

## Karyomorphological observations on some taxa of Asteraceae of Nepal

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### Abstract

Chromosome number and karyomorphology of ten taxa of Asteraceae viz. *Ageratum conyzoides* Linnaeus, *Anaphalis triplinervis* (Sims) C.B. Clarke var. *triplinervis*, *Aster peduncularis* subsp. *nepalensis* Grierson, *Bidens pilosa* var. *minor* (Blume) Sherff, *Crassocephalum crepidioides* (Benth) S. Moore, *Eupatorium adenophorum* Sprengel, *Sonchus asper* (Linnaeus) Hill, *Spilanthes calva* de Candolle, *Tagetes patula* Linnaeus, *Rhynchospermum verticillatum* Reinwart collected from different parts of Nepal were observed. Chromosome numbers in somatic cells were recorded to be  $2n = 20$  in *Ageratum conyzoides*, *Anaphalis triplinervis* var. *triplinervis*;  $2n = 40$  in *Aster peduncularis* subsp. *nepalensis*;  $2n = 24$  in *Tagetes patula*;  $2n = 36$  in *Bidens pilosa* var. *minor* and *Spilanthes calva*;  $2n = 40$  in *Crassocephalum crepidioides*;  $2n = 50$  in *Eupatorium adenophorum*;  $2n = 18$  in *Sonchus asper* and *Rhynchospermum verticillatum*. Chromosome number and karyomorphology for *Aster peduncularis* subsp. *nepalensis* studied in this research are new report. Chromosome counts of *Anaphalis triplinervis* var. *triplinervis*, *Eupatorium adenophorum*, in the present investigation new numbers of chromosomes have been reported. Chromosome counts of *Crassocephalum crepidioides*, *Rhynchospermum verticillatum* and *Tagetes patula* tally with previous reports. The chromosome number and karyotype of the taxa in the present study except 3 taxa viz. *A. conyzoides*, *C. crepidioides* and *S. asper* are new record for Nepal.

**Key words:** Asteraceae, Chromosome number, karyotypes

### INTRODUCTION

Asteraceae is one of the largest families of flowering plants which comprise ca. 1500 genera and 23000 species worldwide (Judd *et al.* 1999). In Nepal 111 genera and 417 species are reported from different regions (Press *et al.* 2000; Ohashi 1975; Hara & Williams 1979). They are mostly from temperate to tropical regions.

Cytological studies of different taxa of Asteraceae appear to be less known. However, karyomorphological studies of several species of Asteraceae have been done by several previous workers including Joshi (1988) and Malla *et al.* (1981).

Economically, the plants under present study come under one of the most important families of flowering plants having medicinal, aesthetic, and ornamental values.

Leaf juice of *Ageratum conyzoides*, *Bidens pilosa*, and *Eupatorium adenophorum* is applied externally on cuts and wounds to check bleeding (FRBG 21, 2003). Plant paste of *Spilanthes calva* is applied on snake bite, root paste of *Crassocephalum crepidioides* is applied on cuts and wounds (DPR 28, 2007). Flower paste of *Anaphalis triplinervis* is used as an antiseptic application in wounds for both human and cattle (Bhattarai 1989; Pohl 1990). Plant paste of *Sonchus asper* is applied to wounds and boils (FRBG 21, 2003). *Rhynchospermum verticillatum* is used as ornamental plants. Roots and seeds of *Tagetes patula* are purgative, flowers are of great religious value and are used in worshipping ((DPR 28, 2007).

## MATERIAL AND METHODS

Ten genera of Asteraceae were collected from different parts of the Nepal. Voucher specimens and reference slides are deposited in Central Department of Botany, Tribhuvan University, Kathmandu, Nepal.

Mitotic study was made from the healthy root tips. Roots were excised at 11 to 12 A.M. and were pretreated in 0.002 mol. 8-hydroxyquinoline for 3 hours. The pretreated root tips were fixed in glacial acetic acid and ethanol in 1:3 ratios. Fixed root tips were stained in 2 % aceto-orcein and squashed with 45 % acetic acid. For karyotype analysis, method of classifying chromosomes suggested by Leven *et al.* (1964 – 1965) was followed. Photomicrographs of desired plates were taken; camera lucida drawings and ideograms for each taxon was done.

## RESULTS AND DISCUSSIONS

Photomicrographs of desired plates (PLATE - I: Fig. 1a-10a), Camera Lucida drawings (PLATE - II: Fig. 1b-10b) and Ideograms (PLATE - III: Fig. 1c-10c) for each taxon are given below. Cytological observations are summarized in Table 1.

*Ageratum conyzoids* Linnaeus [2n = 20; Voucher No. 101]

The karyotype consists of 4 different types of chromosomes with centromere at median point, median region, sub-median region and sub terminal region. The chromosome length ranged from 0.2 to 1.3  $\mu\text{m}$  with mean length 1.4  $\mu\text{m}$  and absolute length 14  $\mu\text{m}$ . TF % is 38.5. Karyotype formula is  $M_6+m_4+sm_8+st_2$ .

*Anaphalis triplinervis* (Sims) C.B. Clarke var. *triplinervis* [2n = 20; Voucher No. 102]

The karyotype consists of 3 different types of chromosomes with centromere at median point, median region, and sub terminal region. The chromosome length ranged from 0.2 to 1.3  $\mu\text{m}$  with mean length 1.3  $\mu\text{m}$  and absolute length 13.1 $\mu\text{m}$ . TF % is 40.4. Karyotype formula is  $M_8+m_{10}+st_2$ .

*Aster peduncularis* subsp. *nepalensis* Grirson [2n = 40; Voucher No. 103]

The karyotype consists of 3 different types of chromosomes with centromere at median point, median region, and sub median region. The chromosome length ranged from 0.2 to 1.3  $\mu\text{m}$  with mean length 1.1  $\mu\text{m}$  and absolute length 23.6 $\mu\text{m}$ . TF % is 41.5. Karyotype formula is  $M_{18}+m_2+sm_{18}$ .

*Bidens pilosa* Linnaeus var. *minor* (Blume) Sherff [2n = 36; Voucher No. 104]

The karyotype consists of 4 different types of chromosome with centromere at median point, median region, sub median region and sub terminal region. The chromosome length

ranged from 0.2 to 1.3  $\mu\text{m}$  with mean length 1.0  $\mu\text{m}$  and absolute length 19.2  $\mu\text{m}$ . TF % is 38.5. Karyotype formula is  $M_{16}+m_2+sm_{16}+st_2$ .

***Crassocephalum crepidioides*** (Bentham) S. Moore [2n = 40; Voucher No. 105] The karyotype consists of 3 different types of chromosomes with centromere at median point, median region and sub median region. The chromosome length ranged from 0.2 to 1.7  $\mu\text{m}$  with mean length 1.0  $\mu\text{m}$  and absolute length 20.6  $\mu\text{m}$ . TF % is 42.2. Karyotype formula is  $M_{22}+m_2+sm_{16}$ .

***Eupatorium adenophorum*** Sprengel [2n = 50; Voucher No. 106]

The karyotype consists of 4 different types of chromosome with centromere at median point, median region, sub median region and sub terminal region. The chromosome length ranged from 0.1 to 0.8  $\mu\text{m}$  with mean length 0.7  $\mu\text{m}$  and absolute length 18.4  $\mu\text{m}$ . TF % is 34.7. Karyotype formula is  $M_4+m_{12}+sm_{30}+st_4$ .

***Rhynchospermum verticillatum*** Reinwardt [2n = 18; Voucher No. 138]

The karyotype consists of 3 different types of chromosomes with centromere at median point, median region and sub median region. The chromosome length ranged from 0.2 to 0.5  $\mu\text{m}$  with mean length 0.5  $\mu\text{m}$  and absolute length 5.1  $\mu\text{m}$ . TF % is 39.2. Karyotype formula is  $M_4+m_8+Sm_6$ .

***Sonchus asper*** (Linnaeus) Hill [2n = 18; Voucher No. 107]

The karyotype consists of 3 different types of chromosomes with centromere at median point, median region, and sub median region. The chromosome length ranged from 0.41 to 2.1  $\mu\text{m}$  with mean length 1.9  $\mu\text{m}$  and absolute length 17.3  $\mu\text{m}$ . TF % is 40.1. Karyotype formula is  $M_6+m_6+s_6$ .

***Spilanthes calva*** de Candolle [2n = 36; Voucher No. 108]

The karyotype consists of 4 different types of chromosomes with centromere at median point, median region, sub median region and sub terminal region. The chromosome length ranged from 0.2 to 1.3  $\mu\text{m}$  with mean length 0.9  $\mu\text{m}$  and absolute length 16.9  $\mu\text{m}$ . TF % was 39.0. Karyotype formula is  $M_{14}+m_2+sm_{18}+st_2$ .

***Tagetes patula*** Linnaeus [2n = 24; Voucher No. 109]

The karyotype consists of 4 different types of chromosomes with centromere at median point, median region, sub median region and sub terminal region. The chromosome length ranged from 0.4 to 1.7  $\mu\text{m}$  with mean length 1.7  $\mu\text{m}$  and absolute length 21.2  $\mu\text{m}$ . TF % was 40.5. Karyotype formula is  $M_8+m_8+sm_6+st_2$ .

Previous cytological works of Mehra & Remanandan (1975), Gaonkar & Torne (1991) showed two different haploid numbers ( $n = 10, 20$ ) in *Ageratum conyzoides*. Maharjan (1977) reported  $n = 20$  and Nirmala & Rao (1981) reported  $2n = 20$  in this taxon. Present study with  $2n = 20$  in this taxon tallies with the result of Nirmala & Rao (1981). According to the results of Morton (1993) and Dey (1979), the diploid number is  $2n = 40$  in this plants. However, Husaini & Iwo (1990) confirmed the number  $n = 18$ . *Ageratum conyzoides* have been found in three cytotypes. Haploid chromosome number  $n = 18$  may be aneuploid situation.

The cytological observation by Love & Love (1982) was with the haploid number  $n = 14$  in *Anaphalis triplinervis* var. *triplinervis*. However in the present study, the diploid number is  $2n = 20$  for this species.

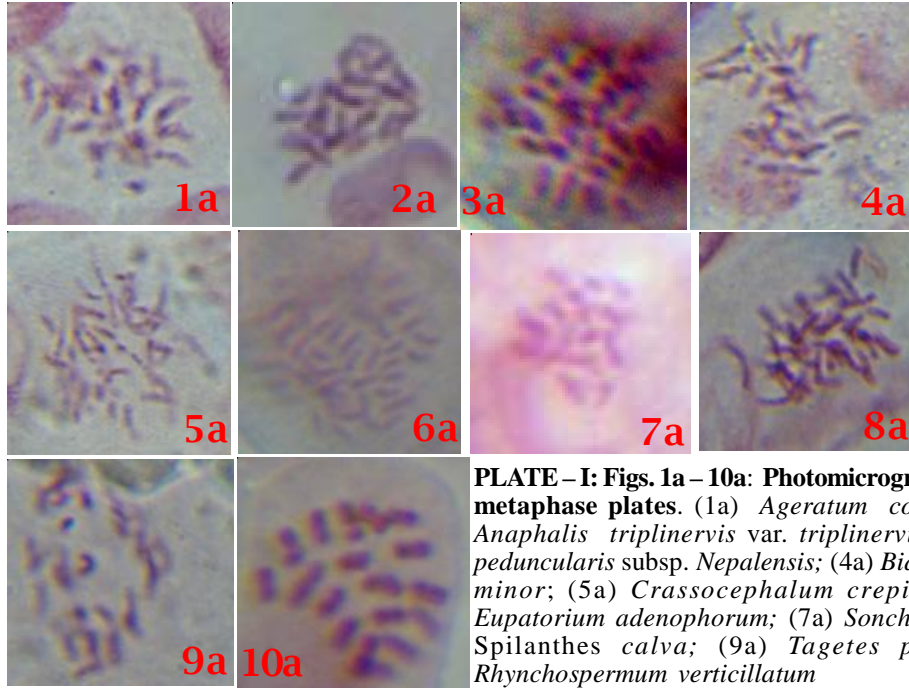
In *Aster peduncularis* subsp. *nepalensis*, the diploid number  $2n = 40$  is observed this perhaps a new record for this taxon.

**Table 1.** Karyomorphology of ten different members of Asteraceae.

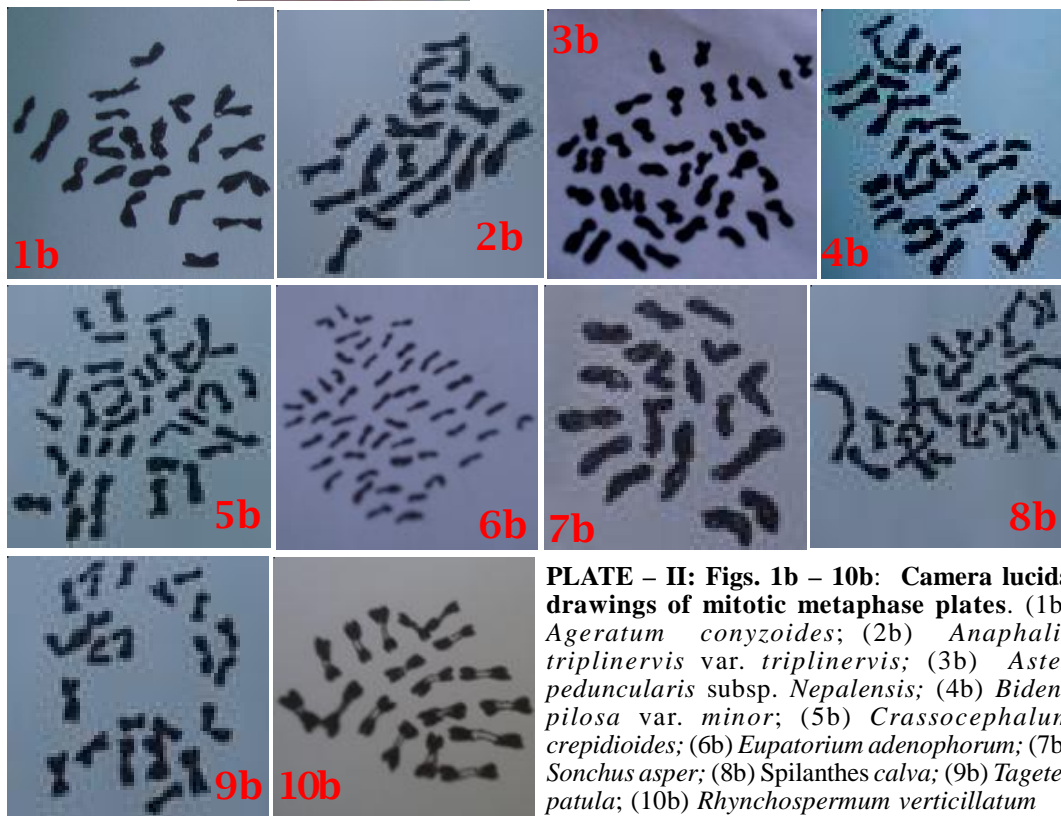
Name of the plants	Chrom. Number (2n)	Range of chrom. Length (µm)	Mean length (µm)	Absolute length (µm)	Karyotype formula	TF%	Fig no.
<i>Ageratum conyzoides</i>	20	0.2-1.3	1.4	14	$M_6+m_4+sm_8+st_2$	38.5	1a, 1b,1c
<i>Anaphalis triplinervis</i> var. <i>triplinervis</i>	20	0.2-1.3	1.3	13.1	$M_8+m_{10}+st_2$	40.4	2a, 2b,2c
<i>Aster peduncularis</i> subsp. <i>nepalensis</i>	40	0.2-1.3	1.1	23.6	$M_{18}+m_4+sm_{18}$	41.5	3a, 3b,3c
<i>Bidens pilos</i> var. <i>minor</i>	36	0.2-1.3	1.0	19.2	$M_{16}+m_2+sm_{16}+st_2$	38.5	4a, 4b,4c
<i>Crassocephalum crepidioides</i>	40	0.2-1.7	1.0	20.6	$M_{22}+m_2+sm_{16}$	42.2	5a, 5b,5c
<i>Eupatorium adenophorum</i>	50	0.1-0.8	0.7	18.4	$M_4+m_{12}+sm_{30}+st_4$	34.7	6a, 6b,6c
<i>Sonchus asper</i>	18	0.41to 2.1	1.9	17.3	$M_6+m_6+s_6$	40.1	7a, 7b,7c
<i>Spilanthes calva</i>	36	0.2-1.3	0.9	16.9	$M_{14}+m_2+sm_{18}+st_2$	39.0	8a, 8b,8c
<i>Tagetes patula</i>	24	0.4-1.7	1.7	21.2	$M_8+m_8+sm_6+st_2$	40.5	9a,9b ,9c
<i>Rhynchospermum verticillatum</i>	18	0.2-0.5	0.5	5.1	$M_4+m_8+Sm_6$	39.2	10a,1 0b,10 c

**Table 2.** Reports on chromosomes counts for the Nepalese taxa of Asteraceae

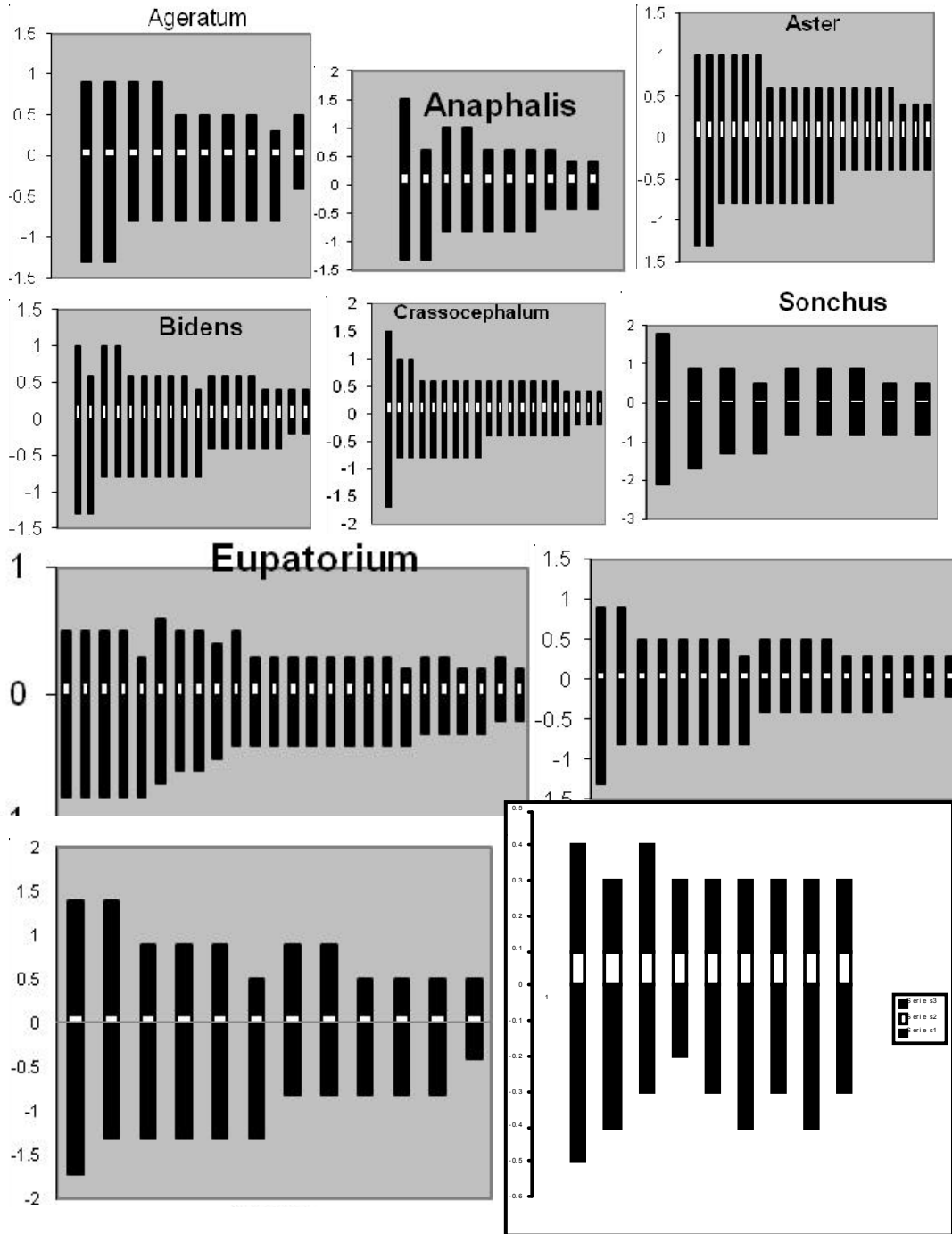
Taxon	Chromosome number	Author	Locality of collection (msl)
<i>Ageratum conyzoides</i>	n=20	Maharjan (1977)	Kirtipur 1300
<i>Ageratum conyzoides</i>	2n=20	Present count	Minbhawan 1280
<i>Anaphalis triplinervis</i> var. <i>triplinervis</i>	2n=28	Sharma (1970)	WCE,1800-3300m
<i>Anaphalis triplinervis</i> var. <i>triplinervis</i>	2n=20	Present count	Phulchowki 1900
<i>Aster peduncularis</i> subsp. <i>nepalensis</i>	2n=40	Present count	T.C. Garden 1300
<i>Bidens pilosa</i>	n=36	Maharjan (1977)	Kirtipur 1300
<i>Bidens pilosa</i> var. <i>minor</i>	2n=36	Present count	T.C. Garden 1300
<i>Crassocephalum crepidioides</i>	2n=20	Maharjan (1977)	Kirtipur 1300
<i>Crassocephalum crepidioides</i>	2n=40	Present count	Baneshwor 1285
<i>Eupatorium adenophorum</i>	2n=50	Present count	Minbhawan 1280
<i>Sonchus asper</i>	2n=18	Joshi (1988)	Kirtipur 1300
<i>Sonchus asper</i>	2n=18	Maharjan (1977)	Kirtipur 1300
<i>Sonchus asper</i>	2n=18	Present count	Kirtipur 1330



**PLATE – I: Figs. 1a – 10a: Photomicrographs of mitotic metaphase plates.** (1a) *Ageratum conyzoides*; (2a) *Anaphalis triplinervis* var. *triplinervis*; (3a) *Aster peduncularis* subsp. *Nepalensis*; (4a) *Bidens pilosa* var. *minor*; (5a) *Crassocephalum crepidioides*; (6a) *Eupatorium adenophorum*; (7a) *Sonchus asper*; (8a) *Spilanthes calva*; (9a) *Tagetes patula*; (10a) *Rhynchospermum verticillatum*



**PLATE – II: Figs. 1b – 10b: Camera lucida drawings of mitotic metaphase plates.** (1b) *Ageratum conyzoides*; (2b) *Anaphalis triplinervis* var. *triplinervis*; (3b) *Aster peduncularis* subsp. *Nepalensis*; (4b) *Bidens pilosa* var. *minor*; (5b) *Crassocephalum crepidioides*; (6b) *Eupatorium adenophorum*; (7b) *Sonchus asper*; (8b) *Spilanthes calva*; (9b) *Tagetes patula*; (10b) *Rhynchospermum verticillatum*



**PLATE – III: Figs. 1c – 10c: Idiograms of studied taxa.** (1c) *Ageratum conyzoides*; (2c) *Anaphalis triplinervis* var. *triplinervis*; (3c) *Aster peduncularis* subsp. *nepalensis*; (4c) *Bidens pilosa* var. *minor*; (5c) *Crassocephalum crepidioides*; (6c) *Eupatorium adenophorum*; (7c) *Sonchus asper*; (8c) *Spilanthes calva*; (9c) *Tagetes patula*; (10c) *Rhynchospermum verticillatum*

Taxon	Chromosome number	Author	Locality of collection (msl)
<i>Spilanthes calva</i>	2n=36	Present count	Godawari 1515
<i>Tagetes patula</i> L.	2n=24	Present report	Minbhawan 1310
<i>Rhynchospermum verticillatum</i>	2n=18	Present report	Godawari 1515
<i>Rhynchospermum verticillatum</i>	2n=18	Peng & Hsu (1977,1978)	Godawari 1515

In *Bidens pilosa* var. *minor* chromosomes recorded previously were  $2n = 36$  (Sharma 1970). Haploid number of chromosomes was  $n = 12$  (Keil & Stuessy 1975) and  $n = 36$  (Maharjan 1977). This taxon has been found in polyploid forms also.

Previous cytological studies recorded for *Crassocephalum crepidioides* were  $2n = 40$  (Henderson 1973; Mathew & Mathew 1988; Baltisberger, 1990; Morton 1993; Daniela 1997) and  $n = 20$  (Henderson, 1973). Present chromosome count in this taxon ( $2n = 40$ ) is confirming the previous observations.

Present observation in *Eupatorium adenophorum* has been encountered with  $2n = 50$ , while in previous report it was  $2n = 51$  (Khonglam & Singh 1980). The previous count may be of an aneuploid form ( $2n+1$ ).

Cytological observations of *Spilanthes calva* by Jose & Mathew (1995) is  $2n = 72$ , this taxon is recorded with  $2n = 36$  in the present study.

Earlier chromosome counts of *Tagetes patula* were  $2n = 20$  (Sharma 1970);  $2n = 24$  (Nirmala & Rao 1986). In the present study, the diploid count is  $2n = 24$  which is similar to the results of Nirmala & Rao (1986).

Present report of *Rhynchospermum verticillatum* is  $2n = 18$  that tallies with the findings of Peng & Hsu (1977, 1978).

The taxon *Eupatorium adenophorum* exhibited advanced character in chromosome structure, which is supported by the results (Table 2). *Bides pilosa*, *Spilanthes calva*, *Crassocephalum crepidioides* and *Tagetes patula* have been found in polyploid forms also.

Also, the taxon *Eupatorium adenophorum* seems to be advanced while *Aster peduncularis*, *Bidens pilosa* and *Rhynchospermum verticillatum* are primitive among all presently investigated taxa according to morphological as well as karyotypic classifications.

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