular India,with adverse impact on the survival of the indigenous species of flora and fauna. The present study used six distinct populations of the species, Anakkatty, Muthanga, Vythiri, Azhnjilam, Thiruvanathapuram and Munnar karyotype analysis. In mitotic metaphase, cells of *S. spectabilis* showed a stable chromosome count of 2n = 28, x =14. The statistical analysis shows that there was a significant difference between the characters studied. The observations were compared with the chromosome characters reported from native *S. spectabilis* population. While it was found that the chromosome number remainsthe same 2n = 28, there were differences with most of the characters studied. The study points to the requirement for more intense taxonomic studies and monitoring of this species and its populations.

Keywords: Cassia, Invasive alien species, Karyotype, *Senna spectabilis*, Western Ghats.

Introduction

Species that cross over their natural distribution and get introduced to new habitats are known as alien species or exotic species. Alien species that colonize or invade their new habitat threatening biological diversity, ecosystem, and human wellbeing, is considered an invasive alien species (IAS). This process is referred to as biological invasion (Gaertner et al., 2009). Biological invasions are regarded as one of the greatest current threats to global biodiversity.Invasive species enter a new environment through many routes. Most are introduced intentionally for a variety of purpose like agriculture, forestry, aesthetic values,etc., while some are introduced accidentally. More than 35% of plants introduced into India are native to South America (Khuroo et al., 2012). Close to 2500 plants are today recognised as IAS (Pagad et al., 2018).

Senna spectabilis (DC.) H.S. Irwin & Barneby, a tree species native to Tropical America, is listed in the global compendium of weeds as an 'environmental weed', 'garden thug', and 'naturalised weed', (Randall, 2007). Senna grows quickly, flowers and sets seed profusely, and coppices when cut (Mungatana and Ahimbisibwe, 2010). *Senna spectabilis* can tolerate a range of soil types and can reportedly adapt to alkaline soil (Gillman and Watson, 2011). The spread of the invasive alien plant *S. spectabilis* is posing a serious threat to wildlife and indigenous flora in the forest areas of the Nilgiri Biosphere Reserve, including the Wayanad Wildlife Sanctuary (WWS), a major

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habitat of Asiatic elephants in India. The region has a nearly 300 sq km stretch, including the Wayanad Wildlife Sanctuary, North and South Wayanad Forest Divisions, and the adjacent Mudumalai, Bandipur, and Nagarhole Tiger Reserves, have been infested by this invasive species (Hrideek et al, 2020). Further, this species has been established all along the Western Ghats, and today distinct discontinuous populations are thriving in various parts of peninsular India.

Ploidy level and karyotype analysis is the first step in the genetic study of plants. Karyotype analysis is a technique to identify and evaluate the size, shape, and number of chromosomes in a biological organism. Chromosome identification is critical for cytological analyses and subsequent studies in genomics, taxonomy, and the evolution of polyploidy, enabling an understanding of the relationship between visible landmarks and genetic or physical map features (Harper and Cande, 2000). It often contributes to the information on genetic barriers to gene flow between species; hence, its role in species diversification has been heavily discussed (Wright, 1978; King, 1993; Rieseberg, 2001). Meiotic analysis, involving studies of the pairing and recombination of chromosomes, the passing of chromosomes to the next generation via gametes, and sometimes revealing structural changes in the chromosomes, is an important tool in the understanding of the nature of genome organization in a species (Griffiths et al., 2015). The construction of species karyotype, with unambiguous identification of individual chromosomes, is critical for integrating genetic and physical map data. Karyotype differences between ecotypes aid in identifying and handling diverse gene pools for devising control strategies of IAS. (Young et al., 2012).

The chromosome number of *Senna* species are 2n = 22, 24, 26, and 28 (Irwin and Barneby1981;1982). Resende et al. (2013) recorded varying chromosome number (n= 12, 13, 14, and 28) in this genus. Based on the centromeric index, 4 types (Type A, B, C and D) of the chromosome were found in *Cassia sp* (Mohanty and Das, 2006), and the authors concluded 2n=28 for *Cassia senna* (*S. spectablis*). The present study is an effort to undertake the karyotype analysis of *S. spectabilis* in different distinct populations of Kerala state to find out the variationsin karyotype, if any, among these populations.

Materials and Methods

The seed samples for the study were collected from different areas of Kerala during the summer of 2019 (Table 1), and seeds germinated in the nursery at Kerala Forest Research Institute, Trichur.

Table 1. Populations of *Senna spectabilis* sampled for Karyotype Analysis

Location	Latitude °N	Longitude °E	Altitude(m) (Msl)
Muthanga wildlife sanctuary	11.66	76.39	875
Vythiri	11.55	76.03	789
Anakkatty	11.11	76.74	527
Thiruvanthapuram	8.50	76.95	6.23
Azhinjilam	11.98	75.86	2.6
Munnar	10.14	77.17	1712

For the cytological observations, vigorously growing root tips were subjected to pre-treatment. Root tip was collected before 10 AM and then pre-treated in 0.2 M of 8-hydroxyquinine (0.29 g of hydroxyl quinoline dissolved in 100ml distilled water) for three hours and fixed overnight in freshly prepared Carnoy's fluid (60% ethanol, 30% chloroform, and 10% glacial acetic acid). The root tip was cut without any prior softening. The entire cross-sections of the root were excised by dabbing the surface with absolute alcohol with a fine brush before cutting since alcohol hardens the tissue immediately. Samples that are water-saturated and very soft are placed for 24 hrs in a solution of 30, 60 and 100 % ethylene glycol 4000 and kept in an oven.

The fixed sample was hydrolysed in 1N HCl for eight minutes at room temperature after washing with distilled water, stained with 1% aceto-orcein

for 15 min, and squashed on a glass slide after slight warming. The squashed cells were observed under a microscope (Olympus BX 61 TRF motorized microscope with cytovision 3.92), and photographs were taken by using cytovision 3.2 software. The observations were repeated several times from different sets of slides. A minimum of five mitotic cells was used to determine chromosome number. Characters such as somatic chromosome number, genomic chromosome length, total form percentage, total chromosome length, genomic chromosome volume were estimated.

Analysis of variance (ANOVA) was carried out to test the significance of variations between the various characters among the populations.

Results and Discussion

Results of the karyotype analysis among the six populations *viz.*, Anakkatty, Muthanga, Vythiri, Azhijilam, Munnar, and Thiruvananthapuram are given in Fig 1. All six populations in mitotic metaphase cells of *Senna spectabilis* showed a stable

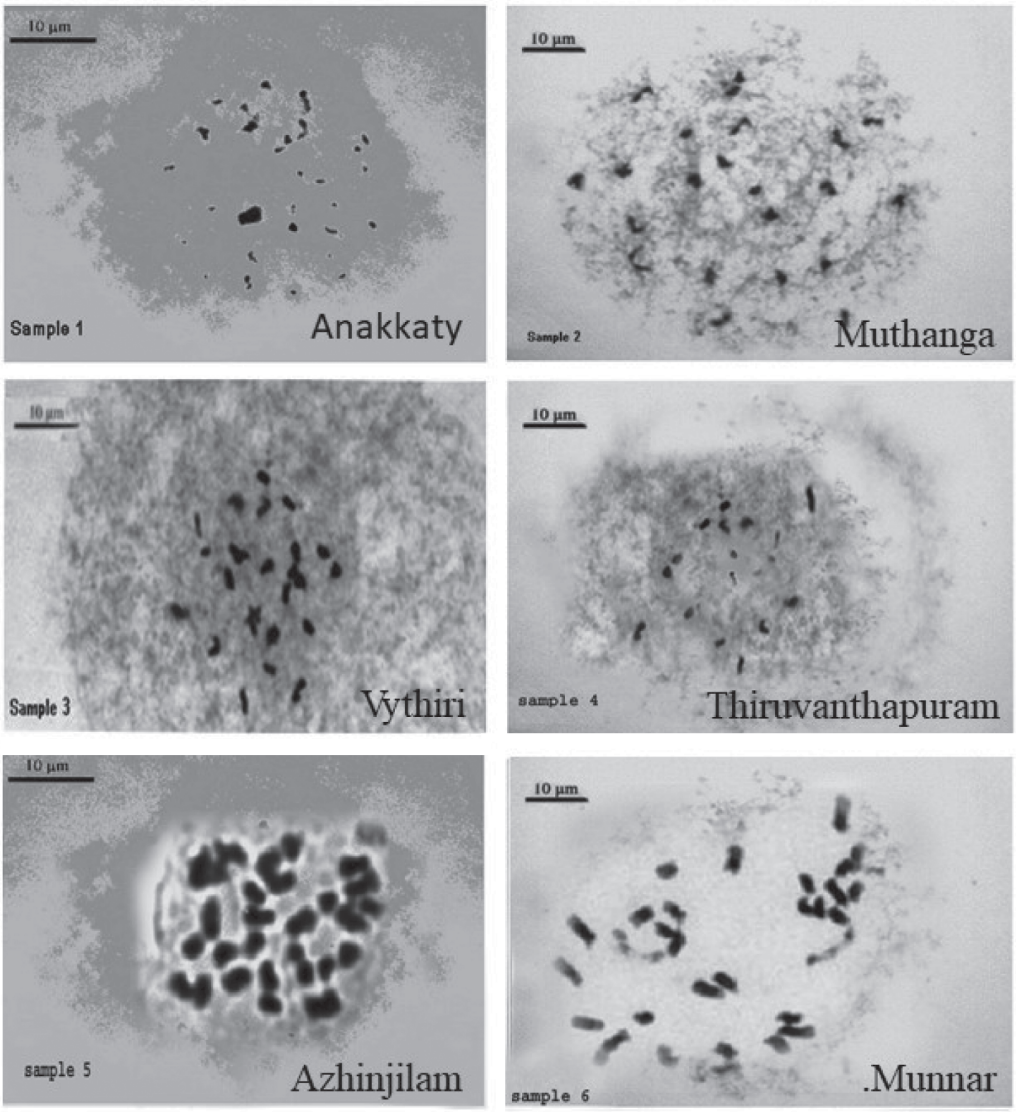

Figure 1. Somatic chromosome plates in the species of *Senna spectabilis* (2n = 28)

Table 2. The Karyotype details of the characters studied

Population	Chromosome number (2n)	Chromosome length (µm)	Total genomic chromosome length (µm)	Total Chromosome volume (µm^3)	Total form %	Arm ratio
Anaikkatty	28	1.34	28.45 ± 0.04	7.88 ± 0.06	36.53 ± 0.01	1.25 ± 0.01
Muthanga	28	1.35	26.33 ± 0.21	8.44 ± 0.02	37.46 ± 0.01	1.26 ± 0.00
Vythiri	28	1.33	27.08 ±0.01	8.73 ± 0.01	37.53 ± 0.01	1.26 ± 0.00
Thiruvanthapuram	28	1.33	27.60 ± 0.02	8.93 ± 0.01	37.62 ± 0.02	1.26 ± 0.00
Azhinjilam	28	1.34	26.92 ± 0.02	8.77 ± 0.00	37.54 ± 0.00	1.26 ± 0.00
Munnar	28	1.34	27.86 ± 0.02	8.42 ± 0.03	36.94 ± 0.04	1.26 ± 0.00
SEM	0.09*	0.03*	0.02*	NS		
Mohanty et al, 2006	28	1.33	22.85	9.50	38.55	1.32

* indicates significant differences at 5 % of significance, NS indicates non significant differences.

chromosome count of 2n= 28, x=14 (Table 2). When compared with the karyotype analysis of native *S. spectabilis* specimen collected from Royal Botanic Gardens, Kew by Mohanty and Das (2006), it was observed that the chromosome number remained the same 2n =28 (Table 2). The chromosome length of the native specimen was 1.33 µm, whereas in populations collected from Kerala did not show significant variation. The sample collected from Muthanga had the highest chromosome length (1.35 µm), and Vythiri and Thiruvananthapuram had the lowest chromosome length (1.33µm). The arm ratio of the mitotic chromosome of the five populations were 1.26, while that of the Anainkatty population was 1.26 (Table 2). While no significant differences were observed among Kerala populations, these values were less than the native specimen record of 1.32. The statistical analysis showed a significant difference between the rest of the characters studied. The characters studied, such as chromosome length, total genomic chromosome length, total chromosome volume, and total form percentage, showed statistically significant variations among the populations.

The total genomic chromosome length of *S. spectabilis* ranged from 26.33µm to 28.45 µm. Compared to other samples, the sample from Anaikkatty had the highest total genomic chromosome length (28.45 µm), and Muthanga had the lowest one (26.33µm).In the case of total genomic chromosome length, present values ranged from 25.85 µm to 28.41µm, where as the plants from the native region had a genomic chromosome length of 22.85 µm. The total chromosome volume of *S. spectabilis* varied from 7.88 µm^3 (Anaikkatty) to 8.93 µm^3 (Thiruvananthapuram). The total chromosome volume with the native specimen was 9.5 µm^3, clearly above the values recorded from Kerala. The total form percentage of *S. spectabilis* collected from six locations ranged from 36.53% (Anainkatty) and 37.62% (Thiruvananthapuram), which was lower than the observation from the native specimen (38.55). Statistical analysis of the observations provides evidence of chromosome variations in the populations in Kerala. Such differences in measurements could result from variations in the level of condensation during processing. Further more, this genus is considered highly unstable too, and these differences could point to the requirement of a larger, more indepth study.

Genus *Senna* was earlier considered as a part of the larger genus *Cassia*. The taxonomically complex genus *Cassia* with close to 500 species exhibits great diversity. There has been a great deal of disagreement about this plant group's delimitation and taxonomic position. Considerable debate in the taxonomic positions of many species in these genera still remains. The genus was initially divided into three subgenera *viz., fistula*, *senna,* and *lasiorhegma* (Bentham, 1871). Britton and Rose (1930) favoured splitting *Cassia* into twenty-eight genera. Irwin and Barneby (1981) recognised *Cassia*, *Senna,* and *Chamaecrita* as three subgenera of the genus *Cassia*. Later, Irwin and Barneby (1982) proposed a new classification elevating both *Senna* and *Chamaecrita*

to generic levels. The confusions on the genus Cassia's taxonomic status remain an enigma since the taxonomic distinctions between and within the three genera are blurred by polyploidy, hybridization, and apoximis (Lewis et al., 1980). For example, Isley (1975) recognised *Cassia excelsa* and *C. spectabilis* as two separate species, while, Irwin and Barneby (1982) considering the overlap of the measurements, recognised them as two varieties of the species *S. spectabilis.* The native Brazilian population was recognised as var *excelsa*, while the more commonly cultivated variety was recognised as var *spectabilis*. (PIER, 2014).

One of the most important features of an invasive species is its broad adaptation to a range of biotic and abiotic factors. This adaptation could be through genetic or plastic changes. Considerable work needs to be done on this invasive population in South India toarrive at conclusive evidence on taxonomic relationships and evolutionary changes in detail.

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