



Research Article

## Cytological Studies on Two Species, *Xanthium strumarium* Linn. and *Bidens pilosa* Linn. Var. *minor* (Blume) Sherff (Asteraceae-Heliantheae) from Nepal

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### Abstract

Karyotype, meiosis and pollen stainability of the taxa *Bidens pilosa* L. var. *minor* (Blume) Sherff and *Xanthium strumarium* L. collected from central part of Nepal was cytologically carried out. Results obtained from this investigation showed that chromosome number in somatic cells were recorded to be  $2n=36$  in *Bidens pilosa* and  $2n=32$  in *Xanthium strumarium* using aceto-orceine squash technique. Likewise haploid chromosome number in reproductive cells were recorded to be  $n=18$  in *Bidens pilosa* and  $n=16$  in *Xanthium strumarium* using aceto-carmin squash technique. The range of chromosome length found to be 0.4 to 2.1  $\mu\text{m}$  in *Bidens pilosa* and 0.4 to 1.6  $\mu\text{m}$  in *Xanthium strumarium*. Karyotype formula was  $M_{16}+m_2+sm_{14}+st_4$  for *Bidens pilosa* and  $M_{18}+sm_{12}+st_2$  for *Xanthium strumarium*. Based on the position of the centromeres, the chromosomes were categorized into four types with centromere at median point, median region, sub median region and terminal region in *Bidens pilosa* whereas three types, namely chromosomes with centromere at median point, sub median region and sub terminal region in *Xanthium strumarium*. In *Bidens pilosa* karyotype is asymmetrical and slightly asymmetrical karyotype is found in *Xanthium strumarium*. Absolute length recorded in *Bidens pilosa* were 19.2  $\mu\text{m}$  and 15.8  $\mu\text{m}$  in *Xanthium strumarium*. Pollen stainability is found to be 94.0 percent in *Bidens pilosa* and 92.7 percent in *Xanthium strumarium*.

**Keywords:** Chromosome- number; Asteraceae; Karyotype; Meiosis; Pollen-stainability.

### Introduction

The taxa *Xanthium strumarium* L. and *Bidens pilosa* L. var. *minor* (Blume) Sherff belong to the family Asteraceae and placed in tribe Heliantheae. The members of both species distributed globally. The genus *Xanthium* includes 25 species (Caius, 1986). In Nepal only one species namely *Xanthium strumarium* has been reported so far (Press *et al.*, 2000). *Xanthium strumarium* is called Bhende kuro in Nepal. Root is bitter tonic and useful in strumous diseases and cancer. (Joshi, 2000; Manandhar, 2002). The genus *Bidens pilosa* includes 230 species (Sherff, 1935). In Nepal two species *Bidens pilosa* and *Bidens biternata* are found (Press *et al.*, 2000). The *Bidens pilosa*, commonly called beggars stick, is known as Kalo kuro in Nepal. The leaf juice is applied in cuts and wounds (Rajbhandari, 2001). Determination of chromosome number, karyotype analysis, meiotic behavior and pollen fertility of the both taxa are objective of this study. The research on chromosome number and karyomorphology is very important as a chromosome number have provided to be of great value in

the determination of tribes in asteraceae. Karyotype analyses have been useful in classifying phylogenetic and evolutionary relationship between some related species and species groups.

### Materials and Methods

The plants were collected from Central parts of 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).

For meiosis study buds of suitable size are fixed in fixative prepared by one part acetic acid and three parts ethanol (1:3) for 24 hours. The buds were fixed between 9-11 a.m. Suitable anthers were dissected from the buds and teased with a needle in a thin film of 1-2 % aceto-carmin. Pollen fertility of investigated taxa was estimated based on stainability test. It was determined by staining pollens in the solution of acetocarmine and glycerine mixed in 1:1 ratio (Müntzing, 1941).

## Result and Discussion

### *Xanthium strumarium* L. (2n=32)

The plant is annual, erect, herb that grows up to 1 meter in height depending on the local conditions. It is commonly known as Cobbler's Pegs or Spanish Needle. Leaves 4-10 cm long opposite, imparipinnate, usually 3-foliolate, segments ovate-lanceolate, serrate and acute (Fig. 1). The petioles are slightly winged. Flowers are borne in small heads on relatively long peduncles. The heads bear about four or five

broad white petals of ray florets, surrounding a disc of tubular yellow florets. The fruits are slightly curved, stiff, rough black rods about 1 cm long with typically two to three stiff, heavily barbed awns at their distal ends. Achenes quadrangular, black. Pappus setae 2-4, retrorsely bristly. Flowering time is May to November and fruiting time October to February.

Chromosome number determined for this taxon is 2n=36. The somatic chromosome number determined from the root tip cell is shown in Fig. 2 and camera lucida drawing is in Fig. 3. Its ideogram is represented in Fig. 4. The chromosome measurements are given in Table 1.

The karyotype consists of three types of chromosomes with centromere at median point, sub- median region and sub-terminal region. The chromosome length ranged from 0.4 to 1.6 µm with mean length 0.9 µm and absolute length 15.8. µm. TF % is 43.0 Karyotype formulas is  $M_{18+} sm_{12+} st_2$ . Absolute length of chromosome found in this species is 15.8. µm.

Meiosis is normal. Daikinesis with sixteen bivalents at Metaphase-I (Fig. 5), Anaphase-I with two laggards (Fig. 6), normal Telophase-I (Fig. 7), Anaphase-II (Fig. 8), late Telophase-I (Fig. 9), Telophase-II (Fig.10),tetrads with abortive microspores (Fig. 11), triporate circular pollens (Fig. 12) are observed. Pollen stainability is 92.7 %.

**Table 1:** Chromosome measurement in *Xanthium strumarium* L.

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	10.1	M
II	0.8	0.8	1.6	1	10.1	M
III	0.8	0.8	1.6	1	10.1	M
IV	0.8	0.4	1.2	2	7.5	sm
V	0.8	0.4	1.2	2	7.5	sm
VI	0.8	0.4	1.2	2	7.5	sm
VII	0.8	0.2	1	4	6.3	st
VIII	0.4	0.4	0.8	1	5.0	M
IX	0.4	0.4	0.8	1	5.0	M
X	0.4	0.4	0.8	1	5.0	M
XI	0.4	0.4	0.8	1	5.0	M
XII	0.4	0.4	0.8	1	5.0	M
XIII	0.4	0.4	0.8	1	5.0	M
XIV	0.4	0.2	0.6	2	3.7	sm
XV	0.4	0.2	0.6	2	3.7	sm
XVI	0.2	0.2	0.4	2	2.5	sm



Fig. 1: Photograph of living plant



Fig. 2: Photomicrograph of mitotic metaphase plate.



Fig. 3: Camera lucida drawing of the Fig 2

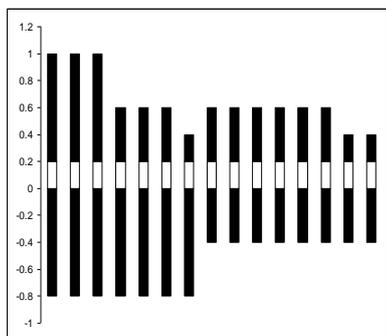


Fig. 4: Ideogram of Fig 3.

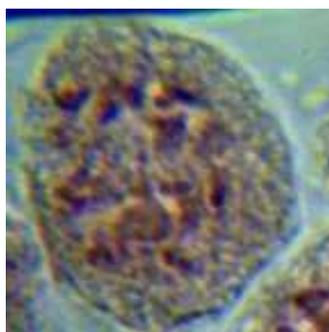


Fig. 5: Diakinesis showing 16 bivalents.



Fig. 6: .Telophase-I.

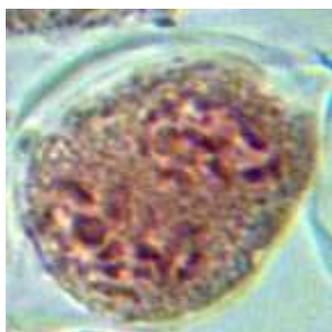


Fig. 7: .Telophase-I.

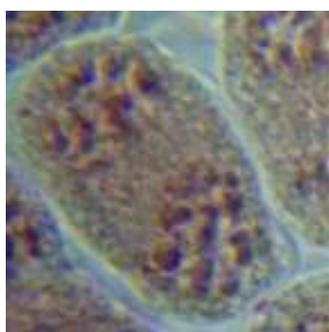


Fig. 8: .Telophase-I.



Fig. 9: .Telophase-I.

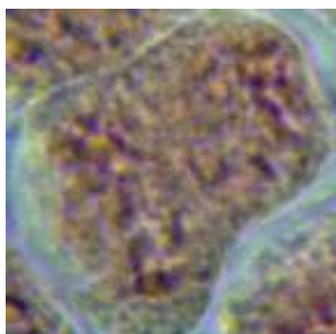


Fig. 10: Anaphase- II.



Fig. 11: Pollen-Tetrad

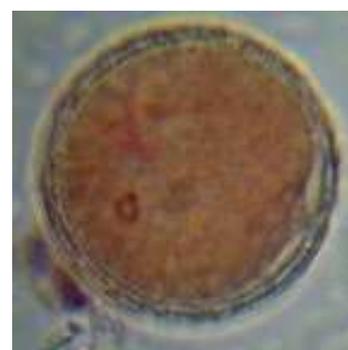


Fig. 12: Pollen grain.

Chromosome count  $2n=32$  in present study for *Xanthium strumarium* is different from the reports of  $2n=34$  by Mohamed (1997),  $2n=36$  by Mathew and Mathew (1988), Jose and Mathew (1995) and Joshi (1988). The present

haploid chromosome number is  $n=16$  for *Xanthium strumarium* is different from earlier reports ( $n=18$ ) was reported by Sarkar *et al.* (1982), Mathew and Mathew (1988), and Gupta and Gill (1989). Karyotype formula

$M_2+m_{26}+sm_8$  ( $2n=36$ ) was reported by Joshi (1988) for this species. *Xanthium strumarium* is an ascending aneuploid plant with  $2n=32$  in present investigation. Thus *Xanthium strumarium* seem to be normal hexaploid form of basic number  $x=6$  with haploid number=16. The two basic chromosome number  $x=8$  and 9 are also suggested by Darlington and Wylie (1955) for this species.

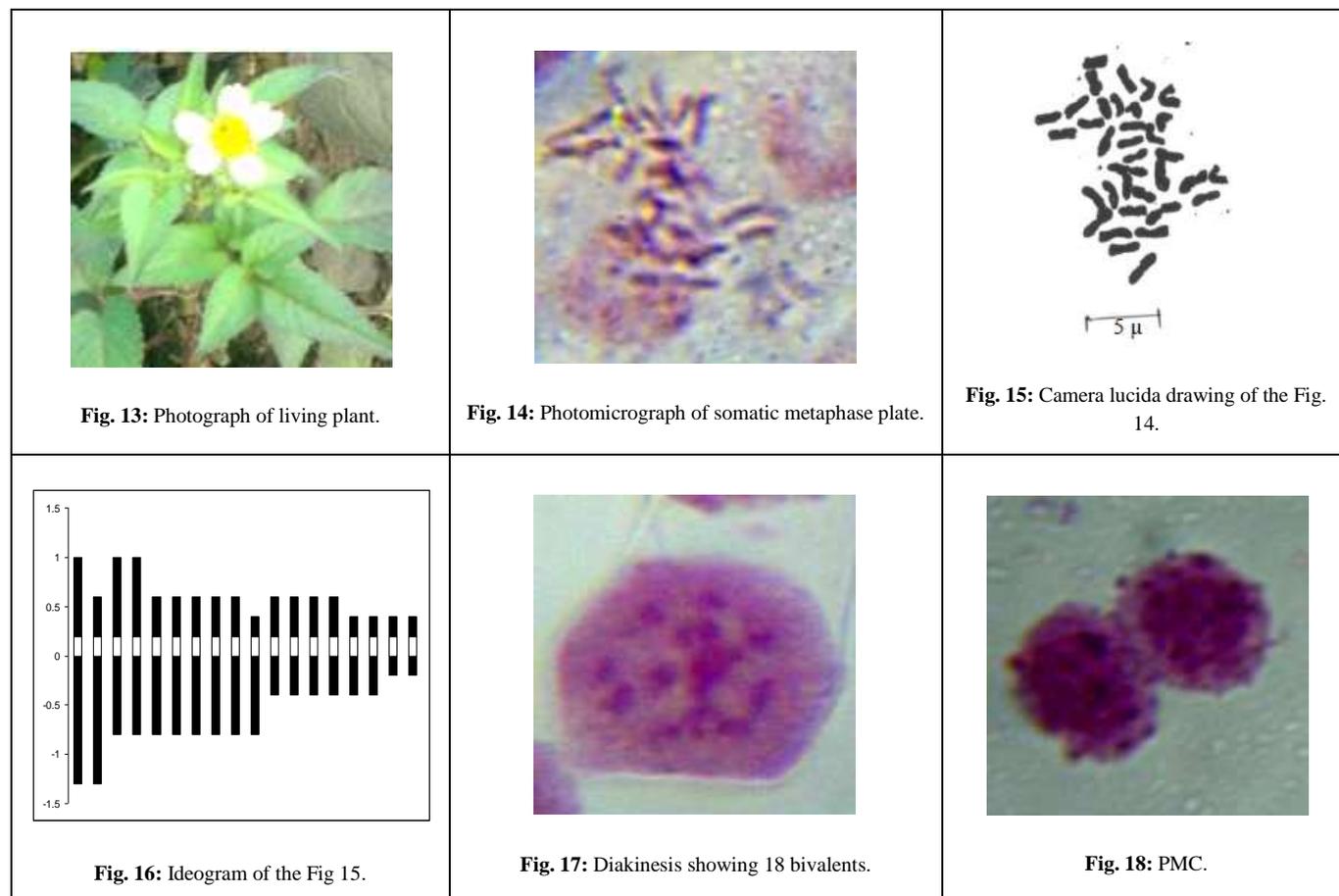
***Bidens pilosa* L. var. *minor* (Blume) Sherff**

The plant is annual, erect, herb that grows up to 1 meter in height depending on the local conditions. It is commonly known as Cobbler's Pegs or Spanish Needle. Leaves 4-10 cm long opposite, imparipinnate, usually 3-foliolate, segments ovate-lanceolate, serrate and acute. The petioles are slightly winged. Flowers are borne in small heads on relatively long peduncles. The heads bear about four or five broad white petals of ray florets, surrounding a disc of tubular yellow florets. The fruits are slightly curved, stiff, rough black rods about 1 cm long with typically two to three stiff, heavily barbed awns at their distal ends. Achenes quadrangular, black. Pappus setae 2-4, retrorsely bristly. Flowering time is May to November and fruiting time October to February (Fig. 13).

Chromosome number determined for this taxon is  $2n=36$ . The somatic chromosome number determined from the root

tip cell is shown in Fig. 14 and camera lucida drawing is in Fig. 15. Its ideogram is represented in Fig. 16. The chromosome measurements are given in Table 2.

The karyotype consists of four different types of chromosome with centromere at median point, median region, sub median region and terminal region. The chromosome length ranged from 0.4 to 2.1  $\mu m$  with mean length 1.0  $\mu m$  and absolute length 19.2 $\mu m$ . TF % is 38.5. Karyotype formula is  $M_{16}+m_2+sm_{14}+st_4$ . Absolute length of *Bidens pilosa* ( $2n=36$ ) is 19.2 $\mu m$ , Chromosome number in *Bidens pilosa* var. *minor* ( $2n=36$ ) determined in present study tallies with Sharma (1970) but number  $2n=72$  (Banerjee, 1971) and  $2n=48$  (Pilz, 1980) are different from present number. This taxa also has been found in polyploid forms which is evidenced from the haploid number  $n=12$  by Keil and Stuessy (1975). The Basic chromosome number  $x=12$  for *Bidens pilosa* has been proposed by Darlington and Wylie (1955) and Barroso (1991). *Bidens pilosa* ( $2n=36$ ) has been found in hexaploid form with basic chromosome number  $x=6$  in present study. Mariano and Marin-Morales (1999) found in two different polyploid numbers:  $2n=48$  and  $2n=72$  indicating that polyploidy is an important evolutionary process for this genus.



Meiosis is normal in this taxon. Metaphase I with 18 bivalents is shown in Fig. 17. Pollen mother cells (Fig. 18), normal tetrads (Figs. 19) and triporate, echinate, spheroidal pollens (Fig. 20) are observed. Pollen stainability is 94.0 %. *B. pilosa* has two pairs of chromosomes with centromere at sub-terminal regions. It seems that *B. pilosa* is advance than the taxa *Xanthium strumarium*. This advanceness reflects on the morphology of *Bidens pilosa* that has pinnate leaf, spiny achens, slightly hard stem. The basic chromosome number of *Bidens* has been reported by Darlington and Wylie (1955) as  $x=12$ . In the present study the chromosome number  $2n=36$  in *Bidens pilosa* is recorded. The number  $n=12$  could be haploid number for *Bidens pilosa* this was also confirmed by Barroso (1991). Meiotic behavior of *Bidens pilosa* is found to be completely normal in present study. The present haploid number for this taxa is  $n=18$ . Previously haploid number  $n=12$  was reported by Löve (1978), Gill (1978), and Keil and Stuessy (1977). Higher ploid  $n=36$  was noted by Nirmala and Rao (1981), Sharma (1970), Keil and Stuessy (1977), Banerjee (1971), Robinson *et al.* (1981). Polyploid origin of the species is supposed to have played a great role in evolution.



Fig. 19 pollen tetrad.

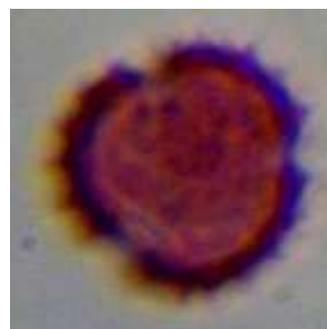


Fig. 20. Pollen grain

**Table 2:** Chromosome measurement in *Bidens pilosa* L. var. *minor* (Blume) Sherff

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	10.9	m
II	1.3	0.4	1.7	3.2	8.8	st
III	0.8	0.8	1.6	1	8.3	M
IV	0.8	0.8	1.6	1	8.3	M
V	0.8	0.4	1.2	2	6.2	sm
VI	0.8	0.4	1.2	2	6.2	sm
VII	0.8	0.4	1.2	2	6.2	sm
VIII	0.8	0.4	1.2	2	6.2	sm
IX	0.8	0.4	1.2	2	6.2	sm
X	0.8	0.2	1.0	4	5.2	st
XI	0.4	0.4	0.8	1	4.1	M
XII	0.4	0.4	0.8	1	4.1	M
XII	0.4	0.4	0.8	1	4.1	M
XIV	0.4	0.4	0.8	1	4.1	M
XV	0.4	0.2	0.6	2	3.1	sm
XVI	0.4	0.2	0.6	2	3.1	sm
XVII	0.2	0.2	0.4	1	2.0	M
XVIII	0.2	0.2	0.4	1	2.0	M

## Acknowledgements

I am thankful to Prof. Dr.Pramod Kumar Jha, former the Head, Central Department of Botany,T.U. Kirtipur, Kathmandu, Nepal for providing facilities. Also grateful to Dr.Laxmi Manandhar and Dr.Buddha Laxmi Vaidya for kind suggestions.

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