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Karyotype analysis of Common Cocklebur (*Xanthium strumarium L.*)

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Abstract

Objective: The purpose of this study is to determine the mitotic chromosome number, morphometric parameters, and karyotypes of *Xanthium strumarium* and make contributions to other multidisciplinary studies on the genus.

Material and Methods: The seeds were germinated on moist filter paper in Petri dishes at 25°C. Actively growing root tips were pre-treated with aqueous paradichlorobenzene for 4h at room temperature. Then, the root tips were fixed with acetic alcohol (1:3 glacial acetic acid–absolute ethanol) for at least 24 h at 4°C, hydrolysed in 1 N HCl at 60°C for 3 min, then rinsed in tap water for 3–5 min. Finally, they were stained in Feulgen for 1 h and mounted in 45% acetic acid. Digital microphotographs from at least five well-spread metaphase plates were taken using an Olympus BX51 microscope and were recorded with an Olympus Camedia C-4000 digital camera.

Results: The chromosome number is determined as 2n = 36 for this taxon. The karyotype consists of 16 median region (m) and 2 submedian region (sm) chromosomes. The metaphase chromosome length ranges from 2.30 to 4.03 µm, longest to shortest chromosome ratio is 1.7:1.1, total karyotype length (TKL) 54.76 µm and the karyotype symmetry is type 1A.

Conclusion: The results of this study showed that the chromosome number of Xanthium strumarium is 2n=36. Satellites were not observed in the karyotype of this species. Identifying the chromosome number of this species in this study provides a base for biosystematic studies.

Keywords: Chromosome number, *Xanthium*, Karyotype Analysis.

Introduction

The genus *Xanthium* L. belongs to the family Asteraceae. The members of this genus are distributed globally, however are most frequently in to be found tropical and sub-tropical regions (1). They are wide-spread in America, Canada, Mexico, Malaysia, Indonesia and India. The taxonomic study of the members of this genus is difficult and confusing. For example, Caius reported that the genus *Xanthium* includes 25 species (2). However, according to Weaver and Lechowich, there are 20 species belonging to this genus. Thus confusion about the species number of this genus still exists (1). Love and Dansureau revised the genus *Xanthium* and reduced the number of species to only 2 (*X. strumarium* L. and *X. spinosum* L.). They mentioned that due to phenotypic plasticity in a number of features, the members showed different phenotypes and creating confusion regarding their taxonomic rank (3). Prain described that the 2 species *i.e. X. strumarium* and *X. spinosum* were found in undivided Bengal (4). However, Oudhia and Dixit (1994) reported *X. indicum* and *X. strumarium* from India (5). In Turkey, only two species, namely *X. strumarium* and *X. spinosum* have so far been reported. *X. strumarium* species found in Turkey has two subspecies (subsp. *strumarium* and *cavanillesii*) (6).

The common names are cocklebur, burr, sheep burr, etc. (7-10). The plant is an annual 30-120 cm in height and is a short-day plant that flowers in July-August. Each cocklebur bur contains two seeds. The seeds are covered by a hard green husk with hooked spines (11). The whole plant (*X. strumarium*), especially its leaf, root and fruit, has been used in traditional medicine for the treatment of rhinitis, malaria, rheumatism, tuberculosis, cancer, and ulcers (12-15). Previous studies indicated that plants of the Asteraceae family are characteristically rich in sesquiterpene lactones, an important class of terpenoids, and the *Xanthium*'s species are rich in such medicinal ingredients.

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The pharmacological properties of *X. strumarium* are largely attributed to the presence of xanthanolides (a class of sesquiterpene lactones), which have been reported to possess antifungal, antibacterial, and cytotoxic activities, and exhibit a growth inhibitory activity against insects (16-22).

The plant is used in classical homeopathy (23) and is officially recognized in China and several other countries. The preparation Adenostop is manufactured from cocklebur in Romania and is used to treat prostate adenoma. High anticancer activity of cocklebur (for breast, lung, stomach, and colon cancer) was recently reported (24). This plant is used as a medicine for curing nasal sinusitis, headache, urticaria and arthritis. It has also been reported to possess curative effects against chronicbronchitis, chronic rhinitis, allergic rhinitis, lumbago and other ailments (25) and is used by various native American tribes to relieve constipation, diarrhea and vomiting (26).

The purpose of this study is to determine the mitotic chromosome number, morphometric parameters, and karyotypes of *Xanthium strumarium* and make contributions to other multidisciplinary studies on the genus.

Material and Methods

Plant material was collected from natural habitats during the fruiting season in Elazig in 2015. Voucher specimen was deposited at the Firat University Herbarium (FUH). Karyological studies were conducted on meristematic cells obtained from the root tips.

The seeds were germinated on moist filter paper in Petri dishes at 25°C. Actively growing root tips were pre-treated with aqueous paradichlorobenzene for 4h at room temperature. Then, the root tips were fixed with acetic alcohol (1:3 glacial acetic acid–absolute ethanol) for at least 24 h at 4°C, hydrolysed in 1 N HCl at 60°C for 3 min, then rinsed in tap water for 3–5 min.

Finally, they were stained in Feulgen for 1 h and mounted in 45% acetic acid. Digital microphotographs from at least five well-spread metaphase plates were taken using an Olympus BX51 microscope (Olympus Optical Co. Ltd., Tokyo, Japan), and were recorded with an Olympus Camedia C-4000 digital camera (Olympus Optical Co. Ltd., Tokyo, Japan). The short arm (s), long arm (l) and total lengths (tl) of each chromosome were measured and the relative lengths, arm ratios, and centromeric indices were determined from images of selected cells. Chromosomes were classified according to the nomenclature of Levan et al. (27). The intra-chromosomal asymmetry index (A1) and the inter-chromosomal asymmetry index (A2) followed those of Romero-Zarco (28). The karyotype symmetry nomenclature followed Stebbins (29). Also, relevant literature the online chromosome number databases, Index to Plant Chromosome Numbers (IPCN) (30) and Index to Chromosome Numbers in Asteraceae (31) were checked.

Results and Discussion

The results of this study showed that the chromosome number of Xanthium strumarium is 2n=36. Karyotype analysis of this species to reveal the many values were calculated. The number of somatic chromosome, ploidy level, karyotype formula, chromosome length range, total karyotype length (TKL), Stebbine C and asymmetry indexes (A1, A2) are presented in Table 1; relative length, arm ration, centromeric index, type, in Table 2.

Haploid ideograms of *X. strumarium* has been shown in Fig. 1 and metaphase chromosomes in Fig. 2. The chromosome number is determined as 2n = 36 for this taxon. The karyotype consists of 16 median region (m) and 2 submedian region (sm) chromosomes. The metaphase chromosome length ranges from 2.30 to 4.03 µm, longest to shortest chromosome ratio is 1.7:1.1, total karyotype length (TKL) 54.76 µm and the karyotype symmetry is type 1A. Satellites were not observed in the karyotype of this species.

Table 1. Somatic chromosome number, ploidy level, karyotype formula, chromosome length range, total karyotype length (TKL), asymmetry indexes (A1, A2) of Romero Zarco (1986) and symmetry classes (SC) of Stebbins (1971) of *Xanthium strumarium*.

Taxon	2n	Ploidy level	Karyotype formula	Chromosome length range (µm)	TKL (µm)	A1	A2	SC
Xanthium strumarium	36	4x	16m+2sm	2.30-4.03	54.76	0.21	0.15	1A

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Table 2. Karyomorphological parameters of *Xanthium strumarium*

Xanthium strumarium				
Pair No	Relative Length	Arm Ration	Centromeric Index	Type
1	7.36	1.16	0.46	m
2	6.89	1.20	0.45	m
3	6.43	1.17	0.45	m
4	6.40	1.38	0,41	m
5	6.16	1.26	0.44	m
6	5.88	1.19	0.45	m
7	5.77	1.44	0.40	m
8	5.67	1.23	0.44	m
9	5.49	1.25	0.44	m
10	5.43	1.78	0.35	sm
11	5.15	1.11	0.47	m
12	5.11	1.22	0.44	m
13	5.01	1.32	0.43	m
14	4.99	1.78	0.35	sm
15	4.94	1.25	0.44	m
16	4.62	1.18	0.45	m
17	4.42	1.15	0.46	m
18	4.21	1.20	0.45	m

Meiotic chromosome number is n=18 this species was reported in the literature (32-36). Besides, in de book of "Flora der Schweiz und angrezender Gebiete" reported that chromosome number of *X. strumarium* n=18.

2n=36 chromosomes of this species was reported in the literature (37-54). Therefore, the present count confirmed the earlier reports on 2n chromosomes number.

Conclusion

According to our knowledge, chromosome number and morphology report for *Xanthium strumarium* does not exist. Identifying the chromosome number of this species in this study provides a base for biosystematic studies.

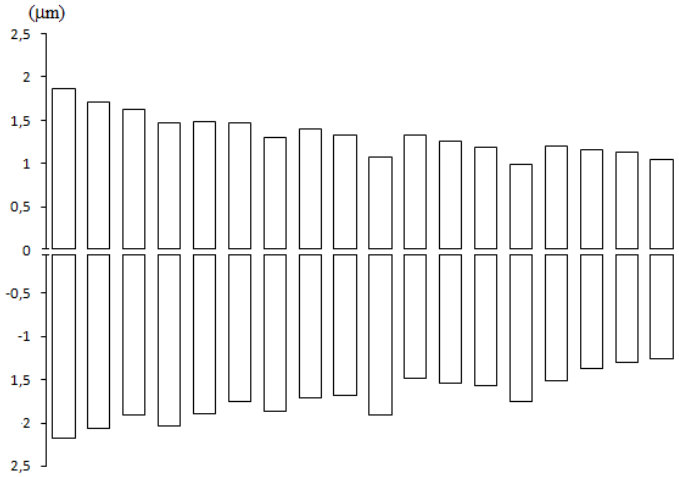

Figure 1. Haploid idiograms of **Xanthium strumarium**.

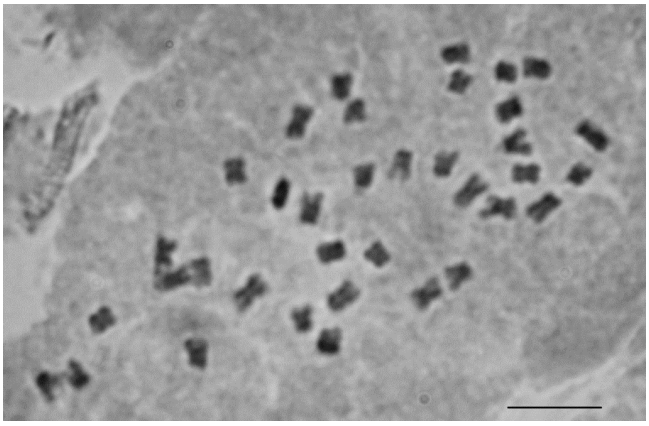

Figure 2. Metaphase chromosomes of **Xanthium strumarium.** Scale bar=10 µm

Conflict of Interest: The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author's Contributions: GD, YK: Collecting of data, writing and revision of article,

Ethical issues: All Authors declare that Originality of research/article etc... and ethical approval of research, and responsibilities of research against local ethics commission are under the Authors responsibilities. The study was conducted due to defined rules by the Local Ethics Commission guidelines and audits.

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