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Research Article

Karyomorphology of Two Species of the Genus Spilanthes (Heliantheae-Asteraceae)

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Abstract

In present investigation karyotype of mitotic chromosomes were determined, two species of the genus Spilanthes, namely *Spilanthes acmella* (L.) Murray and *Spilanthes calva* DC. from plants transplanted in earthen pots collected from different localities from Central Nepal in my home garden. The somatic chromosome number determined in present study were 2n=36 for both species, *Spilanthes acmella* (L.) Murray and Spilanthes *calva* DC. The chromosomes total length ranged from 0.6 to 1.6 µm in *Spilanthes acmella* (L.) Murray and 0.4 to 2.1µm in Spilanthes *calva* DC. The relative length ranged from 2.3 to 12.1 µm in the species *Spilanthes calva* and 2.0 to 8.5 µm in the species *Spilanthes acmella*. The Karyotype formula were $M_{16}+m_2+sm_{16}+st_2$ in *Spilanthes calva* DC. and M_{22} $+sm_{14}$ in *Spilanthes acmella* (L.) Murray cytologically obtained by using 2% aceto-orcein. The karyotype of the two species shows considerable variation exists in morphology of chromosome and structure. The variation in karyomorphology shows evolved nature of this species which play a great role in evolution.

Keywords: Karyomorphology; aceto-orcein; Asteraceae; Chromosome number

Introduction

Cytogenetic studies have long been contributing to increase evolutionary, phylogenetic and taxonomic inferences about various plant groups (Stebbins 1971). Karyotype can be considered as the phenotypic aspect of the chromosome complement at mitotic metaphase. The taxa *Spilanthes acmella* (L.) Murray and *Spilanthes calva* DC. are refers to the important medicinal plant distributed in the tropical and subtropical regions around the world with rich source of medicinal uses. The genus *Spilanthes* had 35 species distributed globally and two species *Spilanthes acmella* and *Spilanthes calva* reported from Nepal (Press *et al*., 2000). The plant *Spilanthes calva*, locally called Marethi in Nepal. Plant paste is applied on snake bite (Manandhar, 2002). *Spilanthes acmella* is called Bhuin timur or Laato Ghaans in Nepal. The plant paste is applied to snake bites. Their flower heads are pungent and chewed in case of toothache (Manandhar, 2002).

Many reports are available on the chromosome numbers and the karyotype of the genus *Spilanthes.* Most of these studies have only been based on chromosome counts. The chromosome number is an important character in plant

cytotaxonomy and may provide information on polyploidy (Martin *et al*.2015). The chromosome morphology is commonly used in plant taxonomy and these data are also useful for clarifying the origin, speciation and phylogenetic relationships of plants.

Sousa *et al*. (2012) studied twelve species of *Lippia* L. (Verbenaceae) karyomorphologically from Brazil. Mallick *et al*. (2013) studied ten species of Asteraceae karyomorphologically from Nepal. Basumatari and Das (2017) studied two species of *Bauhinia* Linn. from India. Large numbers of plants species are endemic to Nepal and poorly studied especially at a cytological level. Very often chromosome number variation has been used to understand karyotype evolution and three basic mechanisms could be suggested to explain those variations at genus level. Polyploidization is one the most important mechanisms of angiosperms evolution, probably due to the success of the hybrids comparing with their diploid parents (Stebbins 1971). Another possible important evolutionary event is aneuploidy. The third mechanism is the dysploidy, in contrast to polyploidy, induces an alteration on the average length of the chromosomes. The main objective of present study is to determine somatic chromosome number and karyomorphology of two species of the genus Spilanthes.

Materials and Methods

The plants were collected from Kathmandu, Nepal and transplanted in earthen pots at my home garden. Somatic chromosomes were prepared from healthy root tips. They were pretreated with aqueous solution of 0.002M 8-hydroxyquinoline for three hours. The material was fixed in mixture of absolute ethanol and glacial acetic acid (3:1) for one day. Chromosome preparations were made in Central Department of Botany of Tribhuvan University. In the laboratory root tip materials were hydrolyzed and stained in a mixture of 2% aceto-orcein and 1N HCl (9:1) contained in watch glass and warmed for few seconds and left for 30 minutes to 1 hour. Squashes were made in 45% acetic acid. The observations were done from this preparation to select the plates for photomicrography. The drawings were made at table level using opcolite-1366 Camera Lucida apparatus. Photomicrographs were taken with the help of digital camera of 12.1 megapixel using 10 x eye pieces and 100x objective of trinocular compound microscope. For karyotype studies at least three different preparations were made from root tips. Chromosomes were measured from the drawn figures. The methodology was followed according to Levan *et al*. (1965).

Results and Discussion

Spilanthes calva DC.

The plant is annual, rarely perennial herbs, often prostrate and rooting at nodes, found often in damp places. Leaves opposite, simple, ovate to linear, entire or sinuate, glabrous. Capitula radiate, many-flowered, usually on long peduncles, terminal or axillary. Involucre campanulate, bracts in 2 or 3 rows, outer membranous, shorter than receptacle. Receptacle convex, sometimes elongated, paleates, boat-shaped, enclosing florets. Ray florets female, fertile, corolla yellow or white, strap-shaped, tube hairy, with elliptic, usually 2-lobed lamina somewhat longer than tube. Disc florets bisexual, fertile; corolla yellow, tubular below, expanded above, 4- or 5-toothed. Anthers with small, obtuse apical appendage, base obtuse or minutely sagittate. Style branches of ray florets linear, of disc florets oblong, truncate, minutely penicillate. Cypsela elliptic or obovate, 3- or 4-angled, compressed, margins hyaline or ciliate. Pappus 0 or of 2 or 3 bristles.

The somatic chromosome number determined for this taxon is 2n=36. The somatic chromosome number determined from the root tip cell is shown in Fig. 1b and camera lucida drawing in Fig. 1c. Its ideogram is represented in Fig.1-d. The chromosome measurements are given in Table 1.

The karyotype consists of 4 different types of chromosomes with centromere at median point, median, sub median and sub terminal regions. The chromosome length ranged from 0.4 to 2.1µm with mean length 0.9µm. and absolute length 16.9µm. TF % was 39.0. Karyotype formula is M16+m2+sm16+st2.

Table 1: Chromosome measurement in *Spilanthes calva* DC.

Chromosome Pairs	Long Arm (µm)	Short Arm (µm)	Total Length (µm)	r- value	Relative Length (µm)	Position of Centromere
I	1.3	0.8	2.1	1.6	12.1	m
II	0.8	0.8	1.6	1	9.4	M
III	0.8	0.4	1.2	2	7.1	sm
IV	0.8	0.4	1.2	2	7.1	sm
V	0.8	0.4	1.2	2	7.1	sm
VI	0.8	0.4	1.2	2	7.1	sm
VII	0.8	0.4	1.2	2	7.1	sm

Table 1: Chromosome measurement in *Spilanthes calva* DC. (Contd.).

Chromosome Pairs	Long Arm (µm)	Short Arm (µm)	Total Length (µm)	r- value	Relative Length (µm)	Position of Centromere
VIII	0.8	0.2	1	4	5.9	st
IX	0.4	0.4	0.8	1	4.7	M
X	0.4	0.4	0.8	1	4.7	M
XI	0.4	0.4	0.8	1	4.7	M
XII	0.4	0.4	0.8	1	4.7	M
XIII	0.4	0.2	0.6	2	3.5	sm
XIV	0.4	0.2	0.6	2	3.5	sm
XV	0.4	0.2	0.6	2	3.5	sm
XVI	0.2	0.2	0.4	1	2.3	M
XVII	0.2	0.2	0.4	1	2.3	M
XVIII	0.2	0.2	0.4	1	2.3	M

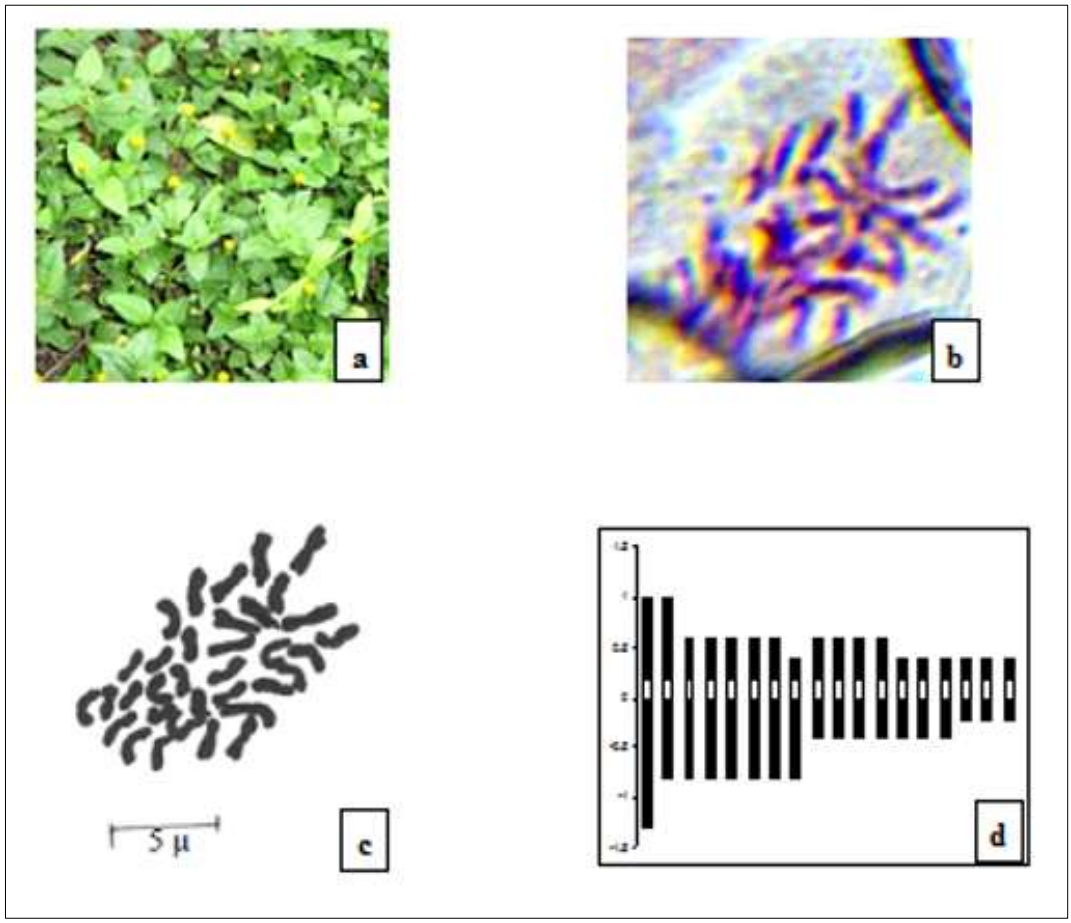

Fig. 1: *Spilanthes calva* DC**. a.** Photograph of living plant of *Spilanthes calva*;
b. Photomicrograph of somatic metaphase plate of *Spilanthes calva***;**
c. Camera lucida drawing of the same; **d.** Ideogram of the above.

Spilanthes acmella (L.) Murray (2n=36)

The plant annual ascending herb, 10 cm tall, is branching more or less, hairy. Leaves triangulate, opposite, 2.5-by1.3-3.8 cm, ovate, acute or sub optuse, irregular crenate-serrate or sometimes entire, glabrous, base usually acute, petioles 0.6-1. 6cm long, pubescent. Heads 0.6-1.3 cm long, ovoid, solitary. Involucral bracts oblong-lanceolate, subacute, pubescent, less than half as long as the head of flowers. Ray -flowers and ligules very often absent, the latter when present minute. Pappus 0. Achenes oblong.

Chromosome number determined for this taxon is 2n=36. The somatic chromosome number determined from the root tip cell is shown in Fig. 2b and camera lucida drawing in Fig. 2c. Its ideogram is represented in Fig. 2d. The chromosome measurements are given in Table 2. Somatic chromosomes are of two types with centromere at median point and sub-median region. The chromosomes total length ranged from 0.6 to 1.6 µm with mean length 1.1 µm and absolute length 20.1 µm. TF% was 43.5 Karyotype formula is M22 +sm14.

Reshmi and Rajalaksmi (2015) studied eight species of Genus *Spilanthes* and found the somatic chromosome number, 2n=52 to 78. Rajalaksmi and Jose studied also four species of *Spilanthes* and somatic chromosome number found to be2n=52 to 78. Length of Chromosome ranged from 0.52-1.6 µm. Karyotype show that considerable variation exists in chromosome morphology and structure according to them. In present investigation somatic chromosome number, 2n=36 determined for both the species, this shows polyploid nature of this genus *Spilanthes* with basic chromosome number x=12. Basic number x=12 also given by Darlington and Wylie (1955) for this genus. The size of chromosome ranged from 0.4 to 2.1 in *Spilanthes calva* and 0.4 to 1.6 in *Spilanthes acmella* in present study.

The karyotype of the two species shows considerable variation exists in morphology of chromosome and structure. The variation in karyomorphology shows evolved nature of this species which play a great role in evolution.

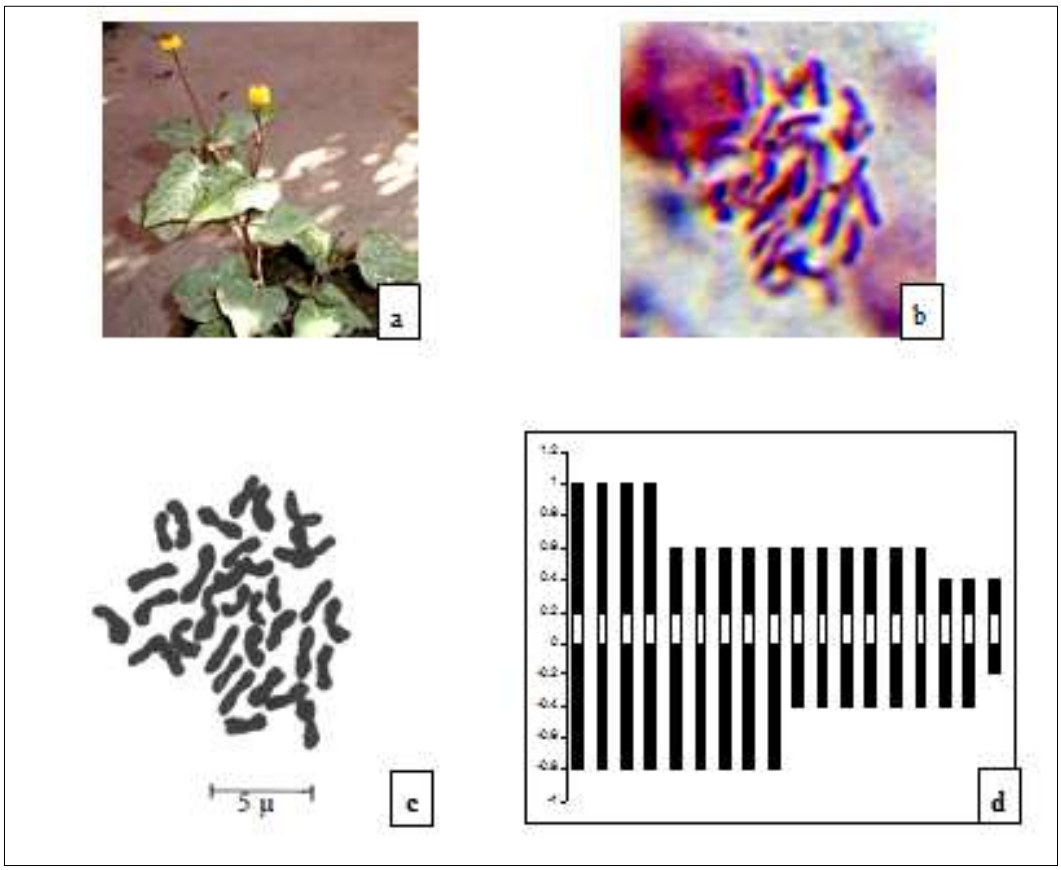

Fig. 2: *Spilanthes acmella* (L.) Murray; **a.** Photograph of living plant;
b. Photomicrograph of somatic metaphase plate.;
c. Camera lucida drawing of the same; **d.** Ideogram of the above.

Table 2: Chromosome measurement in *Spilanthes acmella* (L.) Murray

Chromosome Pairs	Long Arm (µm)	Short Arm (µm)	Total Length (µm)	r - value	Relative Length (µm)	Position of Centromere
I	0.8	0.8	1.6	1	8.5	M
II	0.8	0.8	1.6	1	8.5	M
III	0.8	0.8	1.6	1	8.5	M
IV	0.8	0.8	1.6	1	8.5	M
V	0.8	0.4	1.2	2	6.3	sm
VI	0.8	0.4	1.2	2	6.3	sm
VII	0.8	0.4	1.2	2	6.3	sm
VIII	0.8	0.4	1.2	2	6.3	sm
IX	0.8	0.4	1.2	2	6.3	sm
X	0.4	0.4	0.8	1	4.2	M
XI	0.4	0.4	0.8	1	4.2	M
XII	0.4	0.4	0.8	1	4.2	M
XIII	0.4	0.4	0.8	1	4.2	M
XIV	0.4	0.4	0.8	1	4.2	M
XV	0.4	0.4	0.8	1	4.2	M
XVI	0.4	0.2	0.4	2	0.4	sm
XVII	0.4	0.2	0.4	2	0.4	sm
XVIII	0.2	0.2	0.4	1	2.0	M

Conflict of Interest

The author declares that there is no conflict of interest with present publication.

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