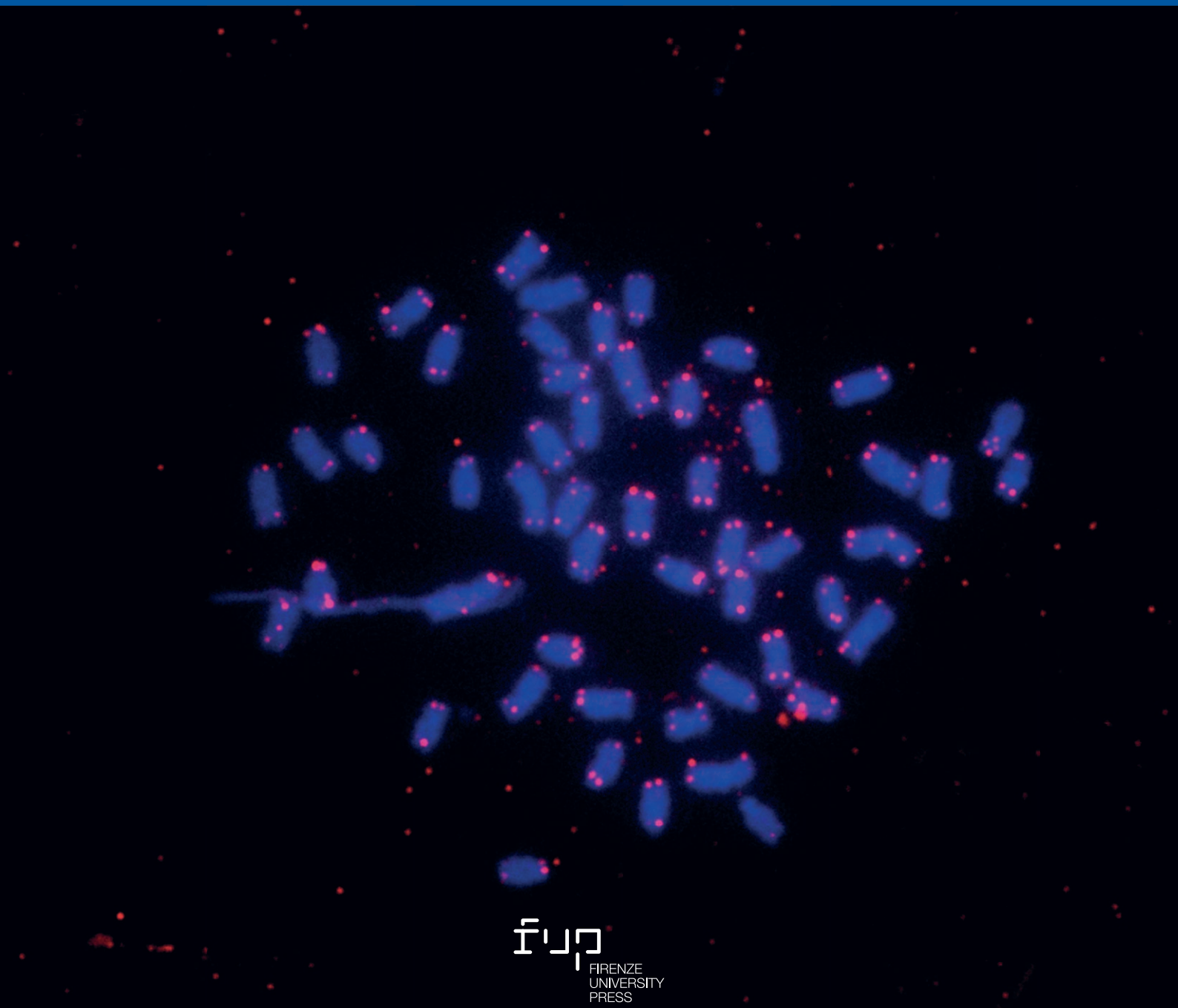
0008-7114

Caryologia

International Journal of Cytology, Cytosystematics and Cytogenetics

2023
Vol. 76 - n. 1



FIRENZE UNIVERSITY PRESS

Caryologia. International Journal of Cytology, Cytosystematics and Cytogenetics
Caryologia is devoted to the publication of original papers, and occasionally of reviews, about plant, animal and human karyological, cytological, cytogenetic, embryological and ultrastructural studies. Articles about the structure, the organization and the biological events relating to DNA and chromatin organization in eukaryotic cells are considered. *Caryologia* has a strong tradition in plant and animal cytosystematics and in cytotoxicology. Bioinformatics articles may be considered, but only if they have an emphasis on the relationship between the nucleus and cytoplasm and/or the structural organization of the eukaryotic cell.

Cover: figure from the article inside by Mauricio Barros Fernandes, Jamille de Araújo Bitencourt, Joandson Calixto dos Santos, José Henrique Galdino, Paulo Roberto Antunes de Mello Affonso. Cytogenetic analysis in *Tetragonopterus franciscoensis* (Characiformes): another piece to the karyoevolutionary puzzle of tetra fishes.

Caryologia

International Journal of Cytology, Cytosystematics and Cytogenetics

Volume 76, Issue 1 - 2023

Firenze University Press

***Caryologia*. International Journal of Cytology, Cytosystematics and Cytogenetics**
Published by
Firenze University Press – University of Florence, Italy
Via Cittadella, 7 - 50144 Florence - Italy
http://www.fupress.com/caryologia

Caryologia
International Journal of Cytology, Cytosystematics and Cytogenetics

Firenze University Press
www.fupress.com/caryologia

OPEN ACCESS

Citation: Funmilola Mabel Ojo, Chinyere Constance Nwokeocha, Julius Olaoye Faluyi (2023). Cytogenetic studies in the *Andropogon gayanus-Andropogon tectorum* Complex in Southwestern Nigeria. *Caryologia* 76(1): 3-13. doi: 10.36253/caryologia-1580

Received: February 20, 2022

Accepted: April 6, 2023

Published: September, 19, 2023

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

ORCID
FMO: 0000-0002-8870-4349

Cytogenetic studies in the *Andropogon gayanus-Andropogon tectorum* Complex in Southwestern Nigeria

Funmilola Mabel Ojo[1,*], Chinyere Constance Nwokeocha[2], Julius Olaoye Faluyi[3]

[1] *Department of Biological Sciences (Botany Programme), Olusegun Agagu University of Science and Technology, Okitipupa, Ondo State, Nigeria*
[2] *Natural History Museum, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria*
[3] *Department of Botany, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria*
*Corresponding author. E-mail: olufunmilola_07@yahoo.co.uk; fm.ojo@oaustech.edu.ng

Abstract. The studies were carried on accessions of the *Andropogon gayanus-Andropogon tectorum* complex (Poaceae) in southwestern Nigeria to determine the chromosome numbers and meiotic dynamics in the species. This involves meiotic chromosome and pollen studies using standard procedures. Studies on the morphology and meiotic behaviour of collections of *A. gayanus* (2n = 40) and *A. tectorum* (2n = 20, 40) have been made. The cytogenetic studies show that the chromosome configuration of *A. gayanus* is a tetraploid (2n = 40) on a basic chromosome number of 10. *A. tectorum* occurs as a regular diploid on a chromosome number of 2n = 20 and it is the only species found in Ekiti State. Accession "Kiwani" was found to be a polyploid. *A. tectorum* with a chromosome number of 2n = 40. This study has also established the stability of the genome of the *Andropogon* complex. In spite of the occurrence of univalent and multiple associations, meiosis ensures a high percentage of pollen grains. The pollens of accession "Kiwani" (> 35 μm) were generally larger than those of other accessions while the other accessions of both *A. gayanus* and *A. tectorum* have modal pollen sizes less than 35 μm, although the *A. tectorum* accessions were generally smaller. The study concluded that the occurrence of the tetraploid (2n = 40 in accession AT10, "Kiwani" can be attributed to apomixis occurring in a lonely plant.

Keywords: *Andropogon gayanus, Andropogon tectorum*, Apomixis, "Kiwani", meiotic study.

INTRODUCTION

The genus *Andropogon* Linn., is a fairly large genus of the grass family, Poaceae, belonging to the tribe Andropogoneae (Hutchinson and Dalziel 1972; Olorode, 1984)). *Andropogon* is a pantropical genus of grasses of about 29 species almost confined to the tropical and warm temperate regions of the world, frequently forming an important part of the savanna vegetation in the tropics. The genus is composed of annual and perennial species frequently

with tall culms, and leaf blades which can be linear to lanceolate or ovate. *Andropogon* is represented by about 14 species in Nigeria (Lowe 1989), although Stanfield (1970) had reported about 12 species.

The spikelets occur in pairs at each node of the raceme, each pair consisting of a pedicellate and a sessile spikelets. The sessile spikelet is bisexual while the pedicellate is unisexual male (Hutchinson and Dalziel 1972). The spikelets are articulated in such a way that at maturity, their pedicel and internodes all break apart leaving no central inflorescence stalk. The sessile spikelet bears a prominent awn which is flexed at an angle to the vertical axis of the glumes. A distinct colour difference exists between the two arms of the awn (Clayton 1969; Stanfield 1970).

Andropogon gayanus Kunth is a tall, tufted perennial grass that grows taller than 3 m. It has many tillers and abundant foliage especially during the rainy season (Chlleda and Crowder 1982). It forms a significant part of the vegetation of many savanna areas throughout Africa south of the Sahara, including South Africa. It is a polymorphic species. In Nigeria, four main varieties were recognized (Clayton, 1962). These are: var. *gayanus* (var. *genius*) Hack; var. *bisquamulatus* (Hochst) Hack var. *squamulatus* (Hochst) Stapf and var. *tridentatus*. Bowden (1963) considered var. *tridentatus* as split from var. *bisquamulatus* thus recognizing only three varieties.

A. gayanus is widespread and abundant in the Northern and Southern Guinea Savanna as well as in the drier areas of the derived savanna whereas *A. tectorum* occupies vast areas in the derived savanna, preferring moderate shade (Stanfield, 1970). However, certain areas in the derived savanna support the growth of both species equally well (Okoli and Olorode 1983). *Andropogon gayanus* is propagated by seeds, which are broadcasted or planted in rows and vegetatively by splitting the tufts. It is relatively free of major pests and diseases and is resistant to grazing and burning. These make it a useful grass for supporting a large number of ruminant animals in Northern Nigeria. It is also one of the high-yielding grasses in West Africa (Bogdan. 1977; Pagot, 1993). The economic importance of *Andropogon gayanus* for livestock grazing is that it is very palatable when young and serves as basic materials for woven houses. *Andropogon gayanus* is a highly-productive grass, which increases fuel loads, produces intense, late dry season fires which seriously damage native woody species; it is also useful as forage in permanent pastures grazed by ruminants. The stems are used for thatching and, when flattened, for weaving coarse grass mats as well as sometimes being planted for erosion control and soil restoration.

Andropogon tectorum Schum. & Thonn. is a perennial grass; caespitose. Culms can be 200–300 cm long without nodal roots or with prop roots. Ligules are eciliate membrane or a ciliolate membrane, 1–2 mm long. Leaf blade base tapers to the midrib and bears false petiole. Leaf-blades are lanceolate; 30–45 cm long; 10 – 25 mm wide; flaccid; light-green, apex is acuminate. *Andropogon gayanus* and *A. tectorum* are important natural fodder grasses in Nigeria. They are also useful in crop rotation, thatching and mat-making (Bowden, 1963) and offer an interesting opportunity for ecological, cytogenetic and evolutionary studies.

There is paucity of information about the genetic studies of the *Andropogon gayanus-Andropogon tectorum* complex aside from the first attempts by Okoli (1978). Further investigation of the precise nature and extent of the apomicitic phenomenon in these species would be both interesting and worthwhile.

MATERIALS AND METHODS

Field trips for plant collections covered agro-ecological zones of the following states: Osun, Ondo, Ogun, Oyo and Ekiti as shown in Figure 1. Whole plants of *Andropogon gayanus* and *Andropogon tectorum* were collected from different locations in Southwest, Nigeria. Accession numbers were given to the specimens. Seeds were also collected for planting and preservation. Garden populations were raised from the vegetative parts of some accessions and they were also maintained in the Botanical Garden of the Obafemi Awolowo University, Ile- Ife, Osun State. The accessions were nurtured to maturity and used for this study. Table 1 shows the locations, coordinates, descriptions of site and collectors of the accessions.

Footnote on Kiwani, this lone plant was discovered on a road side in a gravelly location, the three colleagues on the trip agreed on its pronounced gigas attributes suggesting polyploidy. This was a surprise in a region dominated by diploid *Andropogon tectorum*. After the arguments that ensued, the leader of the trip named the accession *KIWANI* which translates into "what else can this be?' It is denoted as AT10 too in part of the manuscript.

Meiotic chromosome studies

The young flower buds for meiotic chromosome study were collected between 9:00 am and 12:00 noon when the cell activities are believed to be at the peak (Jackson 1962) and stored in 1:3 Acetic acid: Ethanol. The anthers were removed, squashed and stained in FLP Orcein by the squashing technique described by Laose-

Figure 1. Map showing collection site.

bikan and Olorode (1972). The Pollen Mother Cells were examined and scored for chromosome associations. Good meiotic chromosome spreads were photographed at X100 objective under BK Series (Phase Contrast Microscope (PW-BK 5000T) equipped with a DCM 510 5 Megapixel Camera.

Pollen studies

Pollen stainability studies were carried out to assess the fertility of the accessions as well as the pollen size.

Table 1. Accessions of *Andropogon gayanus* (Kunth) - *Andropogon tectorum* (Schum & Thonns) studied and their sources.

Accn	Locations	Coordinates	Descriptions of site	Collectors
AG 1	Asawo, Oyo State	N 08° 59.848` E 04° 17.836`	Ruderal location, regular regrowth forest	Ojo, Faluyi, Azeez and Abraham
AG 2	Budo-Ode, Oyo State	N 08° 20.420` E 04° 14.039`	Derived Savanna	"
AG 3	Between Ogbomosho and Oko, Oyo State	N 08° 59.854` E 04° 17.835`	Ruderal location, regular regrowth forest	"
AG 4	Igbeti 1, Oyo State	N 08° 24.722`E 04° 15.467`	Dry water course, populated by heavy mat of grasses and sedges; chimeric lawn	"
AG 5	Igbeti 2, Oyo State	N 08^0 24.723` E $04^0$ 15.467`	Dry water course, populated by heavy mat of grasses and sedges; chimeric lawn	"
AG 6	Ogbomosho, Oyo State	N 08° 34.794` E 04° 14.669`	Regular regrowth forest with the presence of multiple grass species	"
AT 1	OAUTHC Road, Osun State	N 07° 30.870` E 04° 33.065`	Ruderal location with dwarf morphotypes propagating from crevices	Ojo, Faluyi and Nwokeocha
AT 2	O.A.U. Religious Centre, Osun State	N 07° 30.703` E 04° 33.065`	Ruderal on lateritic soil in company of other grasses, Asteraceae	"
AT 3	O.A.U. International School, Osun State	N 07° 31.793` E 04° 33.261`	Expanse of lateritic soil with close communities of *Andropogon tectorum*	"
AT 4	Ife-Ibadan road, Osun State	N 07° 22.774` E 04° 01.497`	At the fringe of road divider, surrounded by other grasses	Ojo and Faluyi
AT 5	Ondo road, Along Ore, Ondo State	N 07° 49.468` E 04° 52.444`	Ruderal on lateritic soil	Ojo
AT 6	Aladura, Ogun State	N 07° 30.068` E 04° 27.885`	Ruderal on lateritic soil	Ojo and Faluyi
AT 7	Omu-Ayede road, Ekiti State	N 07° 54.374`E 05° 20.167`	Ruderal location with a cluster of *Panicum maximum* and stands of *Chromolaena odorata*	Ojo, Faluyi, Matthew and Abraham
AT 8	Itaji-Oye Road, Ekiti State	N 07° 50.833` E 05° 20.661`	Ruderal location under a tree of *Parkia biglobosa* and *Alchornea laxiflora*	Ojo, Faluyi, Matthew and Abraham
AT 9	Ayede-Oye Road, Ekiti State	N 07° 50.438` E 05° 20.733`	Solitary plant, leaves are broad and usually short; in mesic location	"
AT 10	Erinmo Road, Ekiti State	N 07° 38.203` E 04° 51.755`	Ruderal location on lateritic soil in company of other grasses, Asteraceae	"

Accn: Accession, AG: *Andropogon gayanus*, AT: *Andropogon tectorum*.

Pollen stainability test

Matured pollen grains from each of the accessions were collected between 12:00 pm and 2:00 pm when the spikelets had opened. Pollens were dusted on microscope slides, stained with Cotton Blue in Lactophenol covered with cover slip and left for about 5 to 10 minutes for the pollen grains to properly take up the stain. Each prepared slide was viewed under a compound light microscope at x40 magnification. The pollens were scored as viable and non-viable using twenty fields for each of the accessions. Pollens that were well rounded, deeply-stained and moderately sized, were scored as viable. Those that were small–sized, irregularly-shaped and poorly-stained were scored as non-viable. Percentage pollen fertility were calculated using the formula below:

Pollen size distribution patterns

The diameter of one hundred (100) pollen grains were measured at random for each accession under the compound light microscope using graduated ocular micrometer at X40 objective magnification. Histograms were constructed to show the distribution pattern of the pollen sizes for all accessions.

RESULTS

Figures 4 to 6 show the main features of meiotic chromosomal studies in the accessions of *Andropogon gayanus* and *Andropogon tectorum* studied. In both *A. gayanus* and *A. tectorum* accessions studied, chromosome pairing was perfect in some of the Pollen Mother Cells. Some of the chromosome associations encoun-

Table 2. Morphological Features of the Accessions of *Andropogon gayanus-Andropogon tectorum* Studied.

Accn	Plant form	No. of leaves/ tiller	No. of tillers/ plant stand	Culm characteristics	Petiole
AG1	Broad leaves, heavy tillering, culm diameter fairly big. Plant type robust	6.45±0.15 (6 - 9)	32.85±6.09 (23–44)	Glabrous, green in colour covered with hairy leaf sheath.	Pubescent, grow up to 20 cm
AG2	Narrow leaves, heavy tillering, culm diameter thin. Plant type not so robust	6.33±0.14 (5 – 9)	34.50±5.79 (25-49)	«	Pubescent, grow up to 22 cm
AG3	Broad leaves, intermediate tillering, culm diameter fairly big. Plant type robust	6.20±0.23 (5 – 8)	25.45±8.50 (24-45)	«	Pubescent, grow up to 20 cm
AG4	Broad leaves, heavy tillering, culm diameter big. Plant type not so robust	6.35±0.16 (5–9)	31.23±6.09 (29-43)	«	Pubescent, grow up to 23 cm
AG5	Broad leaves, heavy tillering, culm diameter fairly big. Plant type robust	6.57±0.13 (5 – 9)	32.65±6.79 (27-47)	«	Pubescent, grow up to 22 cm
AG6	Broad leaves, heavy tillering, culm diameter big. Plant type robust	6.32±0.16 (5 – 8)	34.35±7.80 (23-51)	«	Pubescent, grow up to 28 cm
AT1	Narrow leaves intermediate tillering, culm diameter thin. Plant type stout	7.20±0.13 (5 – 9)	23.67±4.21 (26-43)	Glabrous, green in colour covered with hairless leaf sheath	Glabrous, grow up to 58 cm
AT2	Narrow leaves heavy tillering, culm diameter big. Plant type not so robust	7.31±0.21 (6 – 9)	31.33±7.88 (28-41)	Glabrous, green in colour covered with hairy leaf sheath	Glabrous grow up to 38 cm
AT3	Broad leaves, heavy tillering, culm diameter fairly big. Plant type robust	7.15±0.16 (5 – 8)	33.65±7.50 (27-40)	«	Glabrous grow up to 36 cm
AT4	Broad leaves, heavy tillering, culm diameter fairly big. Plant type robust	7.23± 0.13 (5 - 9)	31.75±6.85 (23-41)	Glabrous, purple in colour covered with hairy leaf sheath	Pubescent, grow up to 28 cm
AT5	Narrow leaves heavy tillering, culm diameter fairly big. Plant type robust	7.09±0.16 (6 – 9)	31.67± 3.72 (27-42)	Glabrous, purple in colour covered with hairless leaf sheath	Pubescent, grow up to 30 cm
AT6	Narrow leaves intermediate tillering, culm diameter fairly big. Plant type not so robust	7.12±0.15 (5 – 8)	29.50±5.65 (27-44)	Glabrous, green in colour covered with hairless leaf sheath	Glabrous, grow up to 30 cm
AT7	Broad leaves, heavy tillering, culm diameter big. Plant type robust	7.27±0.16 (6 – 9)	30.05±6.10 (21-45)	«	Glabrous, grow up to 28 cm
AT8	Broad leaves, heavy tillering, culm diameter fairly big. Plant type robust	7.40±0.16 (5 – 8)	32.54±5.08 (23-43)	Glabrous, green in colour covered with hairy leaf sheath	Glabrous, grow up to 22 cm
AT9	Broad leaves, heavy tillering, culm diameter big. Plant type robust	7.16±0.19 (5 – 9)	31.45±6.18 (21-42)	«	Glabrous, grow up to 26 cm
AT10	Broad, short leaves, very heavy tillering, culm diameter fairly big. Plant type robust	7.35±0.12 (6 - 8)	49.56±4.75 (39-69)	Glabrous, green in colour covered with hairless leaf sheath	Glabrous, grow up to 45 cm

Accn: Accession, AG: *Andropogon gayanus*, AT: *Andropogon tectorum*, No: Number.

tered were the formation of multivalents (especially in the polyploid accessions) for example formation of quadrivalents as chain of IV (Plates 4A and F), bivalents (frequently encountered) as ring II and rod II (Plates 5E and F) and occasionally occurrence of univalents in Plate 5E.

The following chromosomal configurations and aberrations were encountered in the meiotic chromosomes of the accessions studied: the formation of multivalents occurring especially in the polyploid accession, for instance, formation of quadrivalents resulting in a 5IV + 10II configuration as shown in Plate 4F, univalent resulting in 20I (Plate 5E), precocious movements at Anaphase I (Figure 4C). Normal bivalent pairing and regular segregation to the poles was also observed (Plate 4A and B). Table 3 shows the meiotic chromosome configuration of the accessions studied.

Pollen studies

Pollen stainability test

Generally, the pollen fertility in the accessions studied was high; it ranged from 83.47% to 97.20%. AT10, sample of the accession "Kiwani" collected on the Itawure-Erinmo Road (N 07°38.203` E04°51.755`) Ekiti State has the lowest value of 83.47% while accession AT1, collected along OAU-THC road (N 07°30.870` E 04°33.065`), Ile-Ife, Osun State has the highest value of 97.20% as shown in Table 4.

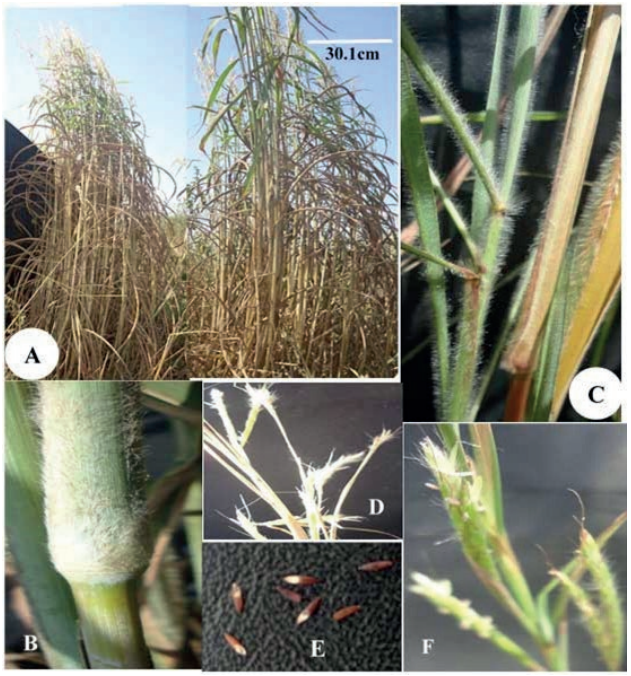


Figure 2. Morphological features of *Andropogon gayanus*. a: Plant form and adaptation; b: Internode; c: Hairy leaf sheath; d: Raceme pairs; e: Seeds; f: Spikelet.

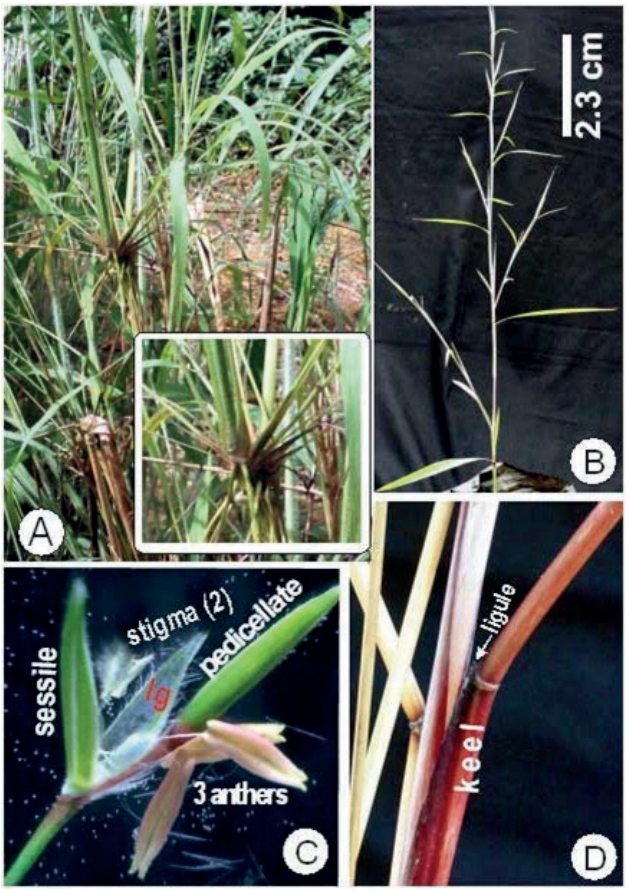


Figure 3. Morphological Features of *Andropogon tectorum*. a: Plant form and adaptation (Insect- Rooting at the node); b: Flowering Scape; c: Spikelet; d: The leaf showing sheath, keel and ligule; lg: Lower glume.

Pollen size distribution patterns

The pollens of AT10 "Kiwani", collected on the Itawure-Erinmo Road (N 07°38.203` E 04°51.755`) in Ekiti State had a majority falling in the large pollen size range while the other accessions of both AT and AG have modal pollen size having the highest frequency; although the AT accessions are generally smaller i.e. < 35 µm and the AG accessions are >35 µm as shown in Figures 7 and 8.

DISCUSSIONS

The key issues involved in occurrence of ploidy levels, meiotic studies within and among the two species of *Andropogon* studied can be summarized as follows:

1. two chromosome numbers were established: 2n = 40 in all accessions of *Andropogon gayanus* which paired as 20 II or at low levels of quadrivalents and univalents e.g. 2 IV + 16 II and 5 IV + 10 II. Segregation problems were very rare at Anaphase.

2. the dominant chromosome number in *Andropogon tectorum* is 2n = 20 which paired usually as 10 II, and occasionally, as 1 IV + 8II as in a AT4 collection from the Ife-Ibadan Road (N 07° 22.774` E 04° 01.497`), 20 I in AT9 accession from a location on the Ayede-Oye Road (N 07° 50.833` E 05° 20.661`) and 18 II + 4 I (2n = 40) in locality Kiwani, AT10 collected on a gravelly location on the Itawure-Erinmo Road (N 07° 38.203` E 04° 51.755`) both in Ekiti State, Nigeria.

The pairing patterns at diakinesis in *A. gayanus* suggests a diploid on a basic chromosome number of x = 20 paired as 20 II or an amphidiploid on a basic chromosome number of 10, while the regular occurrence of quadrivalent associations at low levels (2 IV+16 II, 5 IV + 10 II) suggest a tetraploid as a basic number of x = 10 with the four genomes still exercising considerable homology.

Okoli (1983) reported a chromosome number 2n = 40 for *Andropogon gayanus*, 2n = 20, 30 for *Andropogon tectorum* and their hybrids obtained by open and controlled crosses. The 2n = 20 chromosome in *A. tectorum* paired dominantly as 10 II and occasionally as 1 IV + 8 II which only occurred in a AT4 collection on

Table 3. Meiotic chromosome configurations (Diakinesis) in *Andropogon gayanus-Andropogon tectorum* accessions studied.

Serial No	Accn	Modal chromosome configuration	Diakinesis number
1	AG 1	5.05 ring II + 13.50 rod II	2n = 40
2	AG 2	4.10 ring II + 14.40 rod II + 1.45 Chain IV	"
3	AG 3	3.05 ring II + 14.20 rod II + 0.90 Chain IV	"
4	AG 4	4.55 ring II + 13.70 rod II	"
5	AG 5	3.05 ring II + 14.90 rod II + 1.15 Chain IV	"
6	AG 6	2.45 ring II + 11.90 rod II + 5.05 Chain IV	"
7	AT 1	4.95 ring II + 4.85 rod II	2n = 20
8	AT 2	4.85 ring II + 4.90 rod II	"
9	AT 3	4.95 ring II + 4.35 rod II + 0.45 Chain IV	"
10	AT 4	5.25 ring II + 4.25 rod II + 0.35 Chain IV	"
11	AT 5	4.80 ring II + 4.95 rod II	"
12	AT 6	7.05 ring II + 2.35 rod II	"
13	AT 7	6.85 ring II + 3.05 rod II	2n = 20
14	AT 8	4.05 ring II + 14.50 rod II	2n = 40
15	AT 9	0.95 ring II + 2.40 rod II + 6.45 I	2n = 20
16	AT 10	4.85 ring II + 7.05 rod II + 3.90 Chain IV + 1.90 I	2n = 40

Scores are based on 20 cells per accession.
Accn: Accession, AG: Andropogon gayanus, AT: Andropogon tectorum.

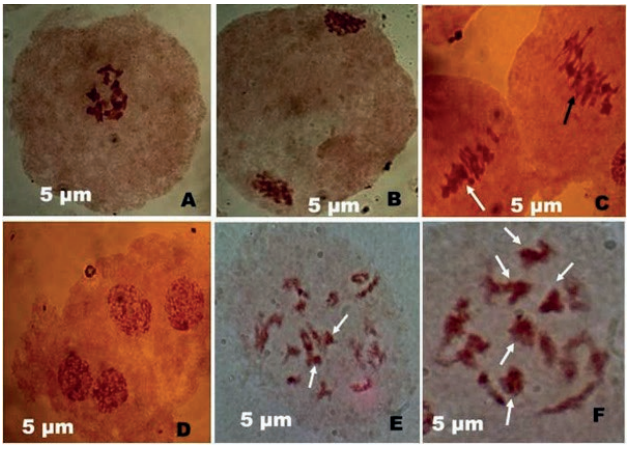


Figure 4. Chromosome Configurations in *A. gayanus* (2n = 40). A: AG 1, Metaphase I showing normal bivalent pairing as 20 II. B: AG 1, Telophase I showing clear segregations to the poles. C: AG 4, Late Metaphase I (white arrow, bottom cell) and Early Anaphase I (black arrow, top cell), showing precocious movements to the poles. D: AG 4, Tetrads of Telophase II. No laggard on restitution nuclei seen. E: AG 2, Diakinesis showing 2 IV + 16 II. White arrows show quadrivalents. F: AG 6, Diakinesis showing 5 IV + 10 II. White arrows show quadrivalents.

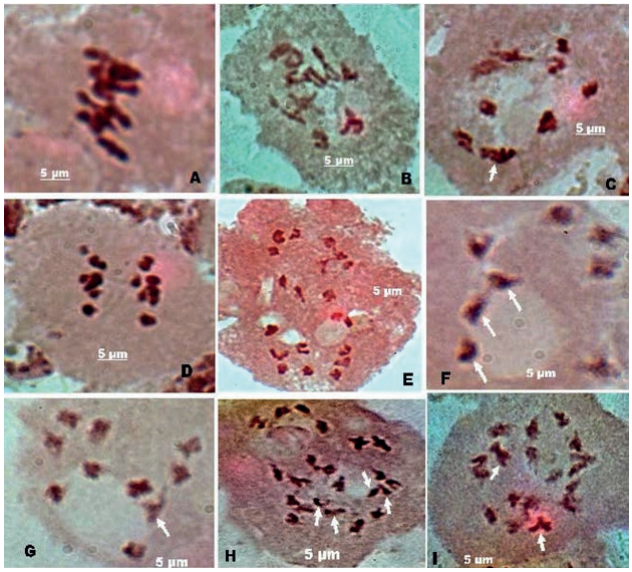


Figure 5. Chromosome configuration in the *A. tectorum* accessions (A-G: 2n = 20, H-I: 2n = 40). A: AT 1, Metaphase I showing normal bivalent pairing as 10 II. B: AT 2, Pachynema-Diplonema showing 10 II. C: AT 4, Diakinesis-Early Metaphase I showing 1 IV + 8 II. The white arrow shows a quadrivalent. D: AT 6, Anaphase I showing normal segregation to the poles. E: AT 9, Diakinesis showing 20 I. F: AT 7, Metaphase I showing three bivalents chromosomes around the nucleolus. G: AT 7, Late Diakinesis/Metaphase I showing 10 II. The white arrow probably shows the satellited pair. H: AT 10, Diakinesis showing 18 II + 4I. The white arrows indicate the four univalents. I: AT 10, Diakinesis showing 16 II + 2 IV. The white arrows indicate the two quadrivalents.

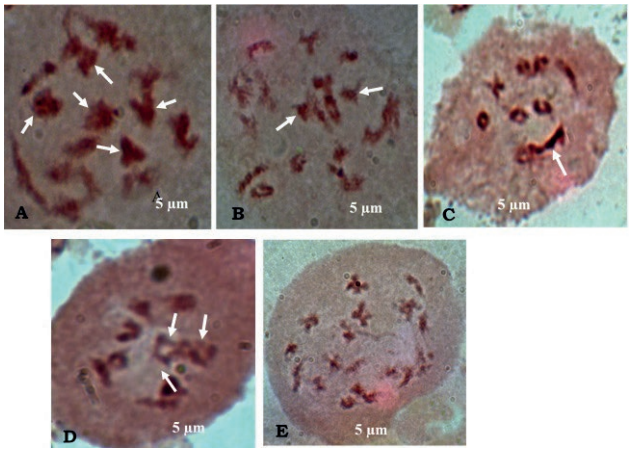


Figure 6. Chromosome Configurations for other accessions (A, B and E: 2n = 40, C and D: 2n = 20). A: AG 3, Diakinesis showing 5 IV + 10 II. White arrows show quadrivalents. B: AG 5, Diakinesis showing 2 IV + 16 II. White arrows show quadrivalents. C: AT 3, Diakinesis I showing 1 IV + 8 II. The white arrow shows a quadrivalent. D: AT 5, Diakinesis I showing fig. 8 and 9 II. White arrow showing fig. 8. E: AT 8, Diakinesis showing 20 II.

Table 4. Pollen size and fertility in the *Andropogon gayanus-Andropogon tectorum* accessions studied.

Characters			Pollen fertility			
Serial No	Accessions	Chromosome No	Mean ± S.D[a] (µm)	C.V. (%)	N[b]	%
1	AG1	2n = 40	43.24±4.75	11	1240	83.47
2	AG2	2n = 40	38.58±4.14	18	1892	87.53
3	AG3	2n = 40	41.25±4.91	12	1089	85.57
4	AG4	2n = 40	37.51±4.17	11	1068	96.34
5	AG5	2n = 40	36.95±4.09	11	1034	95.45
6	AG6	2n = 40	39.38±5.01	15	957	97.04
7	AT1	2n = 20	37.45±2.70	7	1098	96.45
8	AT2	2n = 20	36.52±2.82	7	1152	95.65
9	AT3	2n = 20	36.22±2.79	7	1068	95.77
10	AT4	2n = 20	36.38±2.57	7	1084	94.55
11	AT5	2n = 20	37.98±3.39	8	1194	93.13
12	AT6	2n = 20	37.75±3.23	9	1106	97.20
13	AT7	2n = 20	40.47±4.67	11	1278	95.85
14	AT8	2n = 40	41.95±4.43	12	1327	96.31
15	AT9	2n = 20	40.35±4.25	11	1285	93.54
16	AT10	2n = 40	41.59±4.57	12	1478	96.68

a: based on 100 measurements; b: number on which estimate was based; AG: *Andropogon gayanus*; AT: *Andropogon tectorum*; No: Number; CV: Coefficient of variation; S.D.: Standard Deviation; **%**: Percentage.

the Ife-Ibadan Road (N 07° 22.774` E 04° 01.497`). The occurrence of univalent, as 20 I in AT9 accession on the Ayede-Oye Road (N 07° 50.833` E 05° 20.661`) and 18 II + 4 I in locality Kiwani, AT10 collected on the Itawure-Erinmo Road (N 07° 38.203` E 04° 51.755`) suggests that while these species generally has a chromosome number 2n = 20, there is an isolated instance of the occurrence of 2n = 40 in the Ekiti populations. Accession "Kiwani" featured large biomass typified by a very high number of tillers, broad but short leaves, and dense expression of hairiness and large pollen grains which are typical of polyploidy.

The occurrence of univalent in the AT9 accession on the Ayede-Oye Road (N 07° 50.833` E 05° 20.661`) may be attributed to environmental stress occasioned by the cluster of regrowth forest species that choked the plant. Accession "Kiwani" occurred in an open gravelly ruderal location which had been dug up during road constructions. The plant probably occurred from seed that resulted from the apomictic process.

The cytological evidence from the accessions of *A. gayanus* (2n = 40) studied indicate a regular occurrence of quadrivalent and bivalent associations which suggests that there are four genomes on a basic chromosome number of 10 in *A. gayanus*. The genome can therefore be an allopolyploid (amphidiploid). The configurations 20 II, 2 IV + 16 II and 5 IV + 10 II were recorded in the *A. gayanus* accessions studied.

Okoli (1978) concluded that *A. gayanus* is an allopolyploid based on the karyotypic studies he did. It is significant that Okoli (1978) reported the recovery of a fertile interspecific triploid hybrid between *A. gayanus* and *A. tectorum* in the garden suggesting that such hybrids were through introgression in the wild. No interspecific crosses were formed during the field survey of this study. This does not mean that such hybrids were not occurring; they may be morphologically identical to *A. gayanus* as Okoli (1978) had observed in the artificial triploid hybrids in the garden.

It was observed that the major features of the chromosomes of the accessions studied are regular occurrence of multivalent associations i.e. formation of quadrivalents as chain IV, occurrence of bivalents as ring II and rod II in more frequencies and occasional occurrence of univalents. All the accessions studied conform to the chromosome number of 2n = 40 for *A. gayanus* and 2n = 20 and 2n = 40 for *A. tectorum*. According to Faluyi and Olorode (1987), the occurrence of multivalents and univalent indicate potential for the evolution of aneuploidy and possibilities for change of chromosome number and chromosome repatterning which might lead to genic imbalance and probably viable genetic variability in the species.

The morphological and ecological continuity between *A. gayanus* and *A. tectorum* (Forster, 1962) has led Singh and Godward (1961) to hypothesize that *A.*

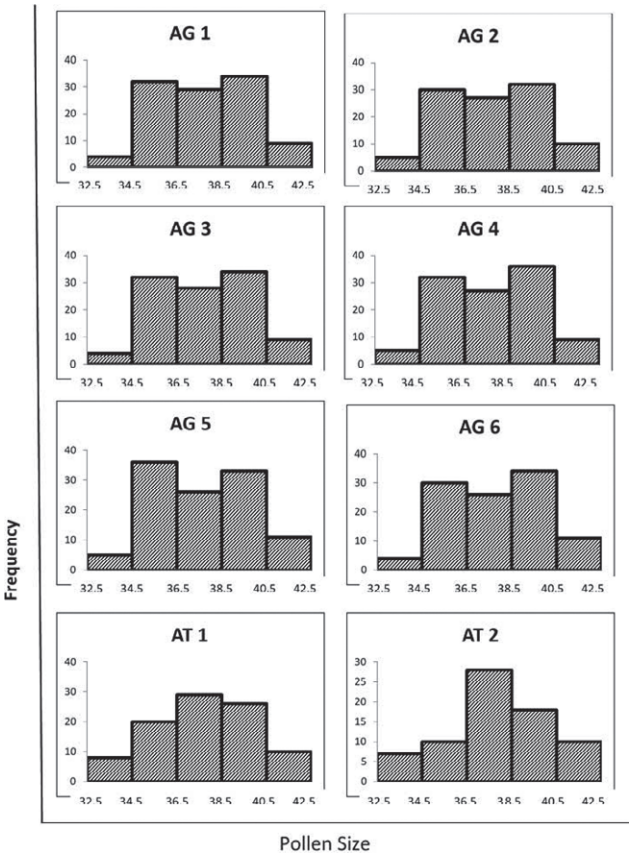


Figure 7. Histogram showing pollen size distribution patterns in the accessions of *Andropogon gayanus-Andropogon tectorum* studied. AG: *Andropogon gayanus*, AT: *Andropogon tectorum*.

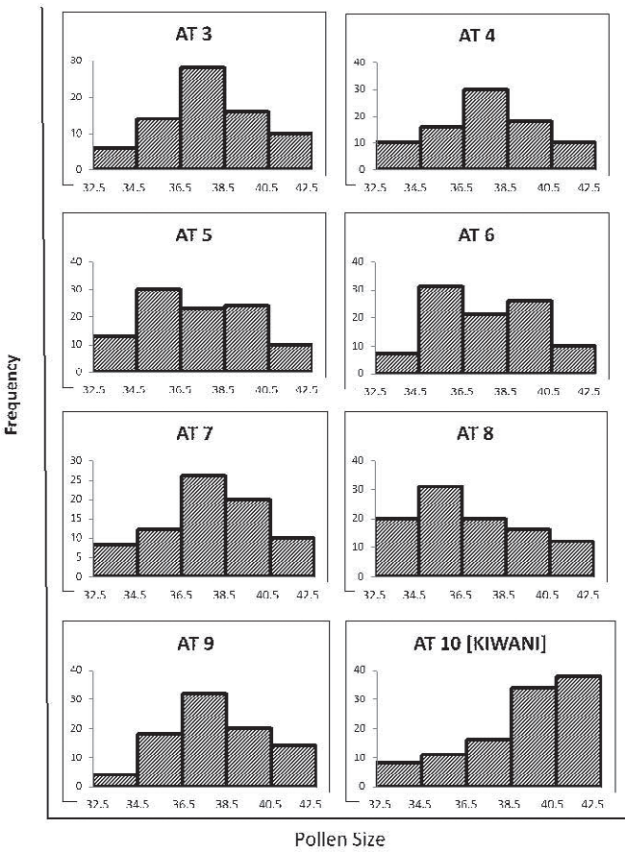


Figure 8. Histogram showing pollen size distribution patterns in the accessions of *Andropogon tectorum* studied. AG: *Andropogon gayanus*, AT: *Andropogon tectorum*.

gayanus (2n =40) resulted from polyploidization of the diploid hybrid between a southern *A. tectorum* and a northern *A. gayanus*. This study pointed to the fact that in *A. tectorum*, the diploid (2n = 20) is ancestral while the higher chromosome numbers are derived. The high incidence of multivalents is suggestive of autoploidy (Stebbins 1950, 1970; Swanson 1968), which means pollen size is high at high ploidy levels. It would be expected that multivalent associations should lead to loss of fertility. The diploid hybrids showed no evidence that the presence of univalent or multivalents were necessarily associated with loss of fertility because all the accessions studied show high pollen viability test and relatively low variability in pollen size. The occurrence of bivalents in the meiotic cells of the species studied suggest that they are allopolyploidy. Also available cytological evidence showed that *A. gayanus* is an allopolyploid while the multivalent associations featuring in diakinesis points to its polyploid (Olorode, 1972).

This study established the occurrence of a tetraploid with a chromosome number of 2n = 40 in one collection of *A. tectorum* from Ekiti State where the chromosome number is predominantly 2n = 20. The details of the cytological studies revealed multiple associations as quadrivalents, bivalents and univalents in the collections of the two species. These events must play major roles in the reproductive and population dynamics of the *Andropogon* complex.

The occurrence of a tetraploid *A. tectorum* is one of the most significant events given the role that this phenomenon and apomixis have been reported to play in the colonization of plants. Hojsgaard (2018) identified the steps involved in the process of polyploidy among which the formation of a triploid arising from a reduced-unreduced gamete fusion is the foundation. This triploid serves as a bridge between parental diploids to produce a derivative tetraploid. The derivative tetraploid produces a new generation of homoploids through a variety of pathways to establish a series of mating partners. The establishment of new polyploids depends on the rate of formation of unreduced (2n) gametes through the apomictic process, frequency-dependent processes that define

minority cytotype disadvantages, small effective population sizes and environmental changes that may increase polyploid establishment rates.

Apomixis is the key process that results in the unreduced gamete which is not a product of the regular meiotic process. The unreduced gamete can lead to a polyploid in one step so that apomicts have an advantage in range expansions on account of fitness, vigour and resistance to adverse environmental conditions. Schinkel et al (2016) monitored the correlations of polyploidy and apomixis with elevation and associated environmental gradients in an alpine plant, *Ranunculus kuepferi* using flow cytometry to quantify apomictic and sexual seed formation while seed set and vegetative growth indices were used as fitness parameters. All parameters were correlated to geographical distribution, elevation, temperature and precipitation. Flow Cytometry in their experiment revealed obligate sexuality and facultative apomixis in diploid populations while tetraploid populations were predominantly facultative to obligate apomicts. Apomictic seed formation correlated significantly to higher elevations where niches were at lower temperatures. Diploid apomixis was not successful in range expansion and obligate sexual polyploids were not observed. Facultative apomixis may have aided the colonization of higher elevations but did not necessarily involve long-distance dispersal.

The prospects for the ecological success of the Andropogon complex in its spread down-south looks very bright from the perspectives of its reproductive biology: production of regular seeds by fertilization, haploid and diploid seeds through apomixis; occurrence of plants as facultative or obligate apomicts, production of propagules by rooting at the nodes, preservation of plants as underground rootstocks during the dry season, even in heavily-grazed locations, induction of flushing by fire and the occurrence of tetraploids and aneuploids through natural processes. Apomixis will help the plants to be able to colonize space because it confers self-compatibility as opposed to sexual reproduction where self-incompatibility might be a hindrance according to Baker's Law (Baker 1967)

The incidence of polyploids in the Ekiti populations of *Andropogon tectorum* and their occurrence in restricted gravelly locations sometimes as large populations as on the Ifaki-Ado Road and the occurrence of collections with marked meiotic disturbances also in some Ekiti accessions have been documented. The establishment of the Andropogon complex in the Derived Savanna of the South West is certainly going to involve many polyploids which will get stable as the new habitats and niches get colonized. There will also be intermediate triploids and aneuploids which will arise from the interbreeding between *Andropogon gayanus* and *Andropogon tectorum* some of which will probably be maintained by apomixis and vegetative propagules. This complex will probably be a major part of the vegetation of the Derived Savanna in the near future based on the resilient reproductive strategies that this study and that of Okoli (1978) have revealed.

CONCLUSIONS

The chromosome numbers reported in this study are based on five consistent counts for all accessions. The collections made reflect a substantial endemicity of *A. gayanus-A. tectorum* and accession "Kiwani" is distinct in every regard. From the meiotic studies, a chromosome number of 2n = 20 and 2n = 40 have been established for the species. The occurrence of the tetraploid, chromosome number of 2n = 40 in accession "Kiwani" has been attributed to apomixis occurring in a lonely plant.

AUTHORS' CONTRIBUTIONS

CCN conceived the study. JOF, FMO, CCN realized the experimental work. FMO acquired funding and Data Resources; FMO, CCN did the Writing of review and editing: JOF, supervised. FMO refined the final revision. All authors read and approved the final version of the main manuscript

ACKNOWLEDGEMENTS

We thank God for the successful completion of this research and the positive impact of my supervisor to achieve this research goal.

FUNDING

The research supported by Bill Dahl Graduate Student Research Grant Award (2019), Botanical Society of America, United States of America and Academic Staff Union of Universities (ASUU) Scholarship Award for 2019/2020 Academic Session.

REFERENCES

Baker, H. G. (1967) Support for Baker's Law-as a rule. Evolution 21: 853–856.

Bogdan, A. V. (1977) Pasture and Fodder Plants (Grasses and Legumes), Longman Press, New York, U.S.A. page 1–39, 229–231.

Bowden, B. N. (1963) Studies on *Andropogon gayanus* Kunth. I. The use of *Andropogon gayanus* in Agriculture. Empire Journal of Experimental Agriculture, 31: 267–273.

Chlleda, H. R. and Crowder, L. V. (1982) Tropical Grassland Husbandry, First edition, Longman, London, p. 346–367.

Clayton, W. D. (1962) A Key to Nigerian Grasses. Government Printer, Kaduna. Nigeria, pp. 86.

Clayton, W. D. (1969) In Hutchinson, J. and Dalziel, J. M. (Flora of West Tropical Africa, Vol. 111(2), 2nd ed. (Revised by F. N. Hepper). Crown Agents for Overseas Governments and Administration, London.

Faluyi J. O., and Olorode, O. (1987) Aspects of the dynamics of meiosis in nymphal and adult populations of *Zonocerus variegatus* L. (Orthoptera: Pyrgomorphidae). Ife Journal of Science, 2 (1 and 2): 51–61.

Forster, W. H. (1962) Investigations preliminary to the production of cultivars of *Andropogon gayanus.* Euphytica, 11: 47–52.

Hojsgaard, D. (2018) Transient Activation of Apomixis in Sexual Neotriploids may retain genomically altered states and enhance Polyploid Establishment. Frontiers in Plant Science, doi:10.3389/fpls.2018.00230

Hutchinson J, Dalziel J. M. (1972) Floral of West Tropical Africa Vol. III Part 2 –Crown Agents, London, p. 459–461.

Jackson, R. C (1962) Documented chromosome numbers of plants. Madrono 16: 266–268.

Laosebikan, B. A. and Olorode O (1972) Morphological variation and cytological aberrations in natural populations of *Zonocerus variegatus* L. (Orthoptera: Pysrogomorphidal) Bull Entomological Society of Nig, 3: 127–133.

Lowe J. (1989) The Floral of Nigerian Grasses. 2nd ed. Ibadan University Press, p. 214.

Okoli, B. E. (1978) Genetic Studies in the *Andropogon gayanus – Andropogon tectorum* (Gramineae) Complex in Nigeria. Ph. D Thesis (Unpublished) submitted to the Department of Botany, University of Ife, Osun State, Nigeria.

Okoli, B. E. (1983) Hybridization, Polyploidy and Apomixis in *Andropogon tectorum* Schum.and Thonn. (Gramineae). New phytologist, 93(4): 591–597.

Okoli B. E. and Olorode O (1983) Cytogenetic studies in the *Andropogon gayanus- A. tectorum* Complex (Gramineae). Botany Journal of Linnean Society, 87: 263–271.

Olorode, O. (1972) Cytological studies in some Nigerian Andropogoneae. Nigeria Journal of Science, 6(1): 13–19.

Olorode, O. (1984) Taxonomy of West African Flowering Plants. (Revised ed.) Longman Group Limited Publishers, London, p 123–127.

Pagot, J. (1993) Animal Production in the Tropics, 1st edition, Macmillan Press, London, pp. 163–181, 224–225.

Schinkel, C. F., Bernhard K., Agnes S. D., Simone K, Manuela W, Stefan D, Elvira H (2016) Correlations of polyploidy and apomixis with elevation and associated environmental gradients in an alpine plant. Annals of Botany

Singh, D. N. and Godward, M. B. E. (1961) Cytological studies on the Gramineae. Heredity, 15: 183–197.

Stanfield, D. P. (1970) The Floral of Nigeria Grasses. (1st ed.) University of Ibadan Press, Ibadan, p. 83.

Stebbins, G. L. (1950) Variation and Evolution in Plants, New York, Columbia University Press, 643.

Stebbins, G. L. (1970) Variation and Evolution in Plants: Progress using the Past Twenty Years, Essays in Evolution and Genetics in Honor of Theodosius Dobzhansky 173–208.

Swanson, C. P. (1968) Cytology and Cytogenetics, 2nd edn. Prentice-Hall Inc., New Jersey.

Caryologia
International Journal of Cytology, Cytosystematics and Cytogenetics

Firenze University Press
www.fupress.com/caryologia

OPEN ACCESS

Citation: Shivali Verma, Irfan I. Sofi, Aijaz H. Ganie, Manzoor A. Shah, Namrata Sharma (2023). Incidence and frequency of desynapsis in *Eremurus persicus* (Jaub. & Spach) Boiss. (Asphodelaceae) – A native and important medicinal plant species of Western Himalaya. *Caryologia* 76(1): 15-26. doi: 10.36253/caryologia-1904

Received: November 23, 2022

Accepted: April 7, 2023

Published: September, 19, 2023

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Incidence and frequency of desynapsis in *Eremurus persicus* (Jaub. & Spach) Boiss. (Asphodelaceae) – A native and important medicinal plant species of Western Himalaya

Shivali Verma[1], Irfan I. Sofi[2], Aijaz H. Ganie[2], Manzoor A. Shah[2], Namrata Sharma[1]

[1] *University of Jammu, India*
[2] *University of Kashmir, India*
E-mail: shivaliverma492@gmail.com; sofi.irfan98@gmail.com; aijazku@gmail.com; mashah@uok.edu.in; namratadni@gmail.com

Abstract. *Eremurus persicus* (Jaub. & Spach) Boiss. (Asphodelaceae); a little known species of genus *Eremurus* grows in arid and semi-arid regions, on rocky mountains in Central Asia and Middle East. The species is native and an important medicinal plant used to treat various diseases. In the current study, we examined male meiosis, karyotypic variability, pollen viability and reproductive output of three populations of the target species. All the studied populations have diploid chromosome count of 2n = 2x =14. Meiotic course in majority of the pollen mother cells (PMCs) in all the three populations is not normal due to the presence of desynapsis at diplotene and metaphase-I. Instead of 7IIs most of the PMCs examined reveal varying number of bivalents and univalents. Anaphasic segregation is also affected in few PMCs. Present study also revealed that the species has asymmetric karyotype of 10 long and 4 short chromosomes. Species exhibits fruit abortion that results in extremely low fruit/seed set. The meiotic abnormalities and low fruit/seed set may be attributed to restricted distribution of this important plant species. Present study may prove helpful in devising the conservation and management strategies for this prized plant species.

Keywords: desynapsis, native, diplotene, metaphase, karyotype, fruit abortion, conservation.

INTRODUCTION

Pairing of chromosomes is the first step that leads to the organized segregation of chromosomes during meiosis and is the basis of continuity of life (Fabig *et al*., 2020). In addition being the first step of reduction division; this is also important as it leads to crossing over and reshuffling of alleles and contributes to the generation of genetic variations (Cai & Xu, 2007). Though it is religiously followed in stable diploid species during zygotene, instances where the deviation from norms occurs are not rare. Chromosomes in some

taxa fail to pair (asynapsis) (Jakson *et al.,* 2002; Ansari *et al.,* 2022) or their pairing breaks precociously (desynapsis) (Golubovskaya,1979; Wani & Bhat, 2017). These anomalies arerecognized when most or all the chromosomes remain as univalents at diakinesis and metaphase-I (Visser *et al*.,1999). Although theoretically the phenomenon of asynapsis & desynapsis are distinct from each other, it is difficult to distinguish them in practice. This is true for those plants where chromosomes are long and pairing cannot be followed during earlier stages (Koul, 1962; Wani & Bhat, 2017).

As a characteristic of a particular variety or ecotype, desynapsis has been reported in several plant genera viz *Zea* (Beadle, 1933); *Nicotiana* (Clausen, 1931); *Datura* (Bergner *et al*., 1934); *Gossypium* (Beasely and Brown, 1942); *Triticum* (Li *et al*.,1945), *Alopecurus* (Jhonson, 1944) etc. But as an inherent feature of a taxa as a whole; it is a rare occurrence. Here we report this phenomenon in *Eremurus persicus* (Jaub. & Spach) Boiss. Described as a feature of meiosis in few plants of this species by Verma (2001) as well, it was found to be a regular feature of this plant species across all the plants in all populations scanned in present study.

Genus *Eremurus* M. Bieb. is commonly known as Foxtail lilies/ Desert candles of family Asphodelaceae comprises of more than 60 species that are native to Central Asia and Caucasia (Kamenetsky & Akhmetova, 1994). The species of the genus can be easily recognized by their long scapes bearing numerous colourful flowers along with rosette of leaves. Most of the species of this genus are important commercially as ornamental plants for landscaping and cut flower markets (Schiappacasse *et al*., 2013). In addition to their ornamental value, *Eremurus* sp. have been used in traditional medicine and are potential sources for anti-inflammatory, anti-bacterial and anti protozoal drugs (Hashemi *et al*., 2014 ; Mati *et al*., 2011). Also bio-oil and adhesives prepared from this genus play an important role in industrial applications (Vala *et al*., 2011). In India Genus *Eremurus* is represented by only two species *viz Eremurus himalaicus* Baker and *Eremurus persicus* (Jaub. & Spach) Boiss.

Eremurus persicus is a perennial herb which perennates through underground fleshy tuberous rootstocks that sprout every year in the month of February- March in the sub-tropical areas of Jammu, UT of J&K, India. The above ground portion of the plant appears in the form of rosettes of leaves which are light to dark green in colour, radical, thick and lanceolate. The inflorescence is raceme which bears flowers in an acropetal manner. Flowers are creamish to light pink in colour, pedicellate, bracteate, bisexual and glabrous. Species holds immense value for its medicinal attributes in addition to being used as a vegetable for its edible leaves. The species is widely distributed in South, East and West of Iran where it is locally known as Sarish (Karl, 1982; Safar *et al*., 2009; Vala *et al*., 2011). It is also reported from Kibri Kuch Ziarat of Pakistan (Khan *et al*., 2011).

In India there are scanty reports of the distribution of the species, however; the species is growing in hilly terrains of Jammu province (Hamal & Karihaloo, 1983). Extensive field surveys, perusal of relevant literature and herbarium studies revealed that the plant species has a restricted distribution in the study area. Only few populations (7 populations) of the species have been located from district Reasi of J&K. Keeping in view the rare distribution and medicinal importance of the target plant species the present study was carried out to provide detailed meiotic, mitotic account, pollen viability and fruit/seed set of *E. persicus*. The knowledge generated in present study may prove helpful in devising conservation strategies for this prized plant species.

MATERIAL AND METHODS

A total of 7 populations of *E. persicus* were located during present investigation, which include: Slal, Kharjala, Slal Kotli, Sarmega I, Sarmega II, Ponsli I and Ponsli II (Figure 1 and 2). Of these three populations *viz.* Slal (Population-I), Sarmega I (Population-II) and Ponsli I (Population-III) were selected for detailed meiotic, mitotic investigation, pollen viability and seed set in the present study. Materials for male meiotic studies were collected from wild plants growing on rocky slopes of hilly areas of Trikuta hills of Reasi. Young unopened flower buds of suitable sizes were collected randomly from different plants of each population during the peak flowering period, i.e., March-April, when minimum and maximum temperature of this area averages 13-25°C. The floral buds were fixed in Carnoy's fixative for 24 h. Subsequently, the materials were transferred in 70% ethanol and stored under refrigerator at about 4°C until analysis. For meiotic preparations, smears of the fixed anthers were made in 1% propiocarmine. Photomicrographs of Pollen Mother Cells (PMCs) for chromosomal counts at different stages and meiotic irregularities were made from the freshly prepared slides using EVOS XL microscope. Chiasmata number was counted for cells at diplotene. Data was used to calculate Recombination Index using the following formulae

RI= Chiasmata frequency per cell + haploid chromosome number of the species

Figure 1. *Eremurus persicus*(a) Habitat, (b&c) A mature flowering plant, (d) Ripen globular fruits.

For Karyological studies, healthy root tips from seedlings were collected and washed with distilled water. These were pre-treated with 0.3% colchicines solution for 4 hours and fixed in acetic acid: ethanol (1:3) for 24 hours. Thereafter, the root tips were transferred to 70% ethanol for further use. For mitotic chromosomal preparations, preserved root tips were washed with water and hydrolyzed in 1 drop of 1N HCL and 9 drops of 1% aceto-orcein at 60°C for 12-15 min. Thereafter these root tips were squashed in 1% acetocarmine. Karyotype formula was determined according to Levan *et al*., 1964. Karyotypes were compared using Stebbins classification, karyotype asymmetry index (As.K %) (Arano, 1963), total form percent (TF %) (Huziwara,1962), Rec and Syi indices (Venora *et al*., 2002), intrachromosomal symmetry index (A1) and interchromosomal asymmetry index (A2) (Romeo-Zarco, 1986), dispersion index (DI) (Lavania & Srivastava, 1999), degree of asymmetry of karyotype (A index) (Watanabe *et al*.,1999), asymmetry index (AI) (Paszko, 2006), the coefficient of variation of chromosome length (CV_{CL}) and the coefficient of variation of centromeric index (CV_{CI}) (Paszko, 2006) and mean centromeric asymmetry (M_{CA}) (Peruzzi *et al*., 2009). The strength of the association between karyotype asymmetry indices was tested using Pearson correlation analysis using PAST software.

To calculate number of pollen grains per anther and/ flower, 30 flowers ready to open with intact anthers were selected. Pollen quantity was estimated by squashing one anther (several times) in 10 drops of distilled water in a cavity block and shaken with a glass rod.

The following equations were used to calculate the number of pollen per flower:

$$\boldsymbol{r} = \boldsymbol{p} \times \boldsymbol{q} \text{ and } \boldsymbol{t} = \boldsymbol{r} \times \boldsymbol{s},$$

where $\boldsymbol{p}$ is the mean pollen count per drop of water; $\boldsymbol{q}$ is the number of water drops taken initially in which one anther was squashed; $\boldsymbol{r}$ is the mean number of pollen per

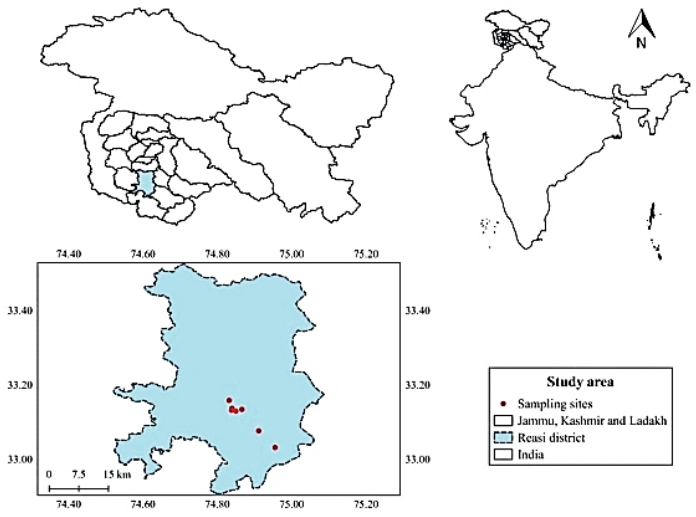


Figure 2. Map showing sampling sites of *E. persicus.*

anther; ***s*** is the mean number of anthers per flower; ***t*** is the total count per flower (Ganie *et al.,* 2021).

For calculation of pollen count per flower the average pollen number in one anther was multiplied by total number of anther per flower

Average ovule number per pistil was counted using dissection microscope (Ganie *et al.,* 2021).

Pollen viability was determined by subjecting the pollen grains to 1% acetocarmine, TTC and FDA. In first method, mature anthers were squashed in 1% acetocarime and kept for 10 minutes and then slides were scanned under microscope, well stained and plump pollen grains were considered viable while as lightly stained and shriveled pollen grains were considered as non-viable. In second method, the ready-to-dehisce mature anthers were placed in 1% tetrazolium chloride for one hour and squashed to check for viability (Rashid *et al.* 2023) and in third method pollen grains were mounted in Fluorescein diacetate (FDA) solution and incubated for 3-5 min. The pollens with fluorescent and non-fluorescent cytoplasm were treated as viable and nonviable, respectively (Rashid *et al.* 2023). In all the three methods, percentage of pollen viability was determined by following formula:

$$\text{Pollen viability} = \frac{\text{Number of pollengrains stained}}{\text{Total number of observed pollen grains}} \text{ X } 100$$

To check the reproductive efficiency on open pollination, 20 different inflorescences were tagged and kept for pollination to take place as it does in nature. These were monitored regularly from the time of flower opening to that of fruit maturation.These flowers were observed after some days to check the percentage fruit set. Percentage fruit set on unassisted selfing was performed by bagging large number of buds from 10 different inflorescences in transparent butter paper bags. Total number of fruits as an estimate of female success was calculated. Mature fruits were harvested shortly before dehiscence and scored for the presence of seeds. Percentage fruit set and seed set was then calculated by using Bharti *et al*., 2021 method.

RESULT AND DISCUSSION

PMC meiosis

All the three populations of *E. persicus* matched in having 14 chromosomes in their PMCs revealing 2n= 2x = 14. PMCs were analysed at diplotene, diakinesis, metaphase-I and anaphase-I. Desynapsis was observed as a regular feature in majority of the cells at diplotene and all the cells at Metaphase-I. Meiotic behavior of three populations differed slightly and the meiotic behavior of the studied populations is given as under:

Population-I (P-I)

In P-1, a total of 97 cells were scanned at different stages of meiosis with 26 cells (26.80%) at diplotene, 15 cells (15.46%) at diakinesis, 39 cells (40.20%) at metaphase-I and 17 cells (17.52%) at anaphase-I (Figure 3 a-e). Out of 26 cells at diplotene only 2 cells were having perfect 7IIs (Figure 3 a). Of observed bivalents, 5 bivalents/chromosomes were large and 2 were small. Interestingly in these bivalents, chromosomes were mostly held by terminal chiasmata only. At metaphase-I, cells were observed with different configurations and with varying number of univalent and bivalents in all PMCs. It has also been observed that 38.46% of cells showed all the 14 chromosomes as univalents (Figure 3 b) while 25.64% of cells showed the presence of 2IIs along with 10Is. Other configurations like 1,3,4 & 5 IIs were also observed. The univalents at this stage are arranged close to each other. At anaphase-I, PMCs usually showed normal segregation of 7:7 chromosomes at each pole (Figure 3 c-e). Chiasmata frequency per PMC calculated at diplotene in this population is 9.15 while RI calculated is 16.5.

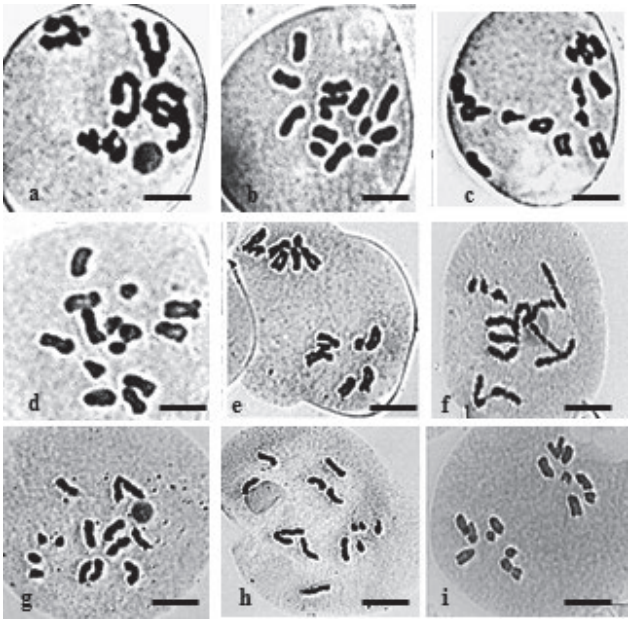


Figure 3. Population I (a-e): (a)A PMC at diakinesis with 7IIs, (b) A PMC at metaphase-I with univalents, (c&d) PMCs at early anaphase stage, (e) A PMC at anaphase-I. **Population II (f-i):** (f) A PMC at diplotene showing terminal chiasma, (g&h) PMCs at metaphase-I with 14 univalents, (i) A PMC at anaphase-I showing normal segregation of 7:7 chromosomes.

Population-II (P-II)

In P-II, 91cells were observed, out of which 33 cells (36.26%) were at diplotene, 37 cells (40.65%) were at metaphase-I and 21cells (23.07%) were at anaphase-I (Figure 3 f-i). In this population, formation of 7IIs was lacking even at the diplotene stage. At this stage only few II's were held by terminal chiasmata. At metaphase-I varied number of IIs and their configurations were observed, in addition varied number of Is were also observed in all the scanned PMCs. At this stage also chromosomes in IIs were associated terminally only. In 51.35% of cells all the 14 chromosomes as Is were observed (Figure 3 g-h) followed by 3,2&1 IIs along with Is. However, at anaphase-I usually normal segregation of 7:7 chromosomes was observed (Figure 3 i). Chiasmata frequency per PMC calculated at diplotene in this population is 6.9 and RI thus comes out to be 13.9.

Population-III (P-III)

In P-III, a total of 318 cells were observed at different stages, 45 cells (14.15%) at diplotene (Figure 4 a, b), 26 cells (8.17%) at diakinesis (Figure 4 c), 91 cells (28.61%) at metaphase-I, 79 cells (24.84%) at anaphase-I, 21 cells (6.60%) at telophase-I, 23 cells (7.23%) at metaphase-II and 33 cells (10.37%) at anaphase-II. In this population maximum frequency of PMCs with Is was observed (Figure 4 d), i.e., 61.53% followed by 2,3 & 5 II's in association with I's respectively. In present study 7.54% of cells scanned at anaphase-I revealed normal segregation of chromosomes with 7:7 distribution at each pole (Figure 4 e). Erratic distribution of 7:6, 6:6 and 6:5 chromosomes at each pole was observed in 7 cells (2.20%), 8 cells (2.51%) and 13 cells (4.08%) respectively. Also 27 cells (8.49%) showed presence of laggards and chromosomal bridges along with clumping of chromosomes (Figure 4 f-m). At anaphase-I and anaphase-II the formation of laggards and bridges was observed as a common feature. Chromosomal bridges were observed at metaphase-II also. All the cells scanned at metaphase-II and anaphase-II showed the presence of laggards and chromosomal bridges (Figure 5 a-d). Therefore the second meiotic division exhibited drastic irregularities in this population. Chiasmata frequency per PMC calculated at diplotene in this population is 5.3 and RI is 12.3.

Detailed studies on meiotic system revealed that in the species, majority of the PMCs at diplotene, diakinesis and metaphase consist of univalents ranging from 2-14 in all the three studied populations. At diplotene loosely bound IIs are seen mostly with terminal and

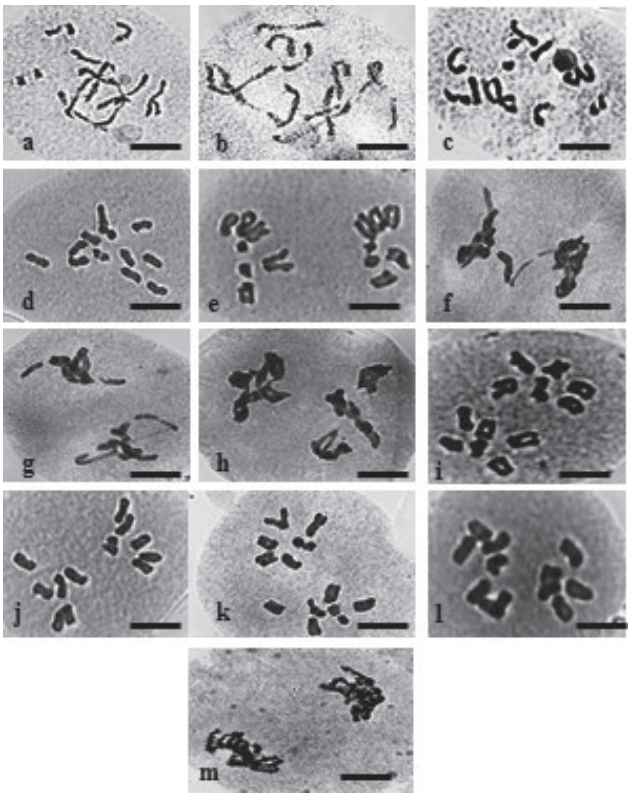


Figure 4. Population III (a-m): (a, b) PMCs at diplotene,(c) PMC at diakinesis(d) PMC at metaphase-I showing univalents,(e) PMC at anaphase-I showing normal distribution of 7:7 chromosomes,(f-h) PMCs at anaphase-I showing laggards and bridges along with clumping of chromosomes, (i) A PMC at anaphase-I showing 7:6 distribution, (j) A PMC with 6:6 distribution at anaphase-I,(k) A PMC showing 6:5 distribution with laggard, (l) A PMC with 6:5 distribution of chromosomes at anaphase-I, (m) A PMC at anaphase-I showing clumping.

rarely with intercalary chiasmata. As meiosis proceeds, intercalary chiasmata are not seen in any of the bivalent and the frequency of univalents goes on increasing. At later stage few bivalents and more frequency of univalents are observed. Subsequently the chromosomes fall apart and remain as univalents during rest of the cell cycle till metaphase-I. In most of the PMCs univalents were found to lie in pairs (distant pairing) were also observed in the present study. The frequency of univalent and bivalents observed in three populations are depicted in Figure 6.

SOMATIC CHROMOSOME COMPLIMENT

All the three populations studied show chromosomes in their somatic compliment confirming 2n=

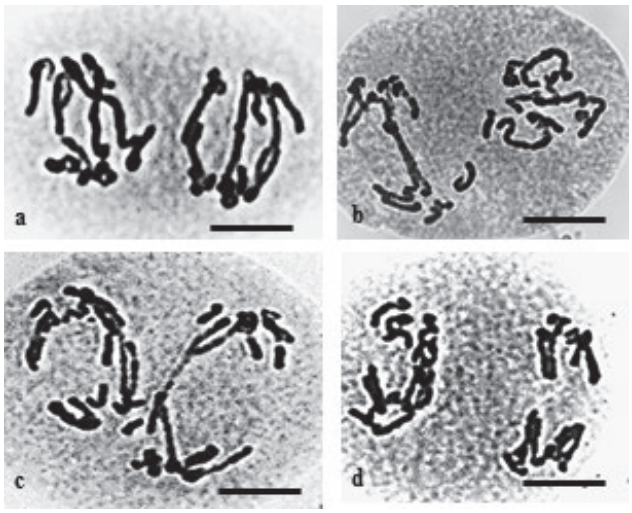


Figure 5. Population III (a-d): (a) A PMC at telophase-I, (b) APMC at metaphase –II with chromosomal bridges, (c, d) PMCsat anaphase-II with laggards and bridges.

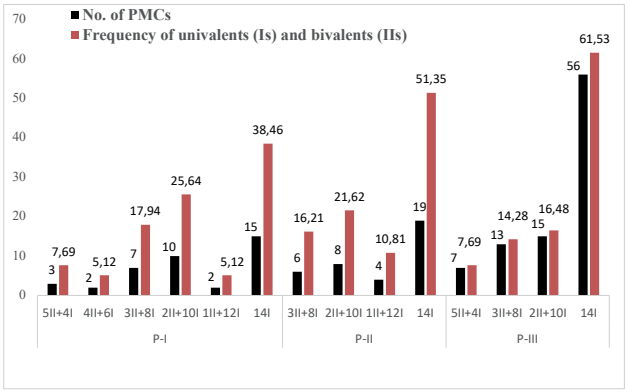


Figure 6. Graphical representation of configurations of univalents and bivalents.

2x = 14 as the diploid chromosome number of the species. These fall into two size groups. Group I includes 10 long chromosomes, all of them being subterminal while group II includes 4 small chromosomes which are submedian and subterminal. The total chromatin length of the somatic compliment for population I, II and III is 160.2, 158.4 and 168.3 µm. The kayotype formula for the three populations is 10ST+2ST+2SM, 10ST+3SM+1ST and 10ST+1ST+3SM respectively (Figure 7). These populations thus varied slightly in their karyotypic formulae too, although the chromosomes were of subtelocentric and submetacentric type only. These fallin 3C category of the Stebbin's (1971) chart of asymmetry. Among all the populations studied, P-III possessed the highest value (17.6 µm) of longest chromosome while P-I (15.54

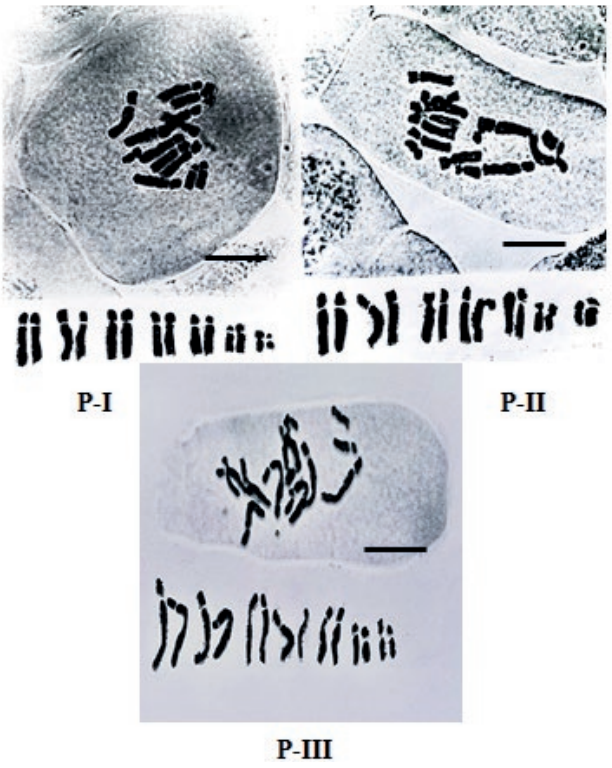


Figure 7. Somatic metaphasic spread of P-I, P-II and P-III showing 14 chromosomes and the karyogram of the same. (Scale = 10µm).

µm) possessed the lowest value for the same. The highest and lowest values for the smallest chromosome occurred in P-III (4.4µm) and P-II (4.95µm) respectively (Table 1). The most asymmetrical karyotype was observed in P-III (Table 2)

P-III possessed the highest value for CV showing the highest variation among its chromosomes compared to the other populations.Pearson coefficient of correlation determined for karyotypic parameters among the populations studied revealed a high correlation significant at $p \leq 0.05$ (Table 3). The AI value and scatter diagram based on the CV_{CL} and CV_{CI} seem best suited to assess overall classification strength and display relationships among *E.persicus* populations with respect to karyotype asymmetry (Table 4; Figure 8 and 9).

Cluster analysis of the populations of *E. persicus* based on relative karyotypic data revealed that P-I and P-II are placed close to each other while P-III joined them with a distance (Figure 10). However, there is morphological similarity between them but due to karyotypic difference it stands separate from the rest of the two populations. This is also in accordance with their meiotic studies where P-III showed high variation in comparison to P-I and P-II.

Table 1. Karyomorphometric analysis of somatic metaphasic chromosomes complement of three populations of *E. persicus.*

	Populations														
	P-I					P-II					P-III				
S. No.	SA (µm)	LA (µm)	TL (µm)	AR	Type	SA (µm)	LA (µm)	TL (µm)	AR	Type	SA (µm)	LA (µm)	TL (µm)	AR	Type
1	3.33	12.21	15.54	3.66	ST	3.85	12.1	15.95	2.49	ST	3.3	14.3	17.6	2.88	ST
2	3.88	10.54	14.42	2.71	ST	3.85	11.55	15.4	2.75	ST	3.3	13.75	17.05	4.16	ST
3	3.33	10.54	13.87	3.16	ST	3.85	11	14.85	2.87	ST	3.85	13.2	17.05	3.42	ST
4	2.77	10.54	13.31	3.80	ST	3.3	11	14.3	3.14	ST	3.3	12.65	15.95	3.83	ST
5	3.33	11.65	14.98	3.49	ST	3.3	11	14.3	2.62	ST	2.75	11.55	14.3	4.2	ST
6	2.77	11.65	14.42	4.20	ST	2.75	12.1	14.85	2.86	ST	1.65	11	12.65	6.66	ST
7	3.33	9.99	13.32	3	ST	3.3	9.35	12.65	4.00	ST	2.2	11	13.2	5	ST
8	3.33	9.43	12.76	2.83	ST	2.75	9.9	12.65	1.71	ST	2.2	11	13.2	5	ST
9	2.22	8.88	11.1	4	ST	2.2	7.7	9.9	1.85	ST	2.2	12.1	14.3	5.5	ST
10	2.77	8.32	11.09	3.00	ST	2.75	8.8	11.55	3.24	ST	2.2	6.05	8.25	1.13	ST
11	1.65	6.05	7.7	3.66	ST	2.2	3.85	6.05	3.5	SM	2.2	6.05	8.25	2.75	ST
12	1.1	6.05	7.15	5.5	ST	1.65	3.3	4.95	3	SM	2.2	3.3	5.5	1.5	SM
13	2.22	3.33	5.55	1.5	SM	1.65	3.3	4.95	3.67	SM	1.65	2.75	4.4	1.66	SM
14	1.66	3.33	4.99	2.00	SM	1.65	4.4	6.05	1.40	ST	1.65	3.85	5.5	2.33	SM
Total	37.69	122.51	160.2			39.05	119.35	158.4			34.65	132.55	168.3		

Table 2. Karyotype formula according to Levan et al. 1964 and characteristics of the studied species SC –the shortest chromosome length; LC – the longest chromosome length; p – mean length of long arm; q – mean of short arm; CL – mean of chromosome; CI – mean centromeric index; ST-subtelocentric; SM- submetacentric; SD- standard deviation.

Population	Range SC-LC (µm)	Ratio LC/SC	p (µm) mean ± SD	q (µm) mean ± SD	CL (µm)	CI (µm)	Karyotype formula
P-I	1.1-12.21	11.1	8.75±2.85	2.69±0.78	11.44±3.49	0.24±0.06	10ST+2ST+2SM
P-II	1.65-12.1	7.33	8.52±27	2.78±0.79	11.31±3.99	0.26±0.06	10ST+3SM+1ST
P-III	1.65-14.3	8.66	9.46±3.98	2.47±0.68	11.94±4.48	0.22±0.07	10ST+1ST+3SM

According to the As. K %, TF% and Syi and Rec indices P- III is more asymmetric than P-I and P-II.

POLLEN COUNT AND VIABILITY

Pollen grains of *E. persicus* are ovate, smooth walled. These are shed at two celled stage. Highest pollen production per anther is observed for POP III ; 6619.4±381.46. Different anthers in a flower display some difference in pollen content, so that the average pollen output per flower comes out to be 38506.6±2298.49 (Table 5). In POP II, pollen production per anther is averaging 6252.9±361.03 and total pollen production per flower comes out to be 37517.4±2166.22. Pollen production per anther averages 5428.36±351.13 in POP I and total pollen production per flower comes out to be 32570.2±2106.79.

Highest pollen stainability by 1% acetocarmine was observed in population II followed by population I and III. However pollen viability by TTC and FDA averages 89.61±0.26 and 90.78±0.53 in population I, 90.53±0.17 and 92.61±0.82 in population II and 90.40±0.33 and 91.74±0.61 in population III by TTC and FDA respectively(Table 5 and Figure 11).

REPRODUCTIVE OUTPUT

All the three populations have very low reproductive output in terms of % fruit set. The plants in their natural populations (open pollination) showed 19.00±3.19, 20.21±2.45 and 18.62±3.46 % of fruit set in P-I, P-II and P-III respectively. Bagged inflorescences do not produce seeds in all the selected populations.Also

Table 3. Pearson correlations for asymmetry indices.

	As.K%	TF%	Rec	SYi	A1	A2	DI	A	AI	CVCI	CVCL	MCA
As.K%	1											
TF%	**-0.99896**											
Rec	-0.93746	**0.952374**										
SYi	**-0.99888**	**0.999999**	**0.952899**									
A1	0.945621	**-0.95949**	**-0.99971**	**-0.95997**								
A2	0.455858	-0.49602	-0.73717	-0.49752	0.720577							
DI	-0.83294	0.806804	0.588219	0.805783	-0.60765	0.112823						
A	**0.996066**	**-0.99907**	**-0.96462**	**-0.99915**	**0.970725**	0.532939	-0.78062					
AI	0.898327	-0.91745	**-0.99507**	-0.91813	**0.992377**	0.800534	-0.50514	0.933725				
CVCI	**0.992863**	**-0.99727**	**-0.97228**	**-0.9974**	**0.977664**	0.558753	-0.761	**0.999525**	0.94431			
CVCL	0.455858	-0.49602	-0.73717	-0.49752	0.720577	**1**	0.112823	0.532939	0.800534	0.558753		
MCA	**0.996066**	**-0.99907**	**-0.96462**	**-0.99915**	**0.970725**	0.532939	-0.78062	**1**	0.933725	**0.999525**	0.532939	1

Significant correlations ($p<0.05$) are in boldface.

Table 4. Karyotypes of *E. persicus* using different methods of evaluating karyotype asymmetry.

Population	ST class	As. K%	TF%	Rec	SYi	A1	A2	DI	A	AI	CVCI	CVCL	MCA
P-I	3C	76.47	23.47	93.28	30.74	0.97	0.30	6.36	0.52	7.5	25	30	52
P-II	3C	75.34	24.65	93	32.62	0.97	0.35	7.59	0.50	8.07	23.07	35	50
P-III	3C	78.75	20.58	83.14	26.10	0.98	0.37	6.16	0.58	11.76	31.81	37	58

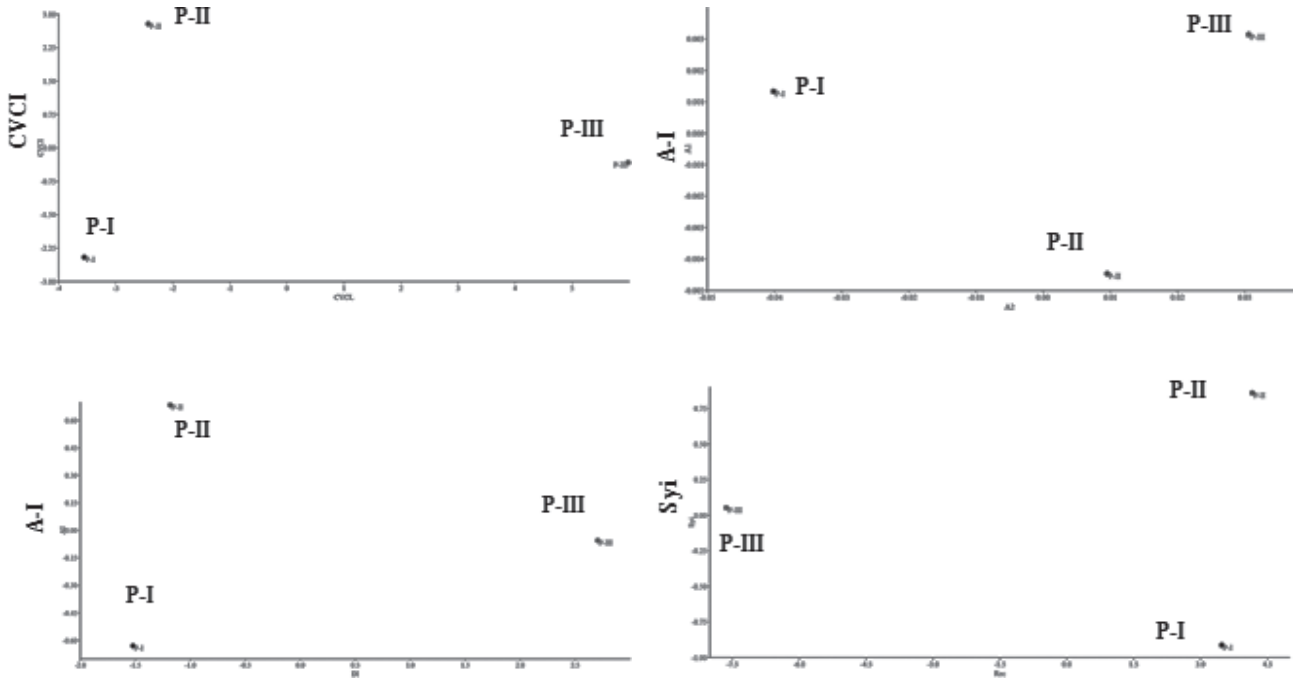


Figure 8-9. Scatter diagrams for P-I, P-II and P-III, (8) A1 against A2 & Rec index against Syi index, (9) CV_{CL} againstCV_{CI} & DI against AI.Degrees of asymmetry according to Stebbins.

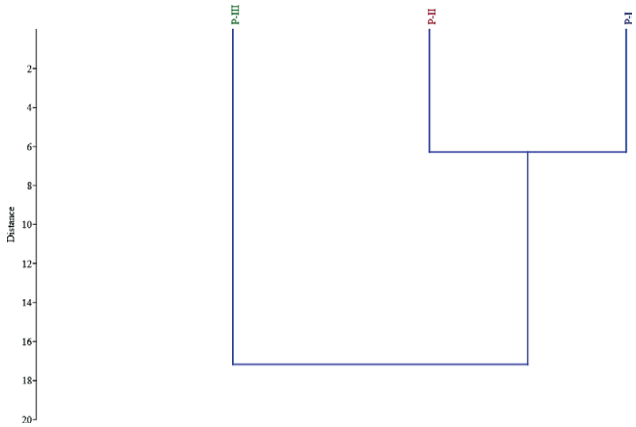


Figure 10. Dendrogram for P-I, P-II and P-III.

the percentage seed set calculated for these populations is very low i.e., 25.79±1.83, 14.88±3.20 and 17.48±3.14in P-I, P-II and P-III respectively. It was observed that fruits which start developing on a scape do not attain maturity and a large amount of the fruits abort during the course of development, in P-I (80.99±3.19%), in P-II (79.50±2.49%) and maximum fruit abortion (81.37±3.46%) was observed in P-III.

The present study revealed that *Eremurus persicus* is diploid with chromosome number of 2n = 2x = 14. The karyotype analysis revealed that various species of the genus *Eremurus* have 2n chromosome counts of 14 and 28 (Hadizadeh *et al.,* 2020). Somatic chromosome analysis of eight species of *Aloe* (Asphodelaceae) showed diploid chromosome number of 2n = 14 (Sánchez-G*et al.* 2018). The other species of the genus growing in India, i.e., *E. himalaicus* is also diploid with chromosome number of 2n = 14 (Kumari *et al.,* 2016). In present study meiotic abnormalities were found in the target species, similarly meiotic abnormalities were also found in *E, himalaicus* growing in Indian Himalayan region (Kumari *et al.,* 2016). The abnormalities are attributed to pervading environmental conditions in the Himalaya (Wani *et al.* 2023). The phenomenon of desynapsis was also found in the PMCs of presently studied plant species, both extrinsic conditions such as temperature and fertilizer quality (Dhesi *et al*., 1975; Rao, 1975) and intrinsic conditions like gene action, loss of chromosome pair, apomixis and structural or numeral changes of chromosomes (Praaken, 1943) are responsible for the desynaptic behavior of plants. Over the years desynapsis has been established as a gene mediated phenomenon in a large number of plant species (Gottschalk & Baquar, 1971), however; the precise mode of action of these genes is not fully understood. Of the three types viz weak, medium and strong or complete desynapsis (Prakken, 1943); in present study medium desynaptic type was found in the target species because of the presence of many univalents and few loose bivalents. In spite of the lack of pairing and the formation of univalents as a consequence of desynapsis, anaphase segregation is normal in majority of cells which needs further investigation. The P-III displays irregular distribution of chromosomes with maximum frequency. Other meiotic irregularities include formation of laggards and bridges at anaphase-I, metaphase-II and anaphase-II. An interesting observation made in the present study revealed the presence of univalents of a pair close to each other, as also the regular anaphasic segregation point towards the species showing "distance pairing" in which the homologs lie

Table 5. Data on Pollen count and pollen viability.

S. No.	Characters (n=30)	POP-I	POP-II	POP-III	p-value
1.	No. of pollen grains per anther	5428.36±351.13* (2896-8983)**	6252.9±361.03 (3202-9883)	6619.4±381.46 (3268-9962)	0.07
2.	No. of pollen grains per flower	32570.2±2106.79 (17376-53898)	37517.4±2166.22 (19212-59298)	38506.6±2298.49 (19608-59772)	0.13
3.	No. of ovules per flower	18.33±0.41 (17-22)	20.13±0.30 (17-22)	20.3±0.33 (17-22)	0.0002***
4.	Pollen viability **a**. (1% acetocarmine)	93.18±0.22* (90-95)**	95.25±0.34 (92-98.78)	91.74±0.27 (89-94)	0.001**
	b. TTC	90.40±0.33 (88-94)	90.53±0.17 (89-93)	89.61±0.26 (88-92.56)	0.04
5.	**c.** FDA	91.74±0.61 (83.6-95)	92.61±0.82 (82-98.7)	90.78±0.53 (82.01-94)	0.16

*Mean± standard error.

**Range.

*Significant at p<0.05 (Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1).

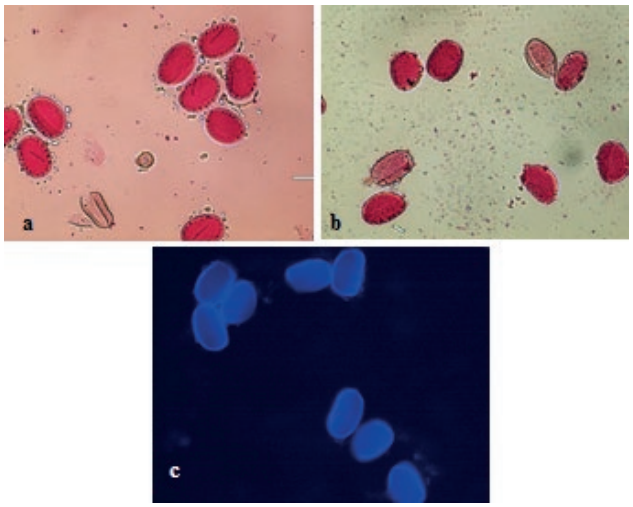


Figure 11. Pollen viability in *Eremurus persicus* (a) 1% acetocarmine, (b) TTC, (c)FDA.

side by side but apparently do not touch. Same has been observed in Human oocytes also (Thermann & Sarto, 1977). Low recombination index of the species due to lack of adequate crossing over can limit genetic variation which may ultimately compromise with the survival of the species (Szczecińska *et al.,* 2016).

The species has asymmetric karyotype of 10 long and 4 short chromosomes. The TF% and Syi and Rec values decreases with increasing asymmetry, while values of others i.e A1 and A2, A and CV_{CL} increase with increasing asymmetry (Zuo& Yuan, 2011) as observed in the present study.

The meiotic abnormalities observed in *E. persicus* may be attributed to high fruit and seed abortion rates. Most conservation biologists believe that before setting up a conservation program for plant species it is better to know about its intrinsic reproductive constraints (Friedman & Ryerson, 2009; Wani *et al,*. 2023). The presently investigated plant species are already facing threats such as habitat fragmentation, overexploitation, and grazing, therefore, the presence of meiotic bottlenecks can add to the factors that lead to a decrease in its population size. Therefore, keeping in view the meiotic abnormalities and anthropogenic threats sustainable conservation strategies are needed to devise for this medicinally important plant species.

ACKNOWLEDGEMENTS

All authors are highly grateful to MoEF&CC, Govt. of India for financial assistance through its project ID NMHS/2017-18/MG43/27 to MAS under the National Mission on Himalayan Studies (NMHS).

REFERENCES

Ansari H. A., Ellison N. W., Verry I. M., Williams W. M. 2022. *Asynapsis and unreduced gamete formation in a Trifolium interspecifc hybrid*. BMC Plant Biology 22:14. https://doi.org/10.1186/s12870-021-03403-w

Arano H. 1963. *Cytological studies in subfamily Carduoideae (Compositae) of Japan.IX. The karyotype analysis and phylogenic considerations on Pertya and Ainsliaea*. Bot. Mag. Tokyo. 76:32-39.

Beadle G.W. 1933. *Further studies of asynaptic maize.*Cytologia. 4: 269-287. https://doi.org/10.1508/cytologia.4:269

Beasely and Brown M.S. 1942. *Asynaptic gossypium plants and their polyploids.* Jour. agric. res. 65: 421-427.

Bergner A. D., Cartledge J. L., Blakeslee A. F. 1934. *Chromosome behaviour due to a gene which prevents metaphase pairing in Datura*. Cytologia. 6: 19-37.

Bharti U, Sharma E, Sharma N. 2021. *Cytological behaviour in relation to reproductive efficiency of some species of Artemisia L.*The International Journal of Plant Reproductive Biology 13(1) :70-76.DOI 10.14787/ijprb.2021 13.1 70-76

Cai X., XuS. S., 2007. *Meiosis-Driven Genome Variation in Plants*. Current Genomics, 2007, 8: 151-161

Clausen R. E. 1931. *Inheritance in Nicotiana tabaccumXI. The fluted assemblage*. Amer. Nat. 65: 316-331. https://doi.org/10.1086/280375

Dhesi J. S., Minocha J. L., Sidhu J. S. 1975. *Effect of minerals on the chiasma frequency in desynaptic pearl millet*. Curr. Sci. 44: 862-863.https://www.jstor.org/stable/i24079462

Fabig G., Kiewisz R., Lindow N., Powers J A., Cota V., Quintanilla L. J., Brugues J., Prohaska S., Chu D. S., Müller-Reichert. T. 2020. *Male meiotic spindle features that efficiently segregate paired and lagging chromosomes*. eLife. 9:e50988.

Friedman W. E., Ryerson K. C. 2009..*Reconstructing the ancestral female gametophyte of angiosperms: Insights from Amborellaand other ancient lineages of flowering plants*. Am. J. Bot. 96: 129–143.

Ganie A.H., Tali B.A., Reshi Z.A., Nawchoo I.A. 2021. *Inflorescence architecture, floral part movements and pollinator attraction by androecia – contrivance for successful mating in Eremurus himalaicus Baker.* Botanica Pacifica. 10(2):29–34.

Golubovskaya I. N. 1979. *Genetic control of meiosis*. Int. Rev. Cytol 58:247–290. https://doi.org/10.1016/S0074-7696(08)61477-1

Gottschalk W., Baquar S. R. 1971. *Desynapsis in Pisum sativum induced through gene mutation*. Can. J. Genet.Cytol. 13: 138-143. https://doi.org/10.1139/g71-022

Hadizadeh H., Bahri B. A., Qi P., Wilde H. D., Devos K.M. 2020. *Intra- and interspecific diversity analyses in the genus Eremurus in Iran using genotyping-bysequencing reveal geographic population structur*e. Horticulture Research. 7:30 https://doi.org/10.1038/s41438-020-0265-9

Hamal I. A., Karihaloo J. L. 1983. *Eremurus persicus Boiss. An addition to the flora of Jammu and Kashmir state*. Indian Journal of forestry.5(3):244.

Hashemi S. J., Asgarpanah J., Alaee Z., Sadeghian S., Hasani H., Azimi A. 2014. *In vitro antifungal activity of four medicinal plants used inIranian traditional medicine.* Res J Pharmacogn. 1: 39-43.

Huziwara Y. 1962. *Karyotype analysis in some genera of Compositae. VIII. Further studies on the chromosome of Aster.* Amer. J. Bot. 49:116-119.

Jakson R. C., Ngo N., Ngo H. 2002. *Chromosome-Specific Desynapsis in the N 2 Race of Haplopappusgracilis (Compositae)*. Amer. J. Bot. 89(5): 777–782.

Johnsson, H.1944. *Meiotic aberrations and sterility in Alopecurus myosuroides*. Hereditas. 30:469-566.

Karl H. R. 1982. *Liliaceae Eremurus* species. Iran. Flora. 1-31.

Kemenetsky R., Akhmetova M.1994. *Floral development of Eremurus altiacus* (Liliaceae). Israel Journal of Plant Sciences. 42: 227- 233.

Khan S. S., Ahmad V. U., Saba N., Tareen R. B. 2011. *Eremurus persicus, a new source of medicinally important compounds.* Pak. J. Bot. 43(5):2311-2313.

Koul A. K., Paliwal R. L. 1962. *An ageotropic mutant of annual teosinte and its cytological behavior.*Agra. Univ. J. Research. 11: 223-232.

Kumari K., Saggoo M. I. S. 2016.*Analysis of Meiotic Behavior in Eremurus himalaicus Baker (Liliaceae): A Rare Endemic Perennial from Kinnaur, Himachal Pradesh, India*. Cytologia. 81(4): 447–453

Lavania U. C., Srivastava S. 1999. *Quantitative delineation of karyotype variation in Papaver as a measure of phylogenetic differentiation and origin*. Curr. Sci. 77:429-435.

Levan A., Fredga K., Sandberg A. A. 1964. *Nomenclature for centromeric position on chromosomes*. Hereditas. 52:201-220.

Li H. M, Pao W. K., Li C. 1945. *Desynapsis in common Wheat*. Amer. J. Bot. 32:92-101.

Mati E., de Bore H. 2011. *Ethnobotany and trade of medicinal plants in the qeysare market, Erbil, Kurdish autonomy region Iraq*. J. Ethnopharmacol. 133: 490-510.

Paszko B. 2006. *A critical review and a new proposal of karyotype asymmetry indices*. Plant Syst. Evol. 258(1-2): 39-48.

Peruzzi L., Leitch I. J., Caparelli K. F. 2009. *Chromosome diversity and evolution in Liliaceae*. Ann. Bot. 103 (93): 459-475.

Prakken R. 1943. *Studies of asynapsis in Rye.*Hereditas 29: 475-495.

Rao P. N. 1975. *Desynapsis in Coix lacryma-jobi caused by genotype-environment-interaction*. Theor. Appl. Genet. 46:315-317.

Rashid S., Rashid K., Wani B.A., Ganie A.H., Nawchoo I.A., Khuroo, A.A. 2023. *Reproductive biology of Actaea kashmiriana, an endemic medicinal plant species from the Himalaya: Implications for conservation and sustainable utilization*. Journal of Applied Research on Medicinal and Aromatic Plants 34, 100461

Romeo Zarco C. 1986.*A new method for estimating karyotype asymmetry*. Taxon.35:526-530.

Safar K.N., Osaloo S. K., Zarrei M. 2009. *Phylogeny of the genus Eremurus (Asphodelaceae) based on morphological characters in the flora Iranica area. Iran*. J. Bot. 15:27-35.

Sánchez G.Y., Raymúndez M .B.,Imery J., Acosta M. C., Moscone E. 2018. *Characterization of eight species of Aloe (Asphodelaceae) from the nucleolar organizing region.* Rodriguésia. 69(2): 363-372.

Schiappacasse F., Szigeti J. C., Manzano E., Kamenetsky R. 2013. *Eremurus as a newcut flower crop in Aysen, Chile: introduction from the northern hemisphere*. Acta Hortic. 1002:115–122.

Stebbins GL. 1971. *Chromosomal evolution in higher plants*. Edward Arnold, London.

Szczecińska M., Sramko G., Wołosz K., Sawicki J. 2016. *Genetic Diversity and Population Structure of the Rare and Endangered Plant Species Pulsatilla patens (L.)Mill in East Central Europe*. PLoS ONE 11(3): e0151730. doi:10.1371/journal. pone.0151730 E

Therman E., Sarto G. E. 1977. *Premeiotic and early meiotic stages in the pollen mother cells of Eremurusand in human embryonic oocytes*. Human Genetics. 35(2):137-151.

Vala M. H., Asgarpanah J., Hedayati M. H., Shirali J., Bejestani F. B. 2011. *Antibacterial and cytotoxic activity of Eremurus persicus (Jaub. and Spach) Boiss.*Afr. J. Microbiol. Res. 5(16): 2341-2352.

Venora G., Blangiforti S., Castiglione R. M., Pignone D., Losavio F., Cremonini R. 2002. *Chromation organization and computer aided karyotyping of Triticum durum Desf.cv. Timilia*. Caryologia 55: 91-98.

Verma S. 2001. *Studies on reproductive biology and ex-situ conservation of some threatenedtaxa of North-West*

Himalaya. Ph.D. Thesis, University of Jammu, Jammu, India.

Visser N. C., Spies J. J., Venter H. J. T. 1999. *The presence of synaptic and chromosome disjunction mutants in Cenchrusciliaris (Poaceae: Paniceae).* Bothalia. 29: 327–334.

Watanbe K., Yahara T, Denda T., Kosuge K.1999. *Chromosomal evolution in the genus Brachyscoma (Asteraceae, Asterae): Statistical tests regarding correlation between changes in karyotype and habit using phylogenetic information.* J. Plant Res. 112: 145-161.

Wani A. A., Bhat T. A. 2017. *Asynapsis and Desynapsis in Plants. In: Bhat T A, Wani A A (eds.), Chromosome Structure and Aberrations*, DOI 10.1007/978-81-322-3673-3_6

Wani B. A, Magray J. A., QadirR., Javid H., Ganie A. H, Nawchoo I. A.2023. *Meiotic Bottlenecks Compromises Reproductive Success by Impairing Pollen Fertility and Seed Set in Swertiathomsonii C. B. Clarke. An Important Endemic Medicinal Herb of Western Himalaya.* Cytologia. 87(4): 323–330

Zuo L., Yuan Q. 2011.*The difference between the heterogeneity of the centromeric index and intrachromosomal asymmetry*. Plant Syst.Evol. 297:141–145.

Caryologia
International Journal of Cytology, Cytosystematics and Cytogenetics

Firenze University Press
www.fupress.com/caryologia

OPEN ACCESS

Citation: Sevgi Unal-Karakus, Muhammet Gaffaroglu, Muradiye Karasu-Ayata (2023). Comparative cytogenetics of four endemic *Capoeta* (Teleostei: Cyprinidae) species from Anatolia, Türkiye. *Caryologia* 76(1): 27-34. doi: 10.36253/caryologia-1982

Received: January 17, 2023

Accepted: July 3, 2023

Published: September, 19, 2023

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

ORCID
SU-K: 0000-0002-6409-7783
MG: 0000-0001-7436-5828
MK-A: 0000-0001-8890-8547

Comparative cytogenetics of four endemic *Capoeta* (Teleostei: Cyprinidae) species from Anatolia, Türkiye

Sevgi Unal-Karakus[1,*], Muhammet Gaffaroglu[2], Muradiye Karasu-Ayata[3]

[1] *Department of Molecular Biology and Genetics, Faculty of Science, Bartin University Bartin, Türkiye*
[2] *Department of Molecular Biology and Genetics, Faculty of Science and Arts, Kirsehir Ahi Evran University, Kirsehir, Türkiye*
[3] *Department of Nutrition and Dietetics, Faculty of Health Sciences, Kirsehir Ahi Evran University, Kirsehir, Türkiye*
*Corresponding author. E-mail: sunal@bartin.edu.tr

Abstract. The genus *Capoeta* is an important taxon covering a wide distribution in Türkiye. However, only a few genetic studies on *Capoeta* species reported from Türkiye. There is no cytogenetical study in *Capoeta aydinensis* Turan, Küçük, Kaya, Güçlü & Bektaş, 2017, *Capoeta bergamae* Karaman, 1969, *Capoeta erhani* Turan, Kottelat & Ekmekçi, 2008 and *Capoeta pestai* (Pietschmann, 1933). Thus, in this study, we karyotyped through classical cytogenetic techniques (Giemsa staining, Ag-NORs, and C-banding) the four endemic *Capoeta* species. The diploid chromosome number invariably was 150 in the four species. However, chromosome morphologies in the karyotypes had some differences between them. The number of biarmed chromosomes in the karyotypes was higher in all studied species. Their karyotypes contained respectively: 54 metacentric, 42 submetacentric and 54 subtelo-acrocentric in *C. aydinensis*, 56 metacentric, 30 submetacentric and 64 subtelo-acrocentric in *C. bergamae*, 50 metacentric, 42 submetacentric and 58 subtelo-acrocentric in *C. erhani* and 44 metacentric, 40 submetacentric and 66 subtelo-acrocentric chromosomes in *C. pestai*. C-bands were on the pericentromeres of most chromosomes in the four species. Three chromosome pairs carry rDNA genes in all studied species. The chromosomal locations of these sites were varied between the species. This study provides new insights into the chromosomal data of the hexaploid cyprinids. Moreover, obtained cytogenetic results should be conclude the cytotaxonomy of the genus *Capoeta* that distributed in Türkiye.

Keywords: Ag-NOR, C-banding, chromosome morphology, chromosome number, scraper.

INTRODUCTION

Türkiye has one of the most diverse and species-rich freshwater ichthyofaunas according to the different eco-regions that formed in Anatolian freshwaters (Küçük et al., 2009; Bektaş et al., 2017). The endemic species are

much higher than in Western Asia or Europe (Küçük et al., 2009). The members of the genus *Capoeta* (Valenciennes, 1842) (Cyprinidae, Barbinae) distribute from East Europe to West Asia, including Anatolia (Bektaş et al., 2017). Seventeen species named *Capoeta antalyensis*, *C. aydinensis*, *C. baliki*, *C. banarescui*, *C. barroisi*, *C. bergamae*, *C. caelestis*, *C. capoeta*, *C. damascina*, *C. ekmekciae*, *C. erhani*, *C. oguzelii*, *C. pestai*, *C. sieboldii*, *C. tinca*, *C. trutta* and *C. umbla* of this genus are presently recognized in the inland waters of Türkiye. Except for six species (*C. barroisi*, *C. capoeta*, *C. damascina*, *C. ekmekciae*, *C. trutta* and *C. umbla*) the other *Capoeta* members are endemic to Anatolia (Bektaş et al., 2019).

Taxonomic problems still exist in Anatolian *Capoeta* species and the species diversity of this genus has not been resolved (Turan et al., 2017). Özuluğ and Freyhof (2008) collected an additional species of *C. trutta* from Seyhan River in Türkiye. *C. turani* was described as a new species from this drainage according to the different morphological characters (Özuluğ and Freyhof, 2008). *C. erhani* was described in Ceyhan River of Türkiye by Turan et al. (2008). It was distinguished from the other members of *C. trutta* in the scope of morphological characters (Turan et al., 2008). Otherwise, *C. pestai* was described from Eğirdir Lake and it was also recorded from Lake Beyşehir. In fact, Beyşehir population of *C. pestai* was described as a new species called *C. mauricii* by Küçük et al. (2009) according to the different morphological characters. However, according to the molecular phylogeny study (cyt b gene sequences) in the genus *Capoeta* by Bektaş et al. (2017), *C. turani* was synonymized to *C. erhani*. Also, *C. mauricii* was synonymized to *C. pestai* (Bektaş et al., 2017). Otherwise, *C. bergamae* distributes in the western basins of Türkiye, as well as *C. aydinensis* was described as a new species in the recent years and is presently known from the Büyük Menderes River drainages (Turan et al., 2017).

The cytogenetic studies have played an important role in describing the main features in cytotaxonomy and for understanding chromosome evolution in fish species (Gaffaroğlu et al., 2020). However, the karyotypes of fishes are poorly studied compared to the other vertebrates in response to the richness of this group. The karyotype of many fish species is still undescribed due to the difficulty of sampling the individuals, the necessity of having alive individuals, in troubling to obtain karyotypes from cell-culture and unsuccessful in obtaining good metaphase spreads (Rossi, 2021). In this context, having too many chromosomes is another reason for this problem .

Knowledge of karyotype is necessary for fish cytogenetics. Detailed investigations of the chromosomes with Giemsa stained karyotypes have only been performed on only seven species namely, *C. trutta*, *C. umbla* (Kılıç-Demirok and Ünlü, 2001), *C. capoeta*, *C. barroisi* (Kaya, 2003), *C. damascina* (Unal and Gaffaroğlu, 2016), *C. antalyensis* and *C. baliki* (Karasu-Ayata et al., 2017) from Türkiye. The chromosomal banding properties have been reported only in *C. damascina* (Unal and Gaffaroğlu, 2016) and *C. antalyensis* (Gaffaroğlu et al., 2012). Due to the lack of chromosomal reports, this study aimed to investigate karyotypes with Giemsa staining, C-banding and Ag-NOR staining in four Anatolian endemic *Capoeta* species.

MATERIAL AND METHODS

Cytogenetic analyses were performed on four *Capoeta* species from Türkiye (Table 1, Figure 1). The alive samples were carried to the laboratory. The individuals were treated in vivo for mitotic chromosome preparation by Bertollo et al. (2015). Chromosome preparations were obtained from the cephalic kidney cells after injection of 0.1% colchicine. After hypnotization with 0.075 M KCl, fixation steps (methanol: acetic acid, 3:1) were repeated at least three times in cell suspension. At least 10 metaphase slide was prepared from each individual. All the experiments followed ethical protocols and after sacrificing, the individuals were deposited in 70% ethanol in the laboratory. The process was approved by the Local Animal Ethics Committee of Türkiye (Protocol Number: 68429034/05/17). The Ag-NORs and C-banding were analysed according to the methods reported by Howell and Black (1980) and Sumner (1972).

At least 100 metaphase spreads per individual were analysed to confirm the diploid chromosome number. Images were photographed using Leica DM 3000 microscope (Leica Microsystems GmbH, Germany) with AKAS software (Argenit Mikrosistem, Türkiye). Chromosomes were measured by digital calliper and classified as metacentric, submetacentric and subtelo-acrocentric according to the arm ratios (Levan et al., 1964). Karyotypes were arranged manually. To count the fundamental arm number (FN) meta- and submetacentrics were considered as biarmed whereas subtelo-acrocentrics as uniarmed.

RESULTS

All studied *Capoeta* species have diploid chromosome number 2n = 150 (*Figs. 2A, 3A, 4A, 5A*) with karyotypes composed of mainly biarmed chromosomes. Karyotype formulas were as follows: 54 metacentric, 42

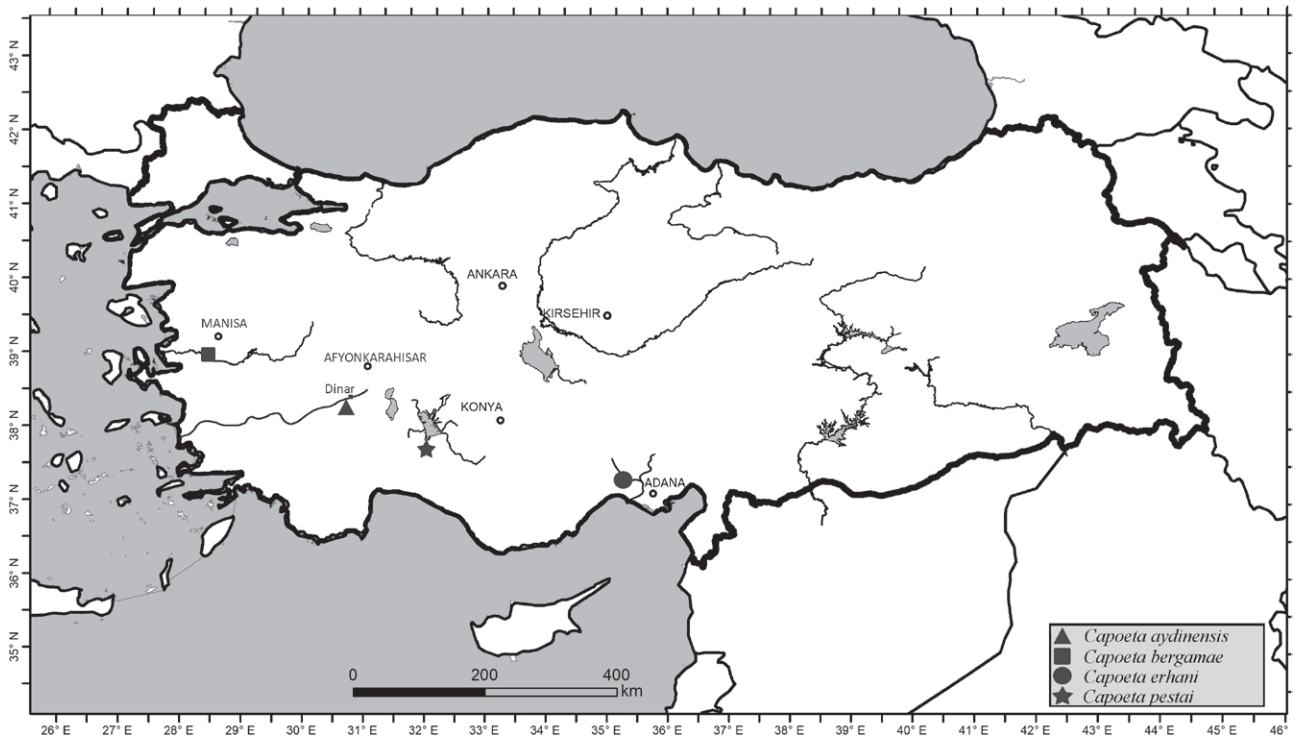


Figure 1. Map of the collected specimens of *Capoeta* species.

submetacentric and 54 subtelo-acrocentric in *C. aydinensis* (*Fig. 2B*); 56 metacentric, 30 submetacentric and 64 subtelo-acrocentric in *C. bergamae* (*Fig. 3B*); 50 metacentric, 42 submetacentric and 58 subtelo-acrocentric in *C. erhani* (*Fig. 4B*) and 44 metacentric, 40 submetacentric and 66 subtelo-acrocentric chromosomes in *C. pestai* (*Fig. 5B*). FN was calculated as 234 in *C. pestai*, 236 in *C. bergamae*, 242 in *C. erhani* and 246 in *C. aydinensis*. Morphologically differentiated sex chromosomes were not detected in all studied species.

In terms of C-bands, *C. aydinensis* contains very few C-bands (*Fig. 2C*). These C-bands were located on the pericentromeres of chromosome pairs 18, 21, 64 and 69 (*Fig. 2D*). Thirteen chromosome pairs of *C. bergamae* has intense pericentromeric C-bands of chromosome pairs 2, 8, 37, 44, 48, 53, 56, 59, 61, 65, 66, 69, 71 (*Fig. 3D*). *C. erhani* has slightly pericentromeric C-bands of chromosome pairs 1, 2, 3, 9, 16, 17, 26, 28, 31, 42, 48, 56, 58, 60, 66 and 73 (*Figs. 4C, D*). Intense pericentromeric C-bands of chromosome pairs 1, 3, 7, 19, 24, 25, 30, 34, 40, 43, 45, 46, 49, 51, 55, 56, 62, 64 and 73 were found in *C. pestai* (*Fig. 5D*). Some of the other chromosomes also have less intense pericentromeric C-bands in *C. pestai* (*Fig. 5C*) and *C. bergamae* (*Fig. 3C*).

Multiple Ag-NORs were found in the studied species. The common Ag-NOR number was six in four *Capoeta* species (*Figs. 2E, 3E, 4E, 5E*). These Ag-NORs were located on the terminal regions of metacentric chromosomes 1 and 5 as a strong signal and additionally weaker signals of chromosomes 12, 25 and 72 in *C. aydinensis* (*Fig. 2F*). Ag-NORs were detected on the terminal regions of the short arms of three submetacentric chromosome pairs 31, 33 and 37 in *C. bergamae* (*Fig. 3F*). Ag-NORs were located on the terminal regions of the short arms of 7th metacentric, 34th and 37th submetacentrics in *C. erhani* (*Fig. 4F*). Ag-NORs were found on the terminal regions of the short arms of three submetacentric chromosome pairs 26, 28 and 30 in *C. pestai* (*Fig. 5F*). Also, Ag-NOR number polymorphisms were detected in *C. bergamae* (*Figs. 6A, B*), *C. erhani* (*Figs. 7A, B, C, D*) and *C. pestai* (*Figs. 8A, B, C, D*) in some silver stained metaphases.

DISCUSSION

In the subfamily Barbinae a large number of species are polyploid. This subfamily may represent a more complicated polyploid system than other vertebrates. Polyploidy (whole genome duplication), has played an important role in the evolution of cyprinids (Yang et al., 2022). From the subfamily Barbinae (which includes

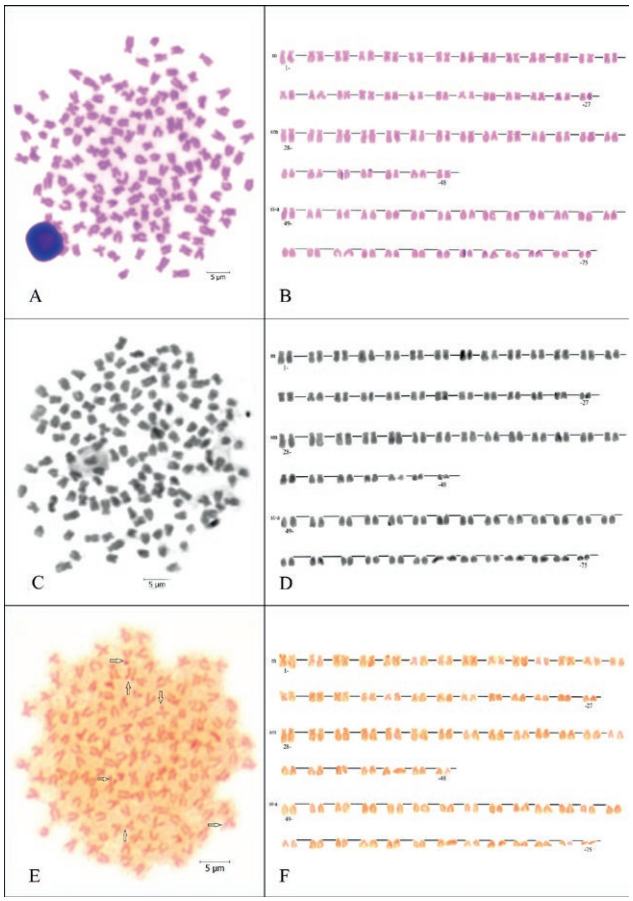


Figure 2. Metaphase plates of *Capoeta aydinensis* by Giemsa stained (A), C-banded (C) and Ag-stained techniques (E) and arranged karyotypes (B, D, F). Arrows indicate the Ag-NORs bearing chromosomes. Scale bars = 5 μm.

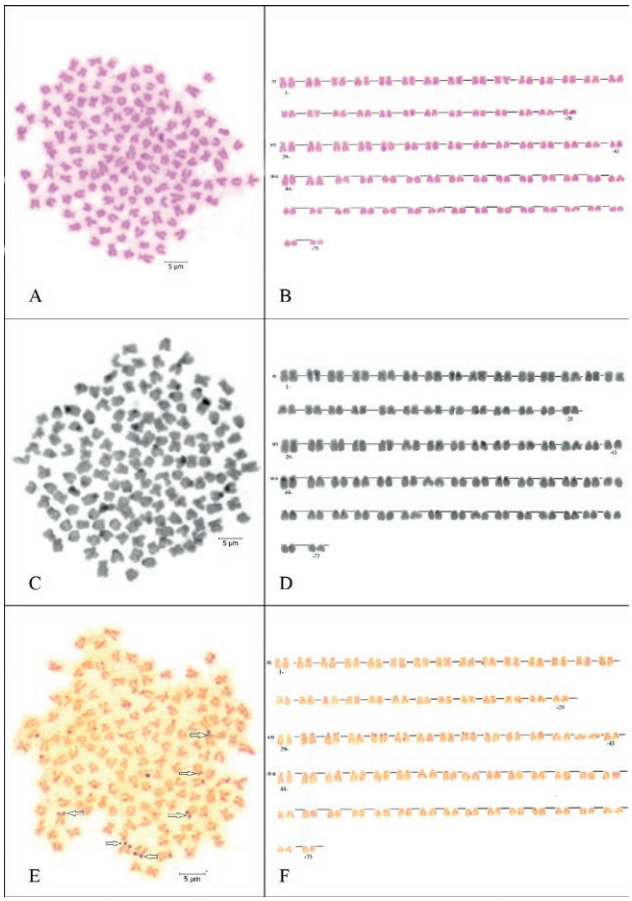


Figure 3. Metaphase plates of *Capoeta bergamae* by Giemsa stained (A), C-banded (C) and Ag-stained techniques (E) and arranged karyotypes (B, D, F). Arrows indicate the Ag-NORs bearing chromosomes. Scale bars = 5 μm.

only four genera), *Barbus* and *Luciobarbus* are tetraploid (2n = 4x) genera (Gaffaroğlu et al., 2013; Karasu-Ayata and Gaffaroğlu, 2019) where the genus *Capoeta* (2n = 6x) is hexaploid (Unal and Gaffaroğlu, 2016). Only the genus *Cyprinion* is diploid (2n = 2x) from this subfamily (Gaffaroğlu and Yüksel, 2004). Yang et al. (2022) reported that according to the mitochondrial and nuclear trees the polyploidy was allopolyploid in the subfamily Barbinae.

Cytogenetic analyses may provide a useful tool for understanding the karyotype changes in the evolution of the species (Gaffaroğlu et al., 2020). Especially according to the high chromosome number (2n = 150) cytogenetic studies are very limited in the genus *Capoeta* from Türkiye (Table 2) and also from the other countries (Arai, 2011). Cytogenetic data are available for only seven Anatolian *Capoeta* species (Kılıç-Demirok and Ünlü, 2001; Kaya, 2003; Unal and Gaffaroğlu, 2016; Karasu-Ayata et al., 2017). The diploid chromosome number has been conserved in the species of the genus *Capoeta* in the previous studies (Table 2). The chromosome number 2n = 6x = 150 in this study is consistent with previous reports (Table 2). However, karyotypes showed a pattern considered basal for the genus, or with small variations due to the pericentric inversions and/or translocations in Anatolian *Capoeta* species (Table 2). The number of biarmed chromosomes is higher than uniarmed chromosomes in the Anatolian *Capoeta* species (Table 2) except *C. trutta* (Kılıç-Demirok and Ünlü, 2001). This feature is detected in this study as well. We conclude that this karyotype structure with mainly biarmed chromosomes is typical for the genus *Capoeta*.

In detail, *C. aydinensis*, *C. bergamae*, *C. erhani* and *C. pestai* show very similar karyotype morphologies with some differences. The number of biarmed chromosomes is as follows 96 in *C. aydinensis*, 92 in *C. erhani*, 86 in *C. bergamae* and, 84 in *C. pestai*. Otherwise, the

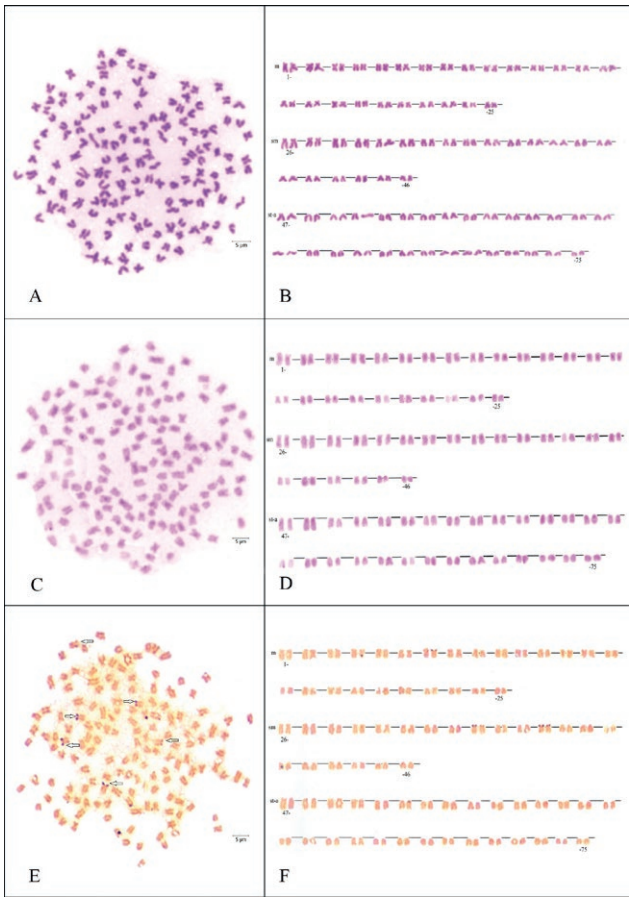


Figure 4. Metaphase plates of *Capoeta erhani* by Giemsa stained (A), C-banded (C) and Ag-stained techniques (E) and arranged karyotypes (B, D, F). Arrows indicate the Ag-NORs bearing chromosomes. Scale bars = 5 μm.

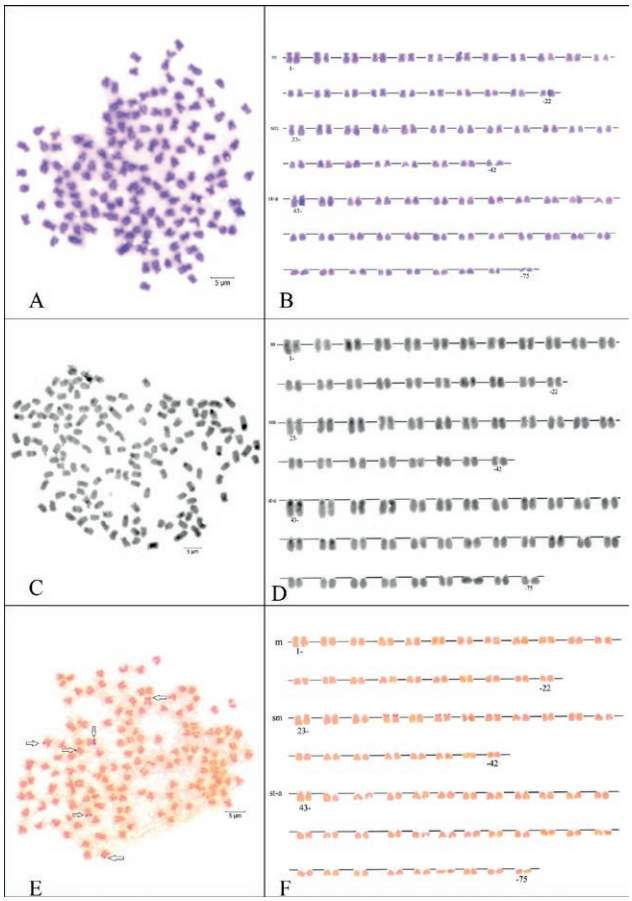


Figure 5. Metaphase plates of *Capoeta pestai* by Giemsa stained (A), C-banded (C) and Ag-stained techniques (E) and arranged karyotypes (B, D, F). Arrows indicate the Ag-NORs bearing chromosomes. Scale bars = 5 μm.

number of uniarmed chromosomes is as follows 54 in *C. aydinensis*, 58 in *C. erhani*, 64 in *C. bergamae* and, 66 in *C. pestai*. The FN ranges from 234 to 246 in this study. Pereira et al. (2012) suggested that distinct FNs with the same chromosome numbers in the species of the genus may be the result of pericentromeric inversions and/or translocations involving centromeres. Karyotypes of four *Capoeta* species in this study showed minor variations in their structures and depending on this having distinct FNs, apparently due to above mentioned chromosomal rearrangements. In addition, karyotypes with higher FNs are regarded to represent a derived condition (Ganai et al., 2011). According to this hypothesis, *C. pestai* should be a more primitive scraper whereas *C. aydinensis* should be the most derived scraper among the four species.

From the other countries *C. capoeta* (Safar, 2000), *C. damascina* (Gorshkova et al., 2002) and *C. sevangi* (Krysanov, 1999) were reported hexaploidy as detected in four studied species. *C. sevangi* differs from *C. aydinensis*, *C. bergamae*, *C. erhani* and *C. pestai* by having 110 uniarmed chromosomes (with FN = 190) (Krysanov, 1999).

Moreover, *C. antalyensis*, *C. baliki* (Karasu-Ayata et al., 2017) and *C. damascina* (Unal and Gaffaroğlu, 2016) showed no sex chromosome differentiation like *C. aydinensis*, *C. bergamae*, *C. erhani* and *C. pestai*.

Cytogenetic studies were mainly limited to detect chromosome number and morphology in the genus *Capoeta* (Table 2). Notably, chromosomal banding data (C-banding and Ag-NORs) revealed in only two *Capoeta* species to date (Gaffaroğlu et al., 2012; Unal and Gaffaroğlu, 2016). C-bands were located mainly on the pericentromeres and terminal regions of some chromosomes in four studied *Capoeta* species. *C. aydinensis* has the least C-bands compared to the other three species. *C. bergamae* and *C. pestai* have more C-banded chro-

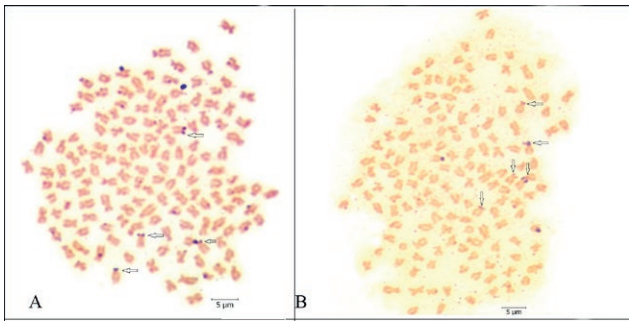


Figure 6. Ag-NOR polymorphisms of *Capoeta bergamae*. Four Ag-NORs (A) and, five Ag-NORs (B). Arrows indicate the Ag-NORs bearing chromosomes. Scale bars = 5 µm.

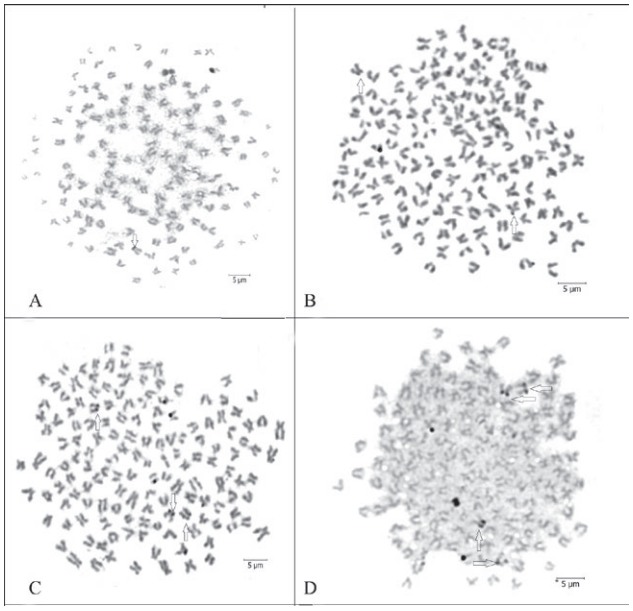


Figure 7. Ag-NOR polymorphisms of *Capoeta erhani*. One Ag-NOR (A), two Ag-NORs (B), three Ag-NORs (C) and, four Ag-NORs (D). Arrows indicate the Ag-NORs bearing chromosomes. Scale bars = 5 µm.

mosomes than *C. aydinensis* and *C. erhani*. Similarly, *C. damascina* (Unal and Gaffaroğlu, 2016) and *C. antalyensis* (Gaffaroğlu et al., 2012) had centromeric C-bands as this study. Heterochromatic blocks that were reported in *C. damascina* (Unal and Gaffaroğlu, 2016) are not observed in this study. Due to the lack of the chromosomal banding data for most of the species of the genus *Capoeta* from different countries, no comparison should be made. However, our results show the basal chromosomal banding information for the genus *Capoeta*.

Ag-NOR numbers have a stable distribution pattern among the four species newly analysed. It is assumed

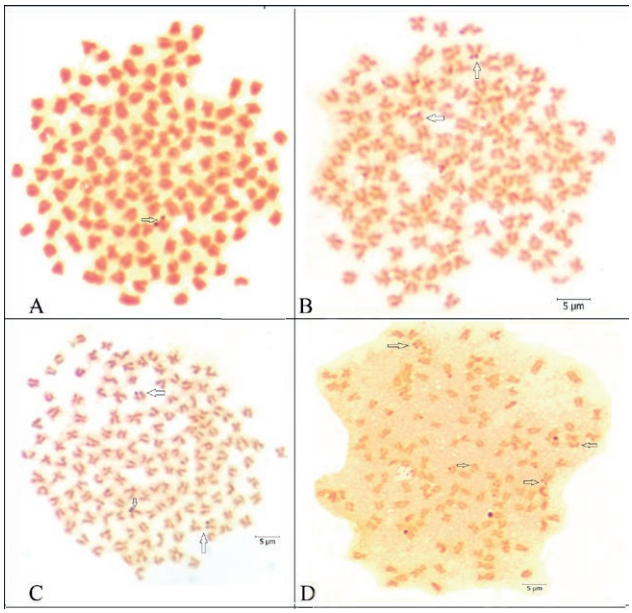


Figure 8. Ag-NOR polymorphisms of *Capoeta pestai*. One Ag-NOR (A), two Ag-NORs (B), three Ag-NORs (C) and, four Ag-NORs (D). Arrows indicate the Ag-NORs bearing chromosomes. Scale bars = 5 µm.

that two Ag-NORs in diploid barbins (Yüksel and Gaffaroğlu, 2006), four Ag-NORs in tetraploid barbins (Karasu-Ayata and Gaffaroğlu, 2019) and six Ag-NORs in hexaploid barbins (Unal and Gaffaroğlu, 2016) are common features. The Ag-NORs observed in the species studied here followed the similar feature observed in the other *Capoeta* species. *C. aydinensis*, *C. bergamae*, *C. erhani* and *C. pestai* are similar to *C. damascina* (Unal and Gaffaroğlu, 2016) and *C. antalyensis* (Gaffaroğlu et al., 2012) in terms of Ag-NOR numbers. Otherwise, *C. bergamae*, *C. erhani* and *C. pestai* are similar to *C. damascina* (Unal and Gaffaroğlu, 2016) in terms of locations of Ag-NORs on the submetacentric chromosomes. *C. antalyensis* (Gaffaroğlu et al., 2012) has Ag-NORs on submeta-subtelocentric chromosomes like *C. aydinensis*. Moreover, Ag-NOR number polymorphism has not been reported in *C. damascina* and *C. antalyensis* (Gaffaroğlu et al., 2012; Unal and Gaffaroğlu, 2016) as observed in *C. bergamae*, *C. erhani* and *C. pestai*. Ribosomal DNA sites are considered as hot spots for chromosomal rearrangements such as duplications, fusions, fissions and inversions. Also, these sites should be correlated with transposable elements or repetitive DNAs (Araya-Jaime et al., 2022). In this context, Ag-NOR number polymorphisms that were detected in the three species in this study should be derived after the above mentioned chromosomal rearrangements.

Table 1. Collection data of the studied species.

Species	Locality	Coordinate
C. aydinensis (2 individuals)	Suçıkan Spring, Dinar, Afyon (Büyük Menderes River)	38°04'N, 30°10'N
C. bergamae (8 individuals)	Dibekdere Stream, Ahmetli, Manisa (Gediz River)	38°33'N, 27°57'E
C. erhani (11 individuals)	Çakıt Stream, Şekerpınarı, Pozantı, Adana (Seyhan River)	37°27'N, 34°52'E
C. pestai (2 individuals)	Kayabaşı Stream, Beyşehir, Konya (South of Beyşehir Lake)	37°29'N, 31°30'E

Table 2. Karyological data for the genus *Capoeta* from Türkiye.

Species	2n	Karyotype formular	FN	References
C. trutta	150	70m-sm+80st-a	220	Kılıç-Demirok and Ünlü, 2001
C. umbla	150	86m-sm+64st-a	236	Kılıç-Demirok and Ünlü, 2001
C. capoeta	150	34m+66sm+12st+38a	250	Kaya, 2003
C. barroisi	150	26m+54sm+26st+38a	230	Kaya, 2003
C. damascina	150	46m+42sm+62st-a	238	Unal and Gaffaroğlu, 2016
C. antalyensis	150	84m-sm+66st-a	234	Karasu-Ayata et al. 2017
C. baliki	150	88m-sm+62st-a	238	Karasu-Ayata et al. 2017
C. aydinensis	150	54m+42sm+54st-a	246	This study
C. bergamae	150	56m+30sm+64st-a	236	This study
C. erhani	150	50m+42sm+58st-a	242	This study
C. pestai	150	44m+40sm+66st-a	234	This study

2n: diploid chromosome number, FN: fundamental number, m: metacentric, sm: submetacentric, st-a: subtelo-acrocentric.

In conclusion, our results provide new data on the cytogenetic features of four *Capoeta* species. The endemic *C. aydinensis*, *C. bergamae*, *C. erhani* and *C. pestai* were analysed for the first time. Karyotype differences that were observed in this study highlight cytogenetics as an important tool for cytotaxonomy. The chromosomal features with classical and molecular cytogenetic techniques of the other *Capoeta* species need to be studied to reveal detailed cytotaxonomy of the genus.

REFERENCES

Arai R. 2011. Fish karyotypes. A check list. – Springer, Japan.

Araya-Jaime CA, Mazzoni Zerbinato de Andrade Silva D, Ribeiro da Silva LR, Neves do Nascimento C, Oliveira C, Foresti F. 2022. Karyotype description and comparative chromosomal mapping of rDNA and U2 snDNA sequences in *Eigenmannia limbata* and *E. microstoma* (Teleostei, Gymnotiformes, Sternopygidae). Comparative Cytogenetics 16(2):127-142.

Bektaş Y, Aksu İ, Kaya C, Turan D. 2019. DNA Barcoding of the Genus *Capoeta* (Actinopterygii: Cyprinidae) from Anatolia. Turkish Journal of Fisheries and Aquatic Sciences 19(9):739-752.

Bektaş Y, Turan D, Aksu İ, Çiftçi Y, Eroglu O, Kalayci G, Beldüz AO. 2017. Molecular phylogeny of the genus *Capoeta* (Teleostei. Cyprinidae) in Anatolia, Turkey. Biochemical Systematics and Ecology 70:80-94.

Bertollo LAC, Cioffi MB, Moreira-Filho O. 2015. Direct chromosome preparation from freshwater teleost fishes. In: Ozouf-Costaz C Pisano E, Foresti F, Toledo LFA (eds) Fish cytogenetic techniques, ray-fin fishes and chondrichthyans, CRC Press, Boca Raton, p 21-26.

Gaffaroğlu M, Karasu-Ayata M, Ünal S, Arslan A. 2013. Chromosomal studies of two different populations (Turkey) of *Luciobarbus escherichii* (Steindachner, 1897). Turkish Journal of Fisheries and Aquatic Sciences 13:875-879.

Gaffaroğlu M, Karasu Ayata M, Yüksel E, Ünal S. 2012. NOR and C-banding in *Capoeta antalyensis* (Battalgil, 1944) (Actinopterygii, Cyprinidae). Ecology 2012 Symposium, 3-5 May 2012, Kilis.

Gaffaroğlu M, Majtanova Z, Symonova R, Pelikanova S, Unal S, Lajbner Z, Rab P. 2020. Present and future salmonid cytogenetics. Genes 11(12):1462.

Gaffaroğlu M, Yüksel E. 2004. Karyotype Analyses of *Cyprinion macrostomus* Heckel, 1843 (Pisces: Cyprinidae). Gazi University Journal of Kırşehir Educational Faculty, 5 (2):235-239.

Ganai FA, Yousuf AR, Dar SA, Tripathi NK, Wani SU. 2011. Cytotaxonomic status of Schizothoracine fishes of Kashmir Himalaya (Teleostei: Cyprinidae). Caryologia 64(4):435-445.

Howell WM, Black DA. 1980. Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method. Experientia 36(8):1014-1015.

Karasu-Ayata M, Gaffaroğlu M. 2019. Chromosomal studies of *Luciobarbus kottelati* (Teleostei, Cyprinidae). Cytologia 89(4):331-334.

Karasu-Ayata M, Ünal S, Gaffaroğlu M. 2017. Karyotypes of *Capoeta antalyensis* (Battalgil, 1944) and *Capoeta baliki* Turan, Kottelat, Ekmekçi & İmamoğlu, 2006 (Actinopterygii, Cyprinidae). Turkish Journal of Fisheries and Aquatic Sciences 17: 269-273.

Kaya F. 2003. The investigation of biometric and karyological characteristics of *Capoeta capoeta* (Guldenstadt, 1773) and *Capoeta barroisi* (Lortet, 1894). Mersin University, Mersin.

Kılıç-Demirok N, Ünlü E. 2001. Karyotypes of cyprinid fish *Capoeta trutta* and *Capoeta capoeta umbla* (Cyprinidae) from the Tigris River. Turkish Journal of Zoology 25:389-393.

Küçük F, Turan D, Şahin C, Gülle İ. 2009. *Capoeta mauricii* n. sp., a new species of cyprinid fish from Lake Beyşehir, Turkey. Zoology in the Middle East 47:71-82.

Levan A, Fredga K, Sandberg AA. 1964. Nomenclature for centromeric position on chromosomes. Hereditas 52:201-220.

Özuluğ M, Freyhof J. 2008. *Capoeta turani*, a new species of barbel from River Seyhan, Turkey (Teleostei. Cyprinidae). Ichthyological Exploration of Freshwaters 19(4):289-296.

Pereira CSA, Rab P, Collares-Pereira MJ. 2012. Chromosomes of European cyprinid fishes: comparative cytogenetics and chromosomal characteristics of ribosomal DNAs in nine Iberian chondrostomine species (Leuciscinae). Genetic. 140:485-495.

Rossi AR. 2021. Fish Cytogenetics: Present and Future. Genes 12:983.

Sumner AT. 1972. A simple technique for demonstrating centromeric heterochromatin. Experimental Cell Research 75:304-306.

Turan D, Kottelat M, Ekmekçi FG. 2008. *Capoeta erhani*, a new species of cyprinid fish fromCeyhan River, Turkey (Teleostei. Cyprinidae). Ichthyological Exploration of Freshwater 19:263-270.

Turan D, Küçük F, Kaya C, Güçlü SS, Bektaş Y. 2017. *Capoeta aydinensis*, a new species of scraper from southwestern Anatolia, Turkey (Teleostei. Cyprinidae). Turkish Journal of Zoology 41:436-442.

Unal S, Gaffaroğlu M. 2016. Karyology of six cyprinid fishes from Seyhan and Ceyhan rivers in Anatolia. Caryologia 69(4):362-369.

Yang L, Naylor G.J.P, Mayden L.R. 2022. Deciphering reticulate evolution of the largest group of polyploid vertebrates, the subfamily cyprininae (Teleostei: Cypriniformes). – Molecular Phylogenetics and Evolution 166:107323.

Yüksel E, Gaffaroğlu M. 2006. NOR phenotype and ploidy level of *Cyprinion macrostomus* (Osteichthyes, Cyprinidae). – Gazi University Journal of Educational Faculty 26(1):17-22.

Caryologia
International Journal of Cytology, Cytosystematics and Cytogenetics

Firenze University Press
www.fupress.com/caryologia

OPEN ACCESS

Citation: Fatemeh Nezhadi, Farzad Fayaz, Ezzat Karami, Hooshmand Safari, Abdol Rahman Rahimi (2023). A karyomorphological comparison of seven species of *Achillea* L. from Kurdistan of Iran. *Caryologia* 76(1): 35-46. doi: 10.36253/caryologia-1988

Received: January 17, 2023

Accepted: June 10, 2023

Published: September, 19, 2023

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

A karyomorphological comparison of seven species of *Achillea* L. from Kurdistan of Iran

Fatemeh Nezhadi[1], Farzad Fayaz[2,*], Ezzat Karami[2], Hooshmand Safari[3], Abdol Rahman Rahimi[2]

[1] *Department of Agronomy and Plant breeding, Sanandaj Branch, Islamic Azad University, Sanandaj, Iran*
[2] *Faculty member of Department of Agronomy and Plant Breeding, Sanandaj Branch, Islamic Azad University, Sanandaj, Iran*
[3] *Faculty member of Forest and Rangeland Research Department, Kermanshah Agriculture and Natural Resources Research and Education and Extension Organization (AREEO), Kermanshah, Iran*
*Corresponding author. E-mail: far.fayaz@gmail.com

Abstract. We conducted the present study on seven important medicinal species of *Achillea* (in a total of 28 populations) in their natural habitats. The results indicated that the populations had a base number (x= 9) and the diploid, tetraploid, and hexaploidy levels were observed. In addition to the inter-species diversity, there was the intra-species genetic diversity as *A. millefolium* (4*x*, 6*x*), *A. vermicularis* (2*x*, 4*x*), *A. tenuifolia* (2*x*, 4*x*), *A. Aleppica* (2*x*), *A. talagonica* (2*x*), *A. biebersteinii*, and *A. wilhelmsii* (4*x*). Furthermore, studies also indicated that 11 out of 28 populations had 1A symmetry, 15 populations had 2B symmetry, a population had 2A, and another population had 2B. Cluster analysis of cytogenetic variables could differentiate only the species *of A. aleppica, A. talagonica* and *A. wilhelmsii* but others could not separate differences between species appropriately, probably due to the superiority of intra-species diversity of populations to inter-species diversity. Furthermore, we found %TF and DRL are useful parameters for differentiating intrachromosomal variation of species.

Keywords: *Achillea*, cytogenetic, cytomorphology, cluster analysis, chromosome structure.

INTRODUCTION

Achillea genus of family Asteraceae is of about 130 species that are distributed from southeastern Europe to southeastern Asia and has spread to North America through Eurasia. Different species of this genus have shown significant adaptation to different environmental conditions and have spread from deserts and coastal areas to rocky regions. The plants of this genus are perennial, allogamous and they are pollinated by insects (Mozaffaria, V. 2003). There are 19 herbaceous species of this genus available in Iran. Other species of this genus also grow in Anatolia, Syria, Caucasus, Lebanon, Palestine, Central Russia, Transcaucasia, Turkmenistan, Afghanistan, Southwest

Asia, and Central Asia in addition to Iran (Ghahreman 1984). Yarrow is a popular medicinal herb that is widely used in traditional medicine to treat diseases, particularly burns and scars (Muẓaffarīyān 1996).

Cytogenetics is study of relationship of chromosome structure with cellular function. Karyotype, the highest level of functional and structural organization of nuclear genome, that is essential for studying chromosomal characterization of plant species (Altınordu et al. 2016). Comparative chromosomal taxon has been providing useful knowledge about patterns and evolutionary mechanisms in speciation (Flavell 2021). Chromosomal features, such as chromosome length, centromere index, number of chromosomes are crucial variables for investigating interrelationships and intrarelationships of taxa. However, in addition of chromosomal morphotype, population geographical origin is an important parameter in interpreting taxon's diversity (Ramsey 2011) .

The basic chromosome number $x = 9$ is commonly reported for *Achillea* but variation in chromosome numbers and different ploidy levels are frequently occurring in this genus. Unfortunately, only few cytological studies have been published concerning karyological aspects on this genus in Iran. Ploidy and number of chromosomes in *A. aleppica* DC. species has been varied from 2*x* to 8*x* and with high symmetrical characteristic karyotype (2A), however in some population asymmetrical chromosomes (2B) have been reported (Rad and Javaheri 2014). *A. vermicularis*, *A. wilhelmsii* and *A. millefolium* species have shown different levels of ploidies 2*x*, 4*x*, 6*x* and 8*x* with 2A symmetrical Stebbins's index (Afshari et al. 2013). Meanwhile, study of nine populations of *A. biebersteinii* species demonstrated a diploid (2*x*); however, chromosomal interspecies variation has been observed and there has been a symmetrical Stebbins's index 1A and 2A (Chehregani Rad et al. 2017). The results of an another study, that was conducted on 14 populations of 8 *Achillea* species showed *A. talagonica* and A. *berbersteinii* species have been 2*x* ploidy, meanwhile *vermicularis* and *wilhelmsii* showed 4*x*, 6*x* ploidy levels respectively (Sheidai et al., 2009). Unfortunately, we have few studies on different species of *Achillea* particularly in Iran. Therefore, to fill this gap, we try to conducted this study on 28 population of seven species of *Achillea* in their natural habitats in Sanandaj find out the cytogenetical characteristics features.

MATERIALS AND METHODS

Plant materials

All of the 28 samples in this study, including seven species of *Achillea* (*A. millefolium, A. vermicularis, A. tenuifolia, A. aelppica, A. biebersteinii, A. wilhelmsii*, *and A. talagonica)*, with four replicates in each species, were collected in west of Iran, Kurdistan, Sanandaj. This region is located at a longitude of 46° 59' 45" E and latitude of 35° 19' 00" N. To identify every species, a sample was collected from each point. Figs 1, 2 represent the exact position of each location and morphological population features. Furthermore, we recorded the geographical position of each location using the GPS. Table (1) presents the latitude and longitude of each site.

Cytogenetical study of species

The seeds obtained from every point were disinfected employing the solution of the Sodium hypochlorite 2%, under sterile conditions, inside a Petri dish, and on the filter paper. Afterwards, the seeds germinated at room temperature. Following two to five days,

Table 1. Details on population sites including geographical coordinates, altitude and origin of samples.

Population	Longitude	Latitude	Altitude/m	Origin
W13	46.71	35.29	2,208	Klatei
W12	46.88	35.36	1,974	Arandan
W11	47.00	35.51	1,999	Sofla Mamox
W14	46.79	35.51	2,026	Gav Dareh
TA1	46.94	35.47	1,628	Chrandoo
TA2	46.98	35.58	1,980	Biaenchob
TA4	46.99	35.51	2,145	Chrandoo
TA3	46.98	35.49	1,919	Sofla Mamox
TE4	46.96	35.46	1,577	Sarab Ghamish
TE2	46.96	35.51	1,841	Chrandoo
TE1	46.99	35.49	1,866	Mamox
TE3	47.02	35.57	1,934	Bazi Rabab
AL3	46.94	35.28	1,829	Hassan Abad
AL4	46.58	35.32	1,607	Goyran
AL1	46.98	35.30	1,653	Pakr kodak
AL2	47.13	35.29	2,095	Salvat Abad
BI3	46.58	35.30	1,436	Danikesh
BI2	46.60	35.32	1,436	Pichon
BI1	46.89	35.13	1,334	Savarian
BI4	46.93	35.46	1,593	Chelgazii
VE4	46.97	35.30	1,838	Dole Rahman
VE1	46.92	35.55	2,334	Ghalvazei
VE3	46.92	35.58	2,152	Sangi Sefied
VE2	46.99	35.51	2,145	Sofla Mamox
MI2	47.07	35.36	1,498	Babareiz
MI1	47.15	35.25	1,985	Salvat Abad
MI4	47.12	35.54	1,642	Jebreillian
MI3	47.11	35.49	1,993	Dolbandi

their roots reached the proper size for sampling (roots with a length of 0.5-1 cm are appropriate for sampling). After applying the pre-treatment, the root samples were exposed to a 0.5% α-bromonaphthalene solution for 4 h, and running water for 30 min to remove the remains of the solution. Subsequently, we performed the fixation. Thus, we used Levitsky solution as a suitable fixator for karyotypic studies, and the samples were in the for 16 h (Levitsky 1931, Levitus et al. 2010). Following the fixation, the samples were rinsed with running water for 3 h to eliminate the residuals of the fix. Then we used squash at an optimal level to separate the cells and put them at the same level, and make staining better. To this end, we removed the roots from 70% ethyl alcohol, rinsed them with running water for 30 minutes, put them in a hydrolyzer (1 M NaOH), and placed them in the oven at 60 °C for 8 min. After hydrolysis, the samples were dried with filter paper and placed in hematoxylin for 3-4 h, to stain the chromosomes (Abbaszade et al. 2017). Chromosomal images were transferred to the monitor and saved with a digital color CCD camera mounted on a light microscope. The chromosomes of each cell were cut in Photoshop and arranged in a separate file. Using Micro Measure software and specifying the beginning to end of chromosomes and their centromere locations, certain characteristics such as short and long arm length, the total chromosome length, and relative chromosome length were calculated. The results were stored in Excel. In the present study, five cells (replications) were selected and evaluated from each slide to measure chromosomal parameters. The parameters calculated for the karyotypes were as follows: Short arm relative length percentage (SA%), Long arm relative length percentage (LA%), total length (TL), Relative length percentage (RL%), Arm ratio (AR), Total form percentage (TF%), Centromere index (CI), Difference of the range of relative length (DRL), Value of relative chromatin (VRC), Intrachromosomal asymmetry Index (A1) and Interchromosomal asymmetry Index (A2) (Altınordu et al. 2016).

According to the number of the replications, we calculated the standard deviation for the traits and the confidence interval for some of them. The chromosome form was determined using a method by Levan (Levan 1964). After measuring the chromosomes, we drew the ideogram associated with the karyotype of the populations based on the lengths of short and long arms, in which the order of chromosomes was considered based on the length of the short arm (from large to small). We utilized the Stebbins method for comparing the karyotypic symmetry in the species (Stebbins 1971).

Statistical data analysis

We performed all the statistical analyses by employing R software. The cluster analysis was performed for cytogenetic data series using the statistical packages, factoextra, FactoMineR, and devtools (Kassambara 2017). To map extract geological we used raster and MapTool packages by using R software. (Bivand and Lewin-Koh 2013) (Fig. 1).

RESULTS

Comparison of cytogenetic parameters between species

Mitotic metaphase chromosomes, ideograms, and morphological diversity of chromosomes were showed in Fig. 3, 4. The comparison of the results of karyotypic characteristics in the populations indicated that the base chromosome number was x=9 in all the populations and there were hexa-, tetra- and diploid levels for the populations. Regarding the ploidy level, there was diversity not only among the species, but also among the populations of the three species, *A. millefolium* (tetra- and hexa-ploidy), *A. vermicularis* (tetra and diploidy), and *A. tenuifolia* (tetra and diploidy). *A. alepine* and *A. talagonica* species were diploid and *A. biebersteinii* and *A. willhelmsii* species were tetraploid (Table 2). Karyotype formulas of inter-species and intra-species populations were different and all the chromosomes were metacentric only in populations AL2, TA1, VE1, TE1, and TE4; the karyotype consisted of a large number of metacentric chromosomes and a small number of chromosomes were submetacentric in other populations. According to the Stebbins' s index, most of the populations were in 1A and 1B, only population BI4 was in 2B, and MI1 in the 2A (Table 2). Therefore, a symmetrical karyotype was observed for the species of this genus. The highest relative amount of chromatin belonged to population AL3 with an average of 4.15 µm whereas the lowest relative amount of chromatin belonged to population TA2 with an average of 2.55. Except for population AL3, the relative chromatin levels of the populations were less than 4 and more than 2 µm. Since the relative difference in the lengths of chromosomes had an inverse relationship with intra-species ploidy levels, the most asymmetric chromosomes among the hexaploidy populations, based on DRL index, belonged to population MI4 with an average of 38.3% (Tables 2, 3). For diploid populations, BI1 population had the highest rate of chromosomal asymmetry with the highest DRL (5.32 %). Among the diploid populations, VE1 population and four populations of *A. talagonica* species had the highest rate of

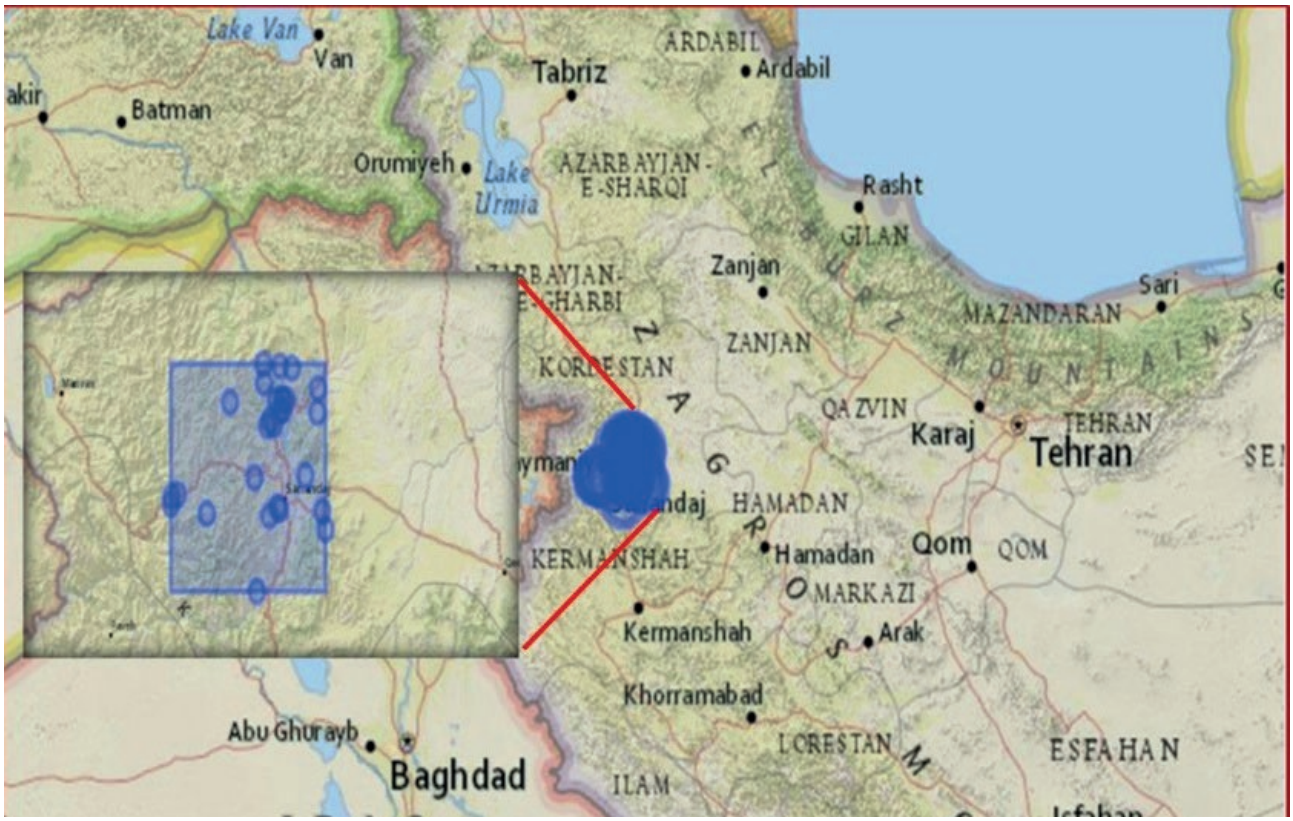


Figure 1. Positions the population samples were collected on the map.

DRL, and the most asymmetric chromosomes. The percentage of overall chromosomes form ranged from 37.6 to 46.11, and the highest percentage of overall chromosome form belonged to populations TE4, TE1, and TA1; thus, they had a more symmetrical karyotype compared to the other populations. On the contrary, AL3, MI2, MI4, and WI4 had the lowest percentage of overall chromosome form; therefore, they had the most asymmetric karyotypes (Tables 2, 3). The lowest A1 belonged to populations TE4, TE1, and TA1; consequently, they had more symmetrical karyotypes than the other populations. Based on index A1, AL3 and Wl4 had the highest chromosomal asymmetry. Hence, it was found that the intra-species diversity was high for A1 and TF%, and the species were indistinguishable based on the parameters. For the A2, the intra-species diversity was somewhat lower, and the species could be divided into three categories; the species of the first class included *A. aleppica* and *A. millefolium*, whose populations had an A2 of less than 0.2 and symmetrical chromosomes based on the index. On the contrary, the populations of two species, *A. talagonica* and *A. vermicularis*, had an index A2 of over 0.2 and asymmetric chromosomes based on the index. However, the populations of other species had higher intra-species diversity compared to the above-mentioned four species populations and also had populations with low inter-chromosomal asymmetry and high A2 (Table 2).

In terms of the CI (Table 3), populations TE4, MI1, TE1, VE3, and TA1 had a centromeric index between 0.42 and 0.46 and they had symmetrical chromosomes based on the index. Meanwhile, populations AL3, BI3, MI2, MI4, WI2, and WI4 with a CI of 0.38 had the most asymmetric chromosomes based on the index. The lowest ratio of long to short arm belonged to TE4, TE1, and TA1 populations with average values of 1.19, 1.37, and 1.4, respectively, and had symmetrical chromosomes based on the index. On the other hand, the highest value for the index with long to short arm ratio between 1.6 and 1.69 belonged to populations AL3, BI3, MI2, MI4, WI4, TE3, and WI2. Thus, they had asymmetric chromosomes. The highest average total chromosome length belonged to population AL3 with an average of 4.15 μm, and other populations had an average total chromosome length between 2.55 and 3.64 μm, among which populations TA2, TE1, TA4, TE4, BI2, VE2, MI1, VE1, BI4,

Figure 2. Shows different morphological attributes of seven species of *Achillea* L. in west of Iran, Kurdistan in their natural habitats. (a) *A. tenifolia* (b) *A. vermicularis* (c) *A. allepica* (d) *A. biebesteinii* (e) *A. wilhielmsii* (f) *A. millefolium* (g) *A. talagonica*.

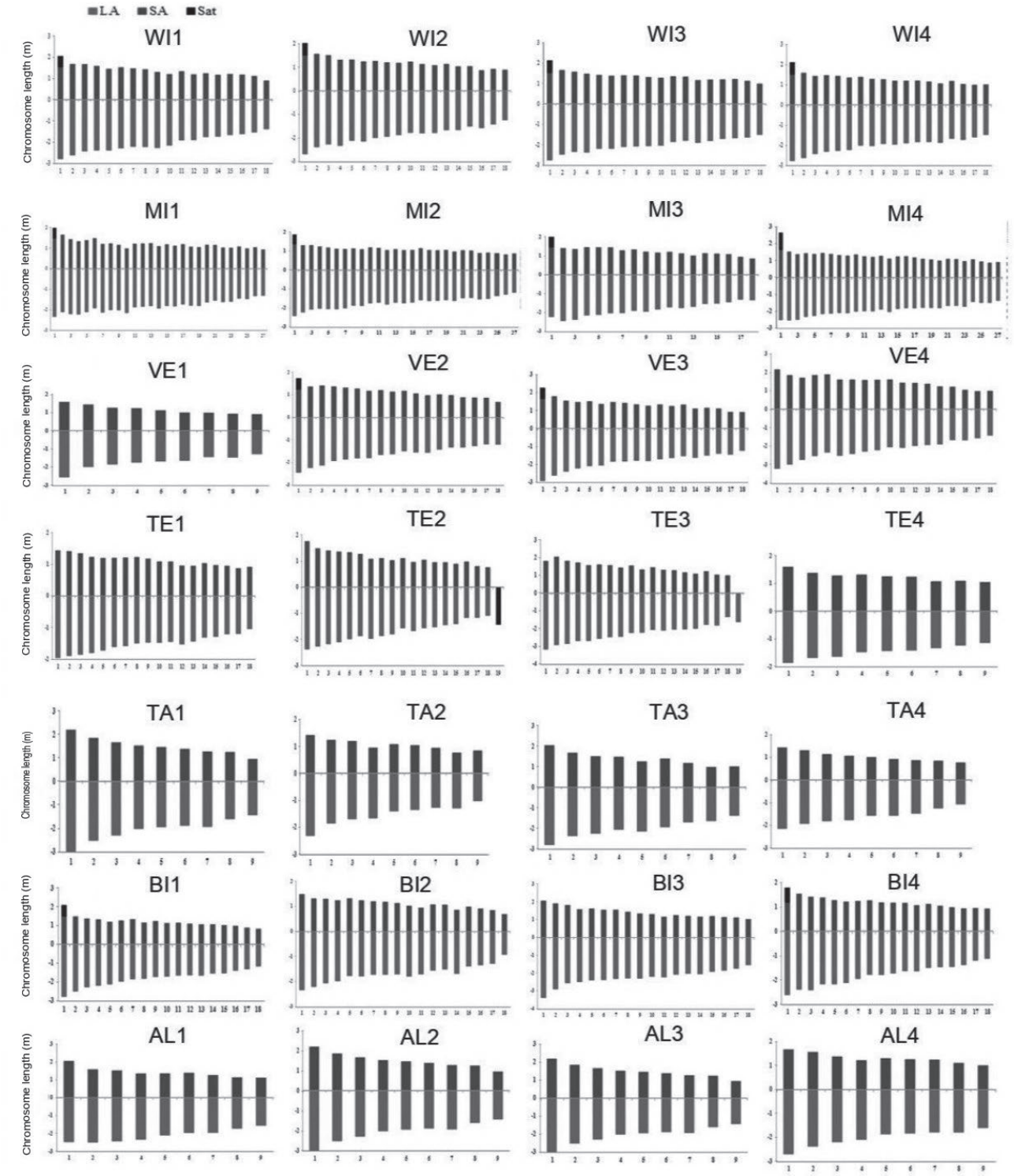


Figure 3. Haploid ideogram of seven species population samples. (Red (SA): Relative length of short arm, Blue (LA): Relative length of long arm, Black (Sat): satellite chromosome, Scale bar = 5 μm, X-axis express No. of chromosome, Y-axis express Relative of long and short arm scale bar = 5 μm).

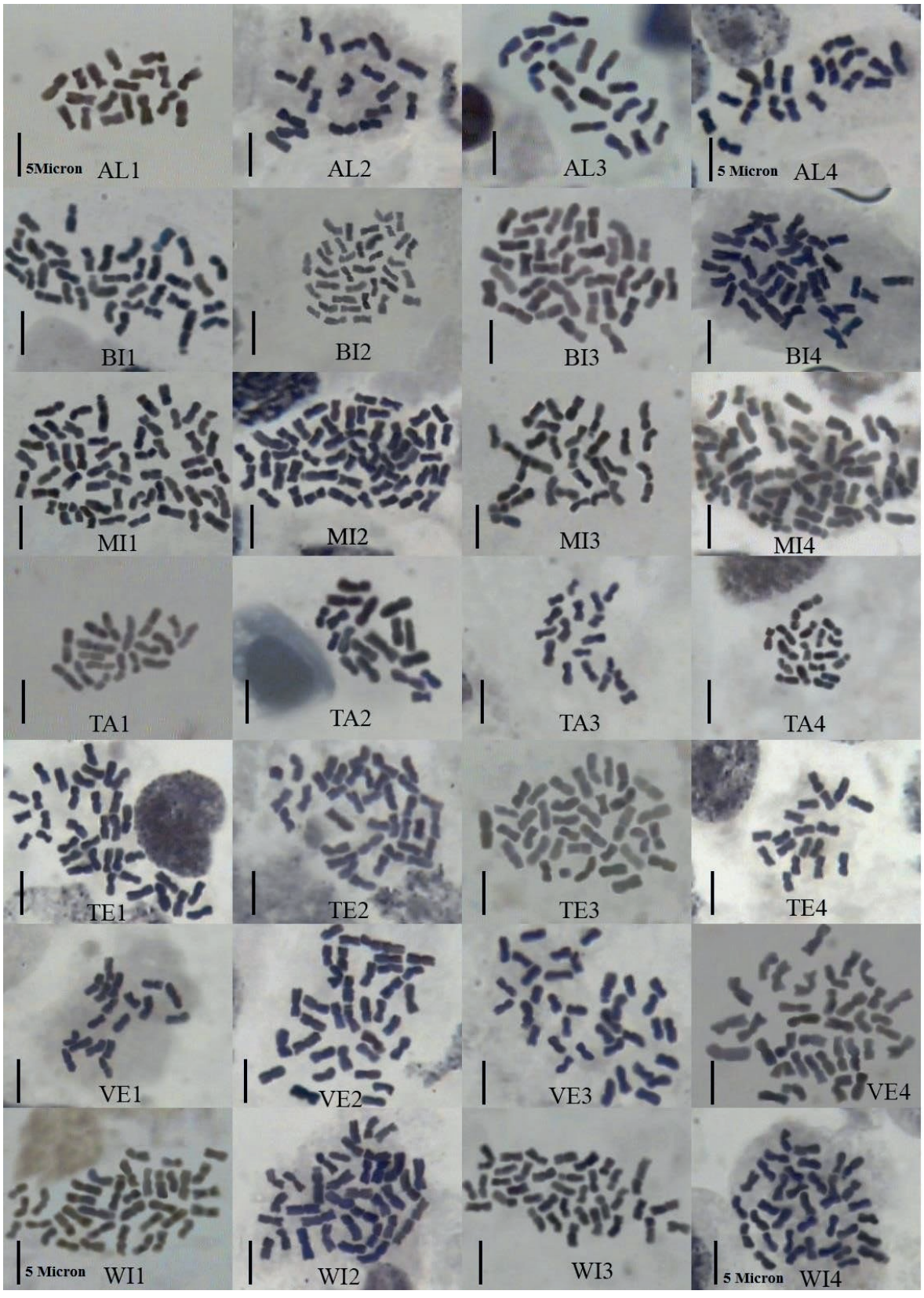


Figure 4. The morphological diversity metaphase chromosomes between and within species (Scale bar = 5μm).

Table 2. Cytogenetic indices data A2, A1, % TF, DRL, VRC, SC, and KF.

Species	KF	2n	SC	VRC	DRL	%TF	A1	A2	Population
A. aleppica	8m+1sm	2*x*=18	1A	3.49	5.90	39.97	0.334	0.170	AL_1
	9m	2*x*=18	1A	3.27	5.67	39.78	0.335	0.162	AL_2
	6m+3sm	2*x*=18	1A	4.15	5.90	37.60	0.395	0.169	AL_3
	8m+1sm	2*x*=18	1A	3.30	5.92	39.27	0.351	0.166	AL_4
A. biebersteinii	14m+4sm	4*x*=36	1B	2.98	5.32	38.42	0.356	0.228	BI_1
	15m+3sm	4*x*=36	1B	2.76	4.43	39.43	0.343	0.190	BI_2
	14m+4sm	4*x*=36	1B	3.62	4.39	38.44	0.376	0.195	BI_3
	14m+4sm	4*x*=36	2B	2.97	4.39	38.62	0.343	0.217	BI_4
A. millefolium	20m+7sm	6*x*=54	2A	3.02	2.54	38.80	0.355	0.153	MI_1
	17m+10sm	6*x*=54	1B	2.79	2.94	37.89	0.378	0.167	MI_2
	15m+3sm	4*x*=36	1A	3.06	3.67	39.29	0.337	0.183	MI_3
	20m+7sm	6*x*=54	1B	3.16	3.38	37.85	0.375	0.194	MI_4
A. talagonica	9m	2*x*=18	1B	3.53	8.80	42.02	0.277	0.236	TA_1
	7m+2sm	2*x*=18	1B	2.55	8.07	40.37	0.311	0.224	TA_2
	7m+2sm	2*x*=18	1B	3.38	8.05	40.68	0.315	0.223	TA_3
	6m+3sm	2*x*=18	1A	2.62	7.34	38.86	0.363	0.209	TA_4
A. vermicularis	9m	2*x*=18	1A	2.85	7.53	39.74	0.339	0.210	VE_1
	15m+3sm	4*x*=36	1B	2.76	4.59	39.84	0.321	0.218	VE_2
	17m+1sm	4*x*=36	1B	3.19	4.94	41.20	0.281	0.224	VE_3
	17m+1sm	4*x*=36	1B	3.64	4.49	40.52	0.317	0.218	VE_4
A. tenuifolia	18m	4*x*=36	1A	2.62	3.04	42.50	0.257	0.159	TE_1
	14m+4sm	4*x*=36	1B	2.82	4.50	39.36	0.347	0.223	TE_2
	11m+7sm	4*x*=36	1B	3.70	3.96	38.56	0.369	0.199	TE_3
	9m	2*x*=18	1A	2.69	5.23	46.11	0.141	0.147	TE_4
A .wilhelmsii	15m+3sm	4*x*=36	1B	3.41	4.17	39.00	0.346	0.190	WI_1
	14m+4sm	4*x*=36	1B	3.08	4.61	38.13	0.371	0.205	WI_2
	16m+2sm	4*x*=36	1A	3.35	3.95	39.13	0.342	0.168	WI_3
	14m+4sm	4*x*=36	1A	3.28	4.04	37.75	0.380	0.183	WI_4

and BI1 had an average total chromosome length of less than 3 μm. The other populations had an average total chromosome length between 3 and 3.64 μm. Therefore, it was found that intra-species diversity was high for AR, CI, and TL indices (Table 3). Based on the parameters, the species were indistinguishable. The range of the total chromosome length varied widely from a minimum range of 1.59 μm in the population BI2 to a maximum of 9.85 μm in the population VE1; hence, the longest chromosome was 6.19 times higher than the shortest chromosome.

Results of analysis of cytogenetic variables

Fig. 5 depicts the results of cluster heatmap (based on Euclidian distance and ward method) analysis for cytogenetic variables of different species. A total of 10 attributes were included in the analysis. The results exhibited that the accessions were assigned two main distinct groups. According to the figure 5, in the first cluster *A. talgonica* (2*x*) species was separated from others. The second group consists of all other species with different ploidy levels, but interestingly in contrary side of first group (maximum distance from first group) a diploid species *A. aleppica* (2*x*) was located. In other words, two diploid species were assigned to different sides of clustering. Obviously, cluster analysis could not detect differences between interspecies, however three species including *A. talagonica*, *A. aleppica* and *A. willhelmsii* were clearly discriminated from others. It can be noted that cluster analysis could not detect ploidy pattens among the *Achillea* populations (annotation group in cluster analysis) (Fig.5). Among the attributes a few variables showed the highest interrelationship variation population accessions which are including %TF, LA and DRL.

Table 3. Karyotypes and chromosomal parameters in this study for each species.

Population	CI	AR	%RL	TL	%SA	%LA	Chromosome range length (µm)
AL1	0.4 ± 0.02	1.52 ± 0.1	11.11 ± 1.23	3.49 ± 0.39	4.44 ± 0.59	6.67 ± 0.71	2.63 – 4.48
AL2	0.4 ± 0.01	1.52 ± 0.05	11.11 ± 1.17	3.27 ± 0.34	4.42 ± 0.41	6.69 ± 0.77	2.53 – 4.19
AL3	0.38 ± 0.01	1.69 ± 0.09	11.11 ± 1.23	4.15 ± 0.46	4.18 ± 0.44	6.93 ± 0.81	3.18 – 5.39
AL4	0.39 ± 0.01	1.56 ± 0.07	11.11 ± 1.2	3.3 ± 0.36	4.36 ± 0.46	6.75 ± 0.76	2.58 – 4.33
BI1	0.39 ± 0.01	1.59 ± 0.07	5.59 ± 0.58	2.98 ± 0.31	2.14 ± 0.16	3.36 ± 0.36	1.95 – 4.81
BI2	0.4 ± 0.01	1.56 ± 0.08	5.56 ± 0.49	2.76 ± 0.24	2.19 ± 0.19	3.37 ± 0.31	1.59 – 3.79
BI3	0.38 ± 0.01	1.63 ± 0.06	5.56 ± 0.5	3.62 ± 0.33	2.14 ± 0.21	3.42 ± 0.3	2.52 – 5.38
BI4	0.39 ± 0.02	1.56 ± 0.1	5.56 ± 0.56	2.97 ± 0.3	2.15 ± 0.15	3.35 ± 0.37	2.01 – 4.35
Ml1	0.44 ± 0.01	1.59 ± 0.07	3.7 ± 0.21	3.02 ± 0.17	1.44 ± 0.08	2.24 ± 0.13	2.22 – 4.29
MI2	0.38 ± 0.01	1.63 ± 0.05	3.7 ± 0.23	2.79 ± 0.18	1.4 ± 0.07	2.28 ± 0.14	2.02 – 4.24
Ml3	0.4 ± 0.01	1.53 ± 0.06	5.56 ± 0.47	3.06 ± 0.26	2.18 ± 0.16	3.32 ± 0.28	2.15 – 4.18
Ml4	0.38 ± 0.01	1.63 ± 0.05	3.7 ± 0.27	3.16 ± 0.23	1.4 ± 0.09	2.25 ± 0.14	2.24 – 5.12
TA1	0.42 ± 0.01	1.4 ± 0.05	11.11 ± 1.71	3.53 ± 0.54	4.67 ± 0.75	6.44 ± 0.97	2.34 – 5.14
TA2	0.41 ± 0.02	1.49 ± 0.14	11.11 ± 1.63	2.55 ± 0.37	4.49 ± 0.58	6.63 ± 1.09	1.83 – 3.68
TA3	0.41 ± 0.01	1.49 ± 0.09	11.11 ± 1.62	3.38 ± 0.49	4.52 ± 0.72	6.59 ± 0.92	2.35 – 4.8
TA4	0.39 ± 0.01	1.59 ± 0.07	11.11 ± 1.52	2.62 ± 0.36	4.32 ± 0.61	6.79 ± 0.92	1.8 – 3.54
VE1	0.4 ± 0.01	1.53 ± 0.06	11.11 ± 1.52	2.85 ± 0.39	4.41 ± 0.59	6.7 ± 0.95	4.92 – 9.85
VE2	0.4 ± 0.01	1.52 ± 0.08	5.56 ± 0.56	2.76 ± 0.28	2.21 ± 0.19	3.29 ± 0.33	2.35 – 3.29
VE3	0.42 ± 0.01	1.42 ± 0.07	5.56 ± 0.58	3.19 ± 0.33	2.29 ± 0.19	3.23 ± 0.36	2.11 – 4.95
VE4	0.41 ± 0.01	1.49 ± 0.06	5.56 ± 0.56	3.64 ± 0.37	2.25 ± 0.23	3.3 ± 0.34	2.36 – 5.30
TE1	0.43 ± 0.01	1.37 ± 0.05	5.56 ± 0.41	2.62 ± 0.19	2.36 ± 0.17	3.19 ± 0.25	1.94 – 3.37
TE2	0.39 ± 0.01	1.56 ± 0.07	5.41 ± 0.61	2.75 ± 0.31	2.19 ± 0.23	3.37 ± 0.34	1.82 – 4.1
TE3	0.39 ± 0.01	1.61 ± 0.06	5.39 ± 0.58	3.59 ± 0.39	2.14 ± 0.2	3.41 ± 0.31	1.6 – 4.93
TE4	0.46 ± 0.01	1.19 ± 0.05	11.11 ± 1.06	2.69 ± 0.26	5.12 ± 0.47	5.99 ± 0.61	2.16 – 3.43
WI1	0.4 ± 0.1	1.56 ± 0.06	5.56 ± 0.49	3.41 ± 0.3	2.17 ± 0.16	3.34 ± 0.3	2.25- 4.85
WI2	0.38 ± 0.01	1.6 ± 0.05	5.56 ± 0.53	3.08 ± 0.29	2.12 ± 0.17	3.39 ± 0.31	2.1 – 4.65
WI3	0.39 ± 0.01	1.54 ± 0.05	5.56 ± 0.43	3.35 ± 0.26	2.17 ± 0.13	3.33 ± 0.25	2.46 – 4.84
WI4	0.38 ± 0.01	1.63 ± 0.05	5.56 ± 0.47	3.28 ± 0.28	2.1 ± 0.14	3.4 ± 0.27	2.45 – 4.84

DISCUSSION

There are high diversity and differences in chromosomal length characteristics of the inter and intra-species of this genus Fig. 3, 4. Given that the existence of diversity and difference in chromosome length indicates an advanced karyotype and has chromosomes in different sizes (Afshari et al. 2013), the species of this genus have advanced karyotypes. The existence of x=9 as the base chromosome number on the yarrow genus has been proven in several reports, yet the number of chromosomes and ploidy levels vary among different species of this genus, which could range from 2n=2x= 18 to 2n=8x=72 even though most species are Diploid(Guo et al. 2005, Baltisberger and Widmer 2016). In addition to inter-species diversity in ploidy levels, there are numerous reports of ploidy level diversity in populations within a species. In other words, different ploidy levels are reported for populations of a species (Hoshi et al. 2010, Ebrahim et al. 2012). Accordingly, a range between diploid to hexaploidy has been reported for *A. aleppica* species (Rad and Javaheri 2014); however, all the accessions of the species were diploid in the present study. The tetraploid level was reported for *A. bieberestini* species (Afshari et al. 2013), which was consistent with the present result. Afshari reported diploid and tetraploid levels for *A. millefolium* species. In another study, hexa and octa-ploidy levels were reported for the species (Ebrahim et al. 2012). The two reports were consistent with the present study in terms of *A. millefolium* species. For four populations of *A. talagonica* species, the diploid level was in accordance with results of studies by Sahin et al. (2006). Finally, the results obtained for ploidy levels of two species, *A. vermicularis* and *A. tenuifolia*, were in agreement with other reports (Afshari et al. 2013, Rad and Javaheri

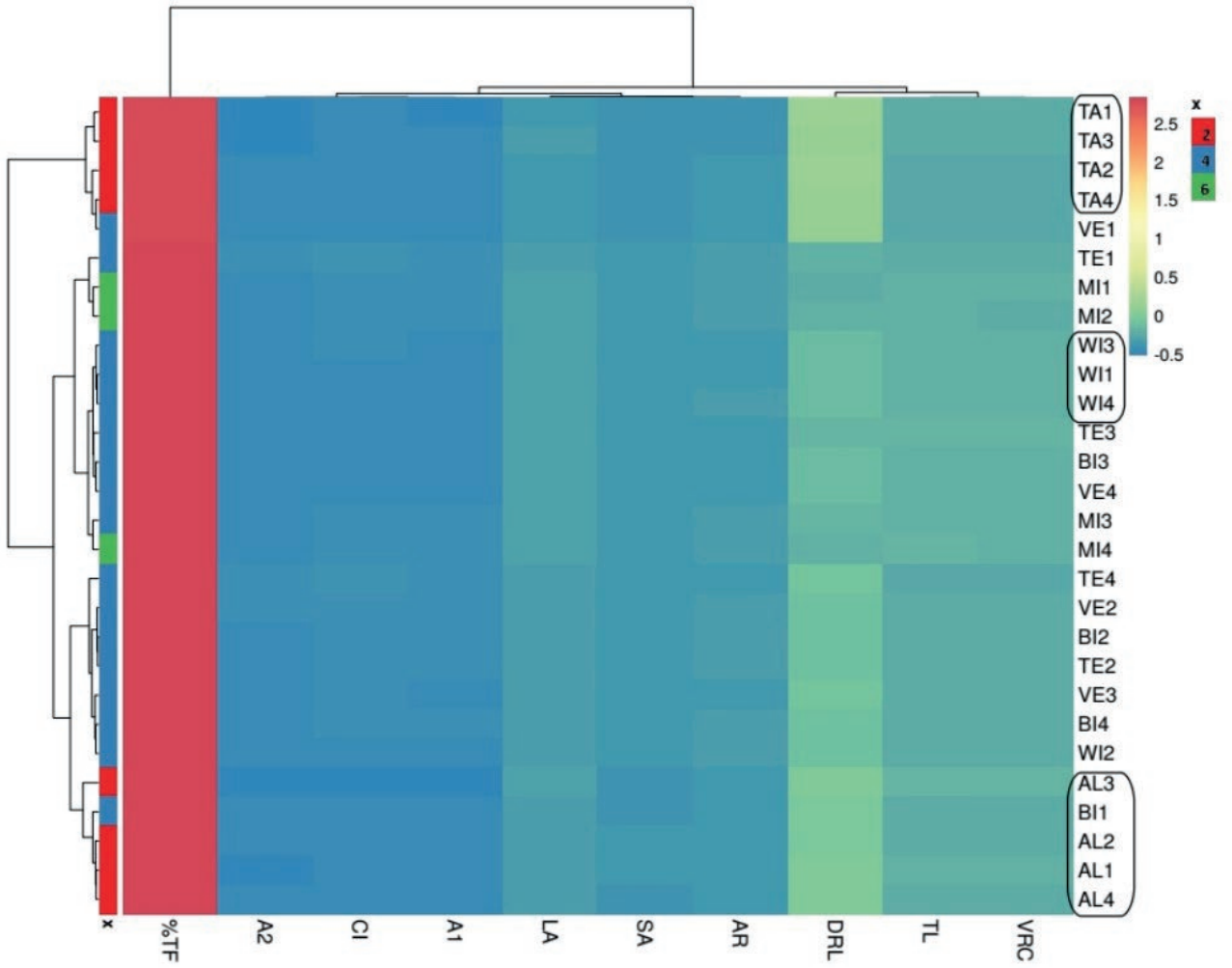


Figure 5. Depicts the results of cluster heatmap (based on Euclidian distance and ward method analysis for cytogenetic parameters).

2014). Therefore, no new reports were found for the ploidy levels of the species.

The karyotypic formulas of all the species consisted of a large number of metacentric chromosomes and a small number of sub-metacentric chromosomes (Table 2). On this basis, the populations of the species of this genus had symmetrical karyotypes, and there were diverse karyotypic formulas for both species and intra-species populations. In several reports on the cytogenetic analysis of species of the yarrow genus, more metacentric chromosomes and less submetacentric chromosomes have been reported (Sahin et al. 2006, Afshari et al. 2013, Rad and Javaheri 2014). Moreover, there were some reports on the subtelocentric chromosomes (Baltisberger and Widmer 2016). However, there were almost symmetrical chromosomes for species of the genus. According to the Stebbins table regarding 28 populations, 11 populations had A1 symmetry, 15 had B1, a population had A2, and a population had B2 symmetry (Table 2); hence, there were more symmetrical chromosomes in the present research than other reports since the karyotype A2 was mostly reported in other reports, and fewer cases had A1 and B1 symmetries (Kiran et al. 2012, Sahin et al. 2006). Accordingly, no obvious differences were reported in karyotype asymmetry between yarrow species; all the species had symmetrical karyotype structures because most chromosomes were metacentric and sub-metacentric (Kiran et al. 2012).

Satellites were observed more in populations with tetra- and hexaploidy levels and on chromosome 1 (Fig. 3). No satellites were observed in diploid populations, and there was only a satellite for each population. Our results were consistent with those of a report by Sahin et al. (2006). On the contrary, no satellites were reported in certain studies (Hoshi et al. 2010, Kiran et al. 2012, Afshari et al. 2013, Rad and Javaheri 2014) whereas one

to three satellites have been reported in some other researches (Afshari et al. 2013). Additionally, more satellites were observed in submetacentric chromosomes and the results were consistent with those of the present study (Hoshi et al. 2010).

There were chromosomes B in two populations of *A. tenuifolium* species (Table 2). A chromosome B was also reported for the species in some populations (Chehregani Rad et al. 2017), and there were some reports on the existence of B chromosome in other species on the genus (Baltisberger and Widmer 2016). Nevertheless, there was no B chromosome in some reports (Kiran et al. 2012).

There was no inter-species diversity for the chromatin content, arm length, and chromosome length. Furthermore, the intra-species populations showed more diversity (Table 3), but there was inter-species diversity for ratios to arms (and large to small); however, the intra-species populations had diversity. Therefore, the evolution and speciation of the genus was through A1 rather than increasing or decreasing the chromatin content and chromosome length. The average length of each chromosome ranged from 2.93 to 3.55 mμ for the species, which was consistent with other reports (Sahin et al. 2006 Afshari et al. 2013) . Meanwhile, the chromosome length range was higher in certain reports than that in the results of the present study, and longer chromosomes were reported for the species (Aksu et al. 2013). Based on the karyotypic characteristics, the *A. aleppica* had more karyotypic evolution in terms of chromatin content, and three species, *A. biebersteinii*, *A. wilhemsii*, and *A. millefolium*, had more complete karyotypes due to the A1 and a higher evolution in terms of chromosome length characteristics and chromatin content. *A. talangonica*, *A. tenifolia*, and *A. vermicularis* had karyotypic evolution due to the chromosomal asymmetry; thus, *A. biebersteinii*, *A. wilhemsii* and *A. millefolium* had more evolved karyotypes than the other species. According to the results, the karyotypic characteristics could not separate the populations of yarrow species due to the intra-species diversity, and the populations of different species were in the same group in several cases. Despite the lack of comprehensive reports on the study of inter and intra-species relationships for the *Achillea* genus based on karyotypic characteristics, the few available reports indicated that the karyotypic characteristics were unable to completely separate the populations of species of the genus (Ebrahim et al. 2012, Kiran et al. 2012).

ACKNOWLEDGMENTS

The authors are grateful to the members of the Cytogenetics Group in Laboratory of Forestry and Plant Resources in Kermanshah, for measuring all the spectra. We also appreciate Dr. Hooshmand Safari for providing technical support on plant bioassays.

REFERENCES

Abbaszade S, Jafari A A, Safari H, Shirvani H. 2017. Genetic Variability and Karyotype Analysis for 13 Accessions of *Lolium multiflorum*. J. Ornam. Plants. 7: 1–8.

Afshari F, Ebrahimi M, Akbari M, Farajpour M. 2013. Cytological investigations and new chromosome number reports in yarrow (*Achillea millefolium* Linnaeus, 1753) accessions from Iran. Comp. Cytogenet. 7: 271–277.

Aksu N, Inceer H, Hayırlıoğlu-Ayaz S. 2013. Karyotype analysis of six *Achillea* L.(Asteraceae, Anthemideae) taxa from Turkey. Caryologia. 66: 103–108.

Altınordu F, Peruzzi L, Yu Y, He X. 2016. A tool for the analysis of chromosomes: KaryoType. Taxon. 65: 586–592.

Baltisberger M, Widmer A. 2016. Chromosome numbers and karyotypes within the genus *Achillea* (Asteraceae: Anthemideae). Willdenowia. 46: 121–135.

Bivand R, Lewin-Koh N. 2013. maptools: Tools for Reading and Handling Spatial Objects. R package version 0.9-2. .

Chehregani Rad A, Salehi H, Mohsenzadeh F. 2017. Karyomorphological Analysis on Several Diploid Populations of *Achillea biebersteinii* Afan. (Asteraceae) from Iran. Proc. Natl. Acad. Sci. India Sect. B - Biol. Sci. 87: 173–180.

Ebrahim F, Pakniyat H, Arzani A, Rahimmalek M. 2012. Karyotype analysis and new chromosome number reports in *Achillea* species. Biologia (Bratisl). 67: 284–288.

Flavell R.B. 2021. Perspective: 50 years of plant chromosome biology. Plant Physiol. 185: 731–753.

Ghahreman A. 1984. Color atlas of Iranian plants. Inst. For. Grasslands, Bot. Div. **5**: 512.

Guo Y, Saukel J, Mittermayr R, Ehrendorfer F. 2005. AFLP analyses demonstrate genetic divergence, hybridization, and multiple polyploidization in the evolution of *Achillea* (Asteraceae-Anthemideae). New Phytol. 166: 273–290.

Hoshi Y, Kondo K, Konishi T, Tannowa T, Smirnov S V, Kucev M, Motohashi T. 2010. A cytogenetic study in three species of *Achillea*. Chromosom. Bot. 5: 87–93.

Kassambara A. 2017. Practical guide to principal component methods in R: PCA, M (CA), FAMD, MFA, HCPC, factoextra. STHDA,

Kiran Y, Turkoglu I, Kirilmaz F, Arabaci T, Sahin A, Bagci E. 2012. Karyological investigation of six *Achillea* L.(Asteraceae) species growing in Turkey. Caryologia. 65: 101–105.

Levan A, Fredga K, Sandberg A. 1964. Nomenclature for centromeric position on chromosomes. Hereditas. 52: 201–220. https://doi.org/10.1111/j.1601-5223.1964.tb01953.x.

Levitsky G.A. 1931. The karyotype in systematics. Bull. Appl. Bot. Genet. Plant Breed. 27: 220–240.

Levitus G, Echenique V, Rubinstein C, Hopp E, Mroginski L. 2010. Biotechnology and Plant Breeding II. Biotechnol. Plant Breed. II.

Mozaffarian, V. 2003. Trees and Shrubs of Iran. Farhange Moaser, Tehran, Iran, 874-877.

Muẓaffarīyān W. 1996. Dictionary of Iranian plant names. Farhang Moaser Publ.

Rad AC , Javaheri F. 2014. Variation of karyomorphological characters in *Achillea aleppica* DC. (Asteraceae) in the western part of Iran. Chromosom. Bot. 9: 13–22.

Ramsey J. 2011. Polyploidy and ecological adaptation in wild yarrow. Proc. Natl. Acad. Sci. U. S. A. 108: 7096–7101.

Sahin A, Kiran Y, Arabaci T, Turkoglu I. 2006. Karyological notes on eight species of *Achillea* L.(Asteraceae, Santolinoideae) from Turkey. Bot. J. Linn. Soc. 151: 573–580.

Sheidai M, Azanei N, Attar F. 2009. New chromosome number and unreduced pollen formation in *Achillea* species (Asteraceae). Acta Biol. Szeged. 53: 39–43.

Stebbins GL. 1971. Chromosomal evolution in higher plants. Chromosom. Evol. High. plants.

Table 1. Details on population sites including geographical coordinates, altitude and origin of samples

Table 2. Cytogenetic indices data A2, A1, % TF, DRL, VRC, SC, and KF.

Table 3. Karyotypes and chromosomal parameters in this study for each species.

Caryologia
International Journal of Cytology, Cytosystematics and Cytogenetics

Firenze University Press
www.fupress.com/caryologia

OPEN ACCESS

Citation: Ayşe Yavuz Kocaman, Berna Yakar (2023). Genotoxicity of a synthetic plant growth regulator, Forchlorfenuron (CPPU), on human lymphocytes using chromosome aberration assay. *Caryologia* 76(1): 47-56. doi: 10.36253/caryologia-2006

Received: January 21, 2023

Accepted: July 3, 2023

Published: September, 19, 2023

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

ORCID
AYK: 0000-0001-7700-784X
BY: 0000-0003-0506-521X

Genotoxicity of a synthetic plant growth regulator, Forchlorfenuron (CPPU), on human lymphocytes using chromosome aberration assay

Ayşe Yavuz Kocaman[1,*], Berna Yakar[2]

[1] *Department of Biology, Faculty of Science and Letters, Hatay Mustafa Kemal University, 31000 Hatay, Türkiye*
[2] *Department of Biology, Basic and Applied Sciences Institute, Hatay Mustafa Kemal University, 31000 Hatay, Türkiye*
*Corresponding author. E-mail: ayavuz@mku.edu.tr

Abstract. Forchlorfenuron (FCF, also known as CPPU), which belongs to the group of phenylurea cytokinins, is one of the most widely used synthetic plant growth regulators (PGRs) worldwide. Although FCF plays a crucial role in cellular growth and differentiation by promoting cell division in plants, it disrupts higher-order septin assembly in other eukaryotic organisms, including humans. Despite its widespread use, no study has been found investigating the genotoxic effects of this synthetic PGR on humans. Hence, this investigation was designed to examine the potential cyto-genotoxicity of a commercial formulation of FCF on human peripheral blood lymphocytes (PBLs) using chromosome aberrations (CAs) and mitotic index (MI) endpoints. The whole blood cultures were treated with 0.25, 0.50, 1.00, and 2.00 μg/ml concentrations of a commercial form of FCF. According to the results, FCF significantly enhanced the percentage of cells containing structural CAs at the concentrations of 1.00 and 2.00 μg/ml for both treatment times (24 and 48 h), in comparison to the negative control ($P<0.05$). Besides, in cultures exposed to FCF concentrations of 0.50, 1.00, and 2.00 μg/ml, the total CA/cell ratio was significantly higher ($P<0.05$). In addition, FCF was found to have cytotoxic activity on human PBLs at all treatments (except for the lowest concentration at 24 h) by significantly reducing the MI compared to the negative control ($P<0.05$). The findings of this investigation indicate the first time that a commercial formulation of FCF (0.50-2.00 μg/ml) may have genotoxic and cytotoxic potential on human lymphocytes.

Keywords: Forchlorfenuron, CPPU, synthetic plant growth regulator, chromosome aberration, mitotic index, human lymphocytes.

INTRODUCTION

Today, environmental pollutants and their toxic effects on organisms have become one of the most discussed issues worldwide (Briggs 2003; Zhang et al. 2011). The main pollutant groups that cause environmental pollution are chemicals used in agriculture such as herbicides, insecticides, fun-

gicides, and plant growth regulators (PGRs), which are an important part of modern agriculture (Lu et al. 2015; Wang and Yang 2016; Rodrigues et al. 2018).

Synthetic PGRs also known as environmental hormones are a class of agrochemicals that stimulate plant developmental processes such as organ formation, cell division, and growth (Wang et al. 2011). People are potentially exposed to PGRs either directly as workers in greenhouses and agriculture or indirectly through food consumption. Therefore, the uncontrolled and excessive use of PGRs both cause environmental pollution and threaten non-target organisms and ultimately human health. Gangadhar et al. (2020) reported that residues of synthetic PGRs can seriously harm human health.

Forchlorfenuron (FCF, also known as CPPU) which is included in the phenylurea synthetic cytokinin group, is among the most widely used PGRs worldwide to increase fruit size/enlargement (USEPA 2004; Heasley et al. 2014). It is known that synthetic cytokinins (i.e. FCF, thiadizuron, and their derivatives) are cytokinin oxidase/dehydrogenase (CKX) inhibitors. The catabolic cytokinin dehydrogenase CKX, is bound by FCF and is competitively inhibited, leading to an increase in intracellular cytokinin levels and, eventually, larger fruits (Kopecný et al. 2010; Heasley et al. 2014). It was also suggested that FCF promotes plant cell division and lateral growth through its synergistic interactions with endogenous auxins, thereby inducing parthenocarpy, which in turn increases fruit size, fruit set, and fruit cluster weight (USEPA 2004; Kim et al. 2006; Su et al. 2021). It is widely used to enlarge many fruits, such as grapes, apples, and kiwi (Kim et al. 2006; EFSA 2017).

Unfortunately, in 2011, FCF was abused by Chinese farmers to grow larger watermelons; but some of them exploded. After this event, the potential threats it poses for organisms and the environment have started to come to the fore more (Gong et al. 2021). It has been reported that FCF and its metabolite residues have frequently been found in fruits, water, sediments, and aquatic species (Meng et al. 2020; Gong et al. 2021). In addition, FCF is bioaccumulated by humans and can reach concentrations that may be harmful to farm laborers (Shi et al. 2012; Toumi et al. 2018). Tixier et al. (2001) indicated that FCF may be an endocrine disruptor and had certain genotoxic and ecotoxic effects. In a recent study also reported the excessive use of swelling agents such as FCF, and thidiazuron in fruits and vegetables has caused food safety problems (Wang et al. 2023). However, the use of FCF in seedless fruit formation by inducing parthenocarpy is still common (Su et al. 2021).

While FCF has been used as a PGR in many countries, it has also been an experimental molecule extensively used to investigate septin functions (Angelis et al. 2014). Septins, a class of GTP-binding proteins, are found in all eukaryotic organisms with the exception of higher plants, and have many important cellular functions (Henzi et al. 2021). It has been reported that FCF disrupts septin localization in budding yeast and causes defects in cytokinesis (Iwase et al. 2004). The researchers showed the FCF causes the accumulation of thick septin bundles and aggregates by blocking septin filament turnover (Hu et al. 2008).

The stability of the septin cytoskeleton induced by FCF has been shown to mimic the functional effects of septin depletion, such as inhibition of cell division, cell-cell adhesions, calcium ingress, migration, and vesicle trafficking (Hu et al. 2008; Sidhaye et al. 2011; Sharma et al. 2013; Tokhtaeva et al. 2015; Marcus et al. 2016; Zhang et al. 2016). The stabilization of septin filaments by FCF impairs their normal functions (Hu et al. 2008). Because FCF influences different cellular systems via stabilizing septins in metazoans, it appears crucial to look into its potential to be genotoxic to humans.

According to our literature search, only one study was found regarding the genotoxic potential of FCF. It was reported that FCF was not mutagenic using the mouse bone marrow micronucleus test (Lin et al. 2012). However, no studies were found to investigate the genotoxic risks of FCF on humans. Therefore, we believe that it is crucial to identify the potential genotoxic/cytotoxic risks of the commercial formulation of FCF (sitofex, active ingredient 10 g/l FCF), which is a widely used synthetic PGR today.

Hence, this study was carried out to investigate the genotoxic and cytotoxic potential of FCF, in a commercial form, using *in vitro* chromosomal aberration (CA) and mitotic index (MI) assays, on human peripheral blood lymphocytes (PBLs).

MATERIALS AND METHODS

Test samples and chemicals

Samples of whole blood were provided from four healthy, non-smoker, and non-alcoholic different donors (30-32 years old, 2 females, and 2 males). Donors were selected from people who did not use drugs in the last three months and were not exposed to environmental pollutants such as pesticides, dust, and chemicals at a high rate.

This research was carried out in accordance with the Helsinki Declaration and ethical approval has been given from the Institutional Ethics Committee of Hatay Mustafa Kemal University, Türkiye (approval number:

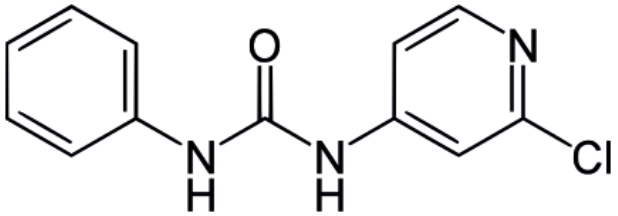


Figure 1. The chemical structure of Forchlorfenuron.

14.02.2019/09). In addition, each participant signed a permission form and provided their informed consent to take part in the study.

A commercial formulation of FCF (Sitofex, containing 10 g/L FCF as the active ingredient) was obtained from the Agrikem company, Türkiye. Its properties are as follows: IUPAC name: 1-(2-chloro-4-pyridyl)- 3-phenylurea; CAS name: N-(2-chloro-4-pyridinyl)-N-phenylurea; Trade names: CPPU, KT-30; Chemical formula: $C_{12}H_{10}ClN_3O$; Molecular weight: 247.68 g/mol; CAS registry number: 68157-60-8. The chemical structure of FCF is given in Figure1. Mitomycin-C (MMC, M-05030), and Colchicine (C-9754, St. Louis, MO) were supplied from Sigma. The other chemicals used in the study were provided by Merck. (Darmstadt, Germany). As the culture media, chromosome medium B (Biochrome F-5023; Berlin, Germany), was used.

Chromosome Aberration (CA) assay

The CA assay was performed according to the protocol of Evans (1984) with minor adjustments (Kocaman and Topaktaş 2007). Heparinized whole blood samples (0.2 ml) obtained from each donor were added to chromosome medium B (2.5 ml) to set up the cultures, which were then incubated at 37 °C for 72 h. The components of the culture medium were (amounts per liter) as follows: minimum essential medium (MEM-joklik) with non-essential amino acids (850 ml), fetal bovine serum (150 ml), heparin (25000 U), penicillin G, sodium salt (75000 U), streptomycin sulfate (50 mg), phytohemagglutinin L (2.5 mg), ascorbic acid (5.0 mg), and glutathione (reduced) (5.0 mg). The commercial FCF formulation was applied to the cultures at concentrations of 0.25, 0.50, 1.00, and 2.00 µg/ml. The highest concentration of FCF (2.00 µg/ml) was determined according to the half-maximal inhibitory concentration (IC_{50}) value that reduced MI by approximately 50% compared to the negative control. The commercial FCF used in this study was in liquid form and its serial dilutions were prepared freshly and under sterile conditions before each experiment by diluting with sterile distilled water (based on the amount of active ingredient it contains). Treatment times were conducted as 24 and 48 h. Parallel tests were also conducted using a negative (untreated) and positive (treated with 0.2 µg/ml MMC) control.

Two hours prior to harvesting, colchicine, at a concentration of 0.06 µg/ml, was given to culture tubes containing the cells. At the 72nd h, the cultures were centrifuged for 5 min at 2000 rpm and the supernatant was removed. The pellets were treated gently with the hypotonic solution (0.4% KCl, at 37 °C) for 15 min. Thereafter, the suspension was centrifuged at 1200 rpm for 10 min and the cells in pellets were fixed three times with a cold fixative (3:1 v/v; methanol: glacial acetic acid, at 22 °C) for 20 min. Finally, slides were prepared by dropping 3-4 drops of the final cell solution on clean, chilled glass slides, and standard procedures were used to stain the slides (5% Giemsa in Sorensen Buffer, for 15-20 min at pH = 6.8), after air-drying.

Microscopic evaluation

In this research, the slides were examined using an Olympus CX21 light microscope at 1000x magnification for CAs, and also 400x magnification for MI. The CAs observed in this study were categorized as structural and numerical (polyploid cells) aberrations according to Mosesso et al. (2013) and Ayabaktı and Kocaman (2020). By considering the same references, structural CAs were further grouped into chromatid (breaks, sister unions, exchanges), and chromosome types (breaks, dicentrics, fragments). In order to score the CAs, a total of 400 metaphases were examined per concentration, for each treatment and donor. Following the scoring of CAs, the percentage of cells having structural CAs, the total number of CAs per cell, as well as the percentage of frequency of each type of CA, were computed.

To calculate MI, the percentage of metaphases within 2000 cells was counted per culture for each treatment and donor (i.e., 8000 cells in total per concentration) using following equation:

$$MI = 100 \times \text{cells in metaphase} / 2000$$

Statistical analysis

For statistical analysis, an experimental unit of four subjects (n=4) was employed. Data were displayed as mean ± standard deviation (SD). The statistical analysis was conducted by utilizing One-Way Analysis of Variance (ANOVA). To compare the experimental groups, a

post-hoc analysis (least significant difference; LSD) test was performed. Relationships between concentration and response were evaluated by correlation and regression coefficients (r^2). The results were considered statistically significant according to the significance level of $P<0.05$.

RESULTS

The effects of the commercial formulation of FCF on human lymphocytes by induced CA are presented in Table 1. According to the results obtained from the *in vitro* CA test; FCF significantly increased the percentage (%) of cells with structural CAs at 1.00 and 2.00 µg/ml for both treatments, 24 and 48 h, in comparison to the negative control ($P<0.05$). Additionally, it was determined that FCF significantly increased total CA/cell at three high concentrations (0.50, 1.00, and 2.00 µg/ml) for both exposure times (24 and 48 h) compared to the negative control ($P<0.05$). However, CA formations caused by FCF in this study were not as effective as the MMC, positive control, and were determined to be significantly lower compared to the MMC at all concentrations and treatment times ($P<0.001$).

Regression analysis results revealed that the percentage of cells including structural CA showed a concentration-dependent increase in just 24 h treatment (for 24 h: $r^2= 0.837$, $P<0.05$ and for 48 h: $r^2= 0.727$, $P>0.05$). Similarly, during the 24 h treatment period, there was a concentration-dependent rise in the frequency of total CAs/cell (for 24 h: $r^2= 0.897$, $P<0.05$ and for 48 h: $r^2= 0.733$, $P>0.05$).

In our study, it was determined that FCF caused especially structural CAs in cultured human lymphocytes. Chromatid breaks (63.07%) (Fig. 2a) and sister chromatid unions (29.41%) (Fig. 2b) were the most frequent chromatid-type structural CAs found in PBLs treated with FCF. However, chromatid exchange (0.65%) (Fig. 2c) was found at a low frequency. In addition, chromosomal type structural CAs caused by FCF were observed at low frequencies as chromosome break (4.25%) (Fig. 2d) and dicentric chromosome (0.33%) (Fig. 2e). As shown in Table 1, in this investigation, only polyploid cells were observed, albeit at a low frequency (2.29%) (Fig. 2f), in the form of numerical CA.

In the present research, MI (percentage of cells in mitosis) values were determined to assess the possible cytotoxic effect of FCF (Table 2). In comparison to the negative control, FCF generally led to a significant decrease in MI for all concentrations (0.25, 0.50, 1.00, and 2.00 µg/ml) and both treatment durations (24 and 48 h) (except for the lowest concentration, 0.25 µg/ml, for 24 h) ($P<0.001$). Although FCF caused decreases in the MI values as the concentration increased; there were no statistically significant concentration-effect relationships ($P>0.05$).

DISCUSSION

Forchlorfenuron is a synthetic phenylurea-derived cytokinin widely used in agriculture as one of the PGRs which regulates the growth and development of plants (USEPA 2004; Heasley et al. 2014). Despite its widespread use, no available studies were found on the genotoxicity and cytotoxicity of FCF on human cells. Hence, the geno/cytotoxic effects of cytofex, a commercial form of PGR containing FCF as the active ingredient, on human PBLs cultures at four distinct concentrations (0.25, 0.50, 1.00, and 2.00 µg/ml) and two exposure times (24 and 48 h) were evaluated using *in vitro* CA test. Evaluation of the increase in CA frequency is an important biomarker of the genotoxic potential and allows the early detection of substances that cause damage to DNA (Bonassi et al. 2008; Murgia et al. 2008). Due to the human lymphocytes being primary cells and having a low spontaneous rate of chromosomal damage they are preferred in *in vitro* studies (Phillips and Arlt 2009).

The findings of the study suggested that FCF could be genotoxic since it markedly enhanced the CA formation in human PBLs. The fact that the percentage of structural CAs significantly increased following exposure to high concentrations (1.00 and 2.00 µg/ml) of FCF supports the clastogenic action of this compound by causing DNA strand breaks. In addition, in cultures treated with FCF, a few polyploid cells were found as an indicator of the aneugenic effect. However, even though at a low frequency, it should not be overlooked that these numerical abnormalities contribute to the increased overall genotoxic effect caused by FCF. As can be seen in Table 1, when evaluated in terms of total CA per cell, the cultures exposed to 0.50 µg/ml of FCF were also found genotoxic. That is, as compared to the negative control, FCF was found to significantly induce the proportion of total CAs/cell at three concentrations (0.50, 1.00, and 2.00 µg/ml), thereby potentially genotoxic to human PBLs.

Contrary to the results of our study, it was reported that an oral LD_{50} dose of FCF 568 mg/kg in male mice and 421 mg/kg in female mice didn't show a mutagenic effect using a mouse bone marrow micronucleus assay (Lin et al. 2012). Bu et al. (2019) reported a study on the effect of FCF on ovarian function of Sprague-Dawley rats. In that chronic toxicity study, Sprague-Dawley rats were fed FCF at dosage levels of 0, 0.6, and 60 mg/kg

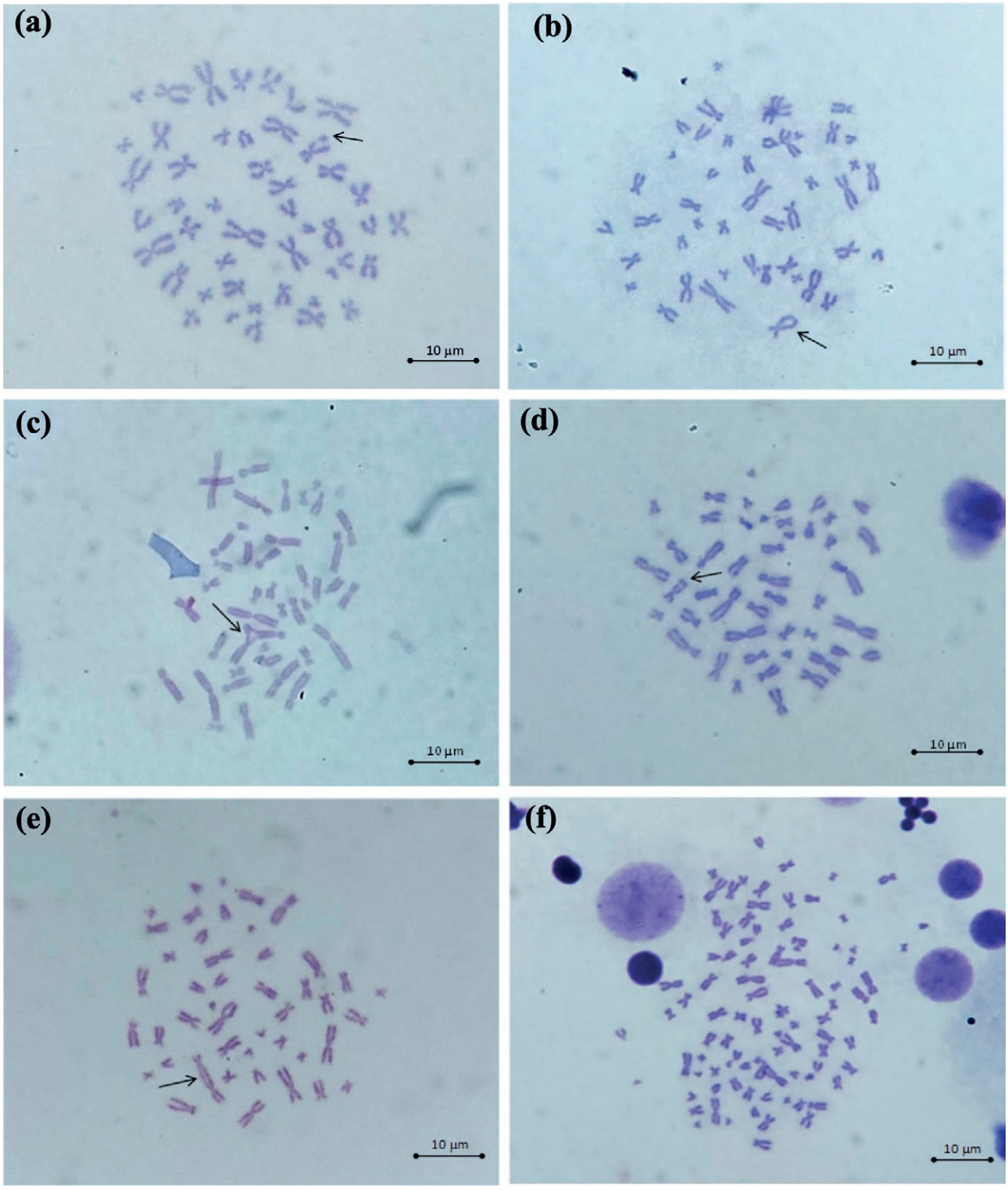


Figure 2. Different types of chromosomal aberrations induced by forchlorfenuron in human lymphocytes (a) chromatid break, (b) sister chromatid union, (c) chromatid exchange, (d) chromosome break, (e) dicentric chromosome., and (f) polyploid cell.

Table 1. Chromosome Aberrations in Human Peripheral Blood Lymphocytes Treated with Different Concentrations of Forchlorfenuron for 24 and 48 h.

Test substance	Treatment		Structural CAs						Numerical CA	Total CA	% cells with structural CAs±SD	Total CAs/ cell±SD
	Time (h)	Conc. (µg/mL)	B'	SU	CE	B"	F	DS	P			
Negative control	-	-	14	9	0	0	0	0	0	23	5.75±0.95	0.058±0.01
Mitomycin-C	24	0.20	53	6	7	14	0	0	0	80	19.50±2.64a_3	0.200±0.02a_3
Forchlorfenuron	24	0.25	20	9	0	0	0	1	1	31	7.25±1.50b_3	0.076±0.01b_3
		0.50	25	10	0	0	0	0	1	36	8.00±1.63b_3	0.090±0.01a_1b_3
		1.00	24	9	1	5	0	0	0	39	9.75±2.36a_1b_3	0.098±0.02a_1b_3
		2.00	25	16	0	2	0	0	1	44	10.25±2.50a_2b_3	0.110±0.05a_2b_3
Mitomycin-C	48	0.20	49	9	12	14	1	1	0	86	20.75±3.86a_3	0.220±0.03a_3
Forchlorfenuron	48	0.25	21	8	0	3	0	0	0	32	7.75±2.87b_3	0.080±0.02b_3
		0.50	24	12	0	0	0	0	1	37	9.00±2.44b_3	0.093±0.02a_1b_3
		1.00	29	11	0	2	0	0	1	43	10.00±2.16a_1b_3	0.108±0.01a_2b_3
		2.00	25	15	1	1	0	0	2	44	10.25±0.95a_1b_3	0.110± 0.01 a_2b_3
#Frequency of aberrations (%):			63.07	29.41	0.65	4.25	–	0.33	2.29			

All data are given as mean±SD; n=4.
In the CA test, 400 cells in total were scored in each concentration.
MMC: Mitomycin; B': Chromatid break; SU: Sister union; CE: Chromatid exchange; B": Chromosome break; F: Fragment; DS: Dicentric chromosome.
a, significant from negative control; b, significant from Mitomycin-C (positive control).
a_1b_1: $P<0.05$; a_2b_2: $P<0.01$; a_3b_3: $P<0.001$.
#: Data from the positive and negative controls were not taken into account when calculating the frequency of aberrations (%).

Table 2. Mitotic Index values in Human Peripheral Blood Lymphocytes Treated with Different Concentrations of Forchlorfenuron for 24 and 48 h.

Test Substance	Treatment		Total Counted Cells	Total Dividing cells	Mitotic Index±SD
	Time (h)	Conc. (µg/ml)			
Negative control	-	-	8000	470	5.88 ± 0.40
Mitomycin-C	24	0.20	8000	281	3.51 ± 0.57 a_3
Forchlorfenuron	24	0.25	8000	464	5.80 ± 0.16 b_3
		0.50	8000	383	4.79 ± 0.59 a_1b_1
		1.00	8000	296	3.70 ± 1.26 a_3
		2.00	8000	305	3.81 ± 0.82 a_3
Mitomycin-C	48	0.20	8000	271	3.39 ± 0.46 a_3
Forchlorfenuron	48	0.25	8000	336	4.20 ± 0.98 a_2
		0.50	8000	276	3.45 ± 0.92 a_3
		1.00	8000	258	3.23 ± 0.66 a_3
		2.00	8000	255	3.19 ± 1.14 a_3

a, significant from negative control; b, significant from Mitomycin-C (positive control).
a_1b_1: $P<0.05$; a_2b_2: $P<0.01$; a_3b_3: $P<0.001$.

b.w for 180 days. The researchers reported severe uterine hydrometra, pathological ovarian alterations, and possible negative effects of FCF on the ovaries and steroidogenesis in rats. Although no other research was found regarding the genotoxic potential of FCF, the genotoxic effects of various synthetic PGRs were described in earlier studies conducted on a variety of test systems, which are consistent with our findings (Kocaman and Bucak 2016; Kocaman and Güven 2016; Özkul et al. 2016; Kocaman and Kılıç 2017; Ozel et al. 2022).

As is well known, while FCF plays a role in cell growth and differentiation by promoting cell division in plants (Vardi-Oknin et al. 2013), it disrupts higher-order septin assembly in other eukaryotic organisms. It was reported that in mammalian cells, FCF specifically and directly changes septin assembly without influencing actin or tubulin polymerization (Hu et al. 2008). At the same time, considering that septin polymers are involved in the organization of various structures such as contractile rings, actin stress fibrils, and mitotic spindle in animals (Kinoshita 2006); in our study, it can be thought that FCF might have inhibited cytokinesis, especially by acting on septins, thus causing the formation of polyploid cells. However, in this study, it should be emphasized that FCF significantly induced structural CAs, especially chromatid breaks and sister chromatid unions indicating that it effectively acts as a clastogenic agent by breaking the phosphodiester backbone of DNA.

In this study, FCF generally showed a cytotoxic effect in human lymphocyte cultures by significantly decreasing the MI compared to the negative control. Zhang et al. (2015) reported that FCF exhibited a cytotoxic effect on Chinese hamster ovary cells, which is in line with the findings of our investigation. Additionally, it was revealed that exposure to FCF derivatives markedly decreased the proliferative capacity of human and mouse malignant mesothelioma cells and led to cytotoxicity (Blum et al. 2019). In recent years, FCF has been employed as an experimental tool for putative therapeutic applications in cancers since it disturbs the higher-order assembly of septins in non-plant eukaryotes (Blum et al. 2019). However, Ivanov et al. (2021) reported that FCF might not be convenient for therapeutic usage in its current form since only active at high concentrations. Animal tissue toxicity and potential off-target effects of FCF have also been documented (Heasley and McMurray 2016; Sun et al., 2020). It was therefore suggested by researchers that caution should be warranted when using FCF to study the biological functions of septins in cellular systems and model organisms (Sun et al. 2020).

The cytotoxic potential of FCF that was observed in this investigation may be due to its disrupting the septin organization in human lymphocyte cells. Hu et al. (2008) reported that FCF suppresses normal septin dynamics in mammalian cells and stabilizes septin polymers, causing alterations in cell shape, mitotic abnormalities, and a reduction in cell motility. Additionally, septins have been associated with the regulation of the cell cycle (Barral et al. 1999; Shulewitz et al. 1999), coordination of the response to DNA damage, and cellular shape (Lew 2003; Keaton and Lew 2006; Enserink et al. 2006; Smolka et al. 2006; Weirich et al. 2008). Blum et al. (2019) reported that the chloride group in the structure of FCF has an important role in binding to septins with a high affinity and thereby inhibiting cell proliferation. It can be thought that the formation of thick septin bundles by stabilizing the septins with the effect of FCF may lead the cell to apoptosis by disrupting the signal transmission traffic.

In conclusion, this study indicated for the first time that commercial formulation of FCF could have genotoxic and cytotoxic effects on human lymphocytes at concentrations ranging from 0.50 to 2.00 µg/ml. It is clear that ensuring the use of PGRs, containing FCF as an active ingredient, in appropriate concentrations in agricultural applications can reduce the risk of this probable toxic substance being released into the environment and the negative effects on human health through residues in vegetables and fruits. It should also be noted that it is important for people exposed to FCF during production or application to wear personal protective gear such as gloves, masks, face shields, and goggles. Finally, it may be said that this study is important in that it encourages further *in vivo* and *in vitro* genotoxicity studies by drawing attention to this widely used PGR.

ACKNOWLEDGMENTS

The authors would like to thank the blood donors who contributed to this study.

REFERENCES

Angelis D, Karasmanis EP, Bai X, Spiliotis ET. 2014. In silico docking of forchlorfenuron (FCF) to septins suggests that FCF interferes with GTP binding. PLoS One. vol. 9(5):e96390. https://doi.org/10.1371/journal.pone.0096390

Ayabaktı S, Kocaman, AY. 2020. Cytogenotoxic effects of venlafaxine hydrochloride on cultured human peripheral blood lymphocytes. Drug Chem Toxicol. 43(2):192–199. https://doi.org/10.1080/01480545.2018.1486410

Barral Y, Parra M, Bidlingmaier, S, Snyder M. 1999. Nim1-related kinases coordinate cell cycle progression with the organization of the peripheral cytoskeleton in yeast. Genes Dev. 13(2):176–187. https://doi.org/10.1101/gad.13.2.176

Blum W, Henzi T, Pecze L, Diep KL, Bochet CG, Schwaller B. 2019. The phytohormone forchlorfenuron decreases viability and proliferation of malignant mesothelioma cells in vitro and in vivo. Oncotarget. 10(65):6944–6956. https://doi.org/10.18632/oncotarget.27341

Bonassi S, Norppa H, Ceppi M, Strömberg U, Vermeulen R, Znaor A, Cebulska-Wasilewska A, Fabianova E, Fucic A, Gundy S, Hansteen IL, Knudsen LE, Lazutka J, Rossner P, Sram RJ, Boffetta P. 2008. Chromosomal aberration frequency in lymphocytes predicts the risk of cancer: Results from a pooled cohort study of 22 358 subjects in 11 countries. Carcinogenesis. 29(6):1178–1183. https://doi.org/10.1093/carcin/bgn075

Briggs D. 2003. Environmental pollution and the global burden of disease, Br Med Bull. 68:1–24. https://doi.org/10.1093/bmb/ldg019

Bu Q, Wang X, Xie H, Zhong K, Wu Y, Zhang J, Wang Z, Gao H, Huang Y. 2019. 180 Day Repeated-Dose Toxicity Study on Forchlorfenuron in Sprague–Daw-

ley Rats and Its Effects on the Production of Steroid Hormones. J Agric Food Chem. 67(36):10207–10213. https://doi.org/10.1021/acs.jafc.9b03855

EFSA (European Food Safety Authority). 2017. Conclusion on the peer review of the pesticide risk assessment of the active substance forchlorfenuron. EFSA Journal. 15:1–18. https://doi.org/10.2903/j.efsa.2017.4874

Evans HJ. 1984. Human peripheral blood lymphocytes for the analysis of chromosome aberrations in mutagen tests. In: Kilbey BJ, Legato M, Nichols W, Ramel C. Eds. Handbook of Mutagenicity Test Procedures. Amsterdam: Elsevier Science. p. 405–427.

Enserink JM, Smolka MB, Zhou H, Kolodner RD. 2006. Checkpoint proteins control morphogenetic events during DNA replication stress in *Saccharomyces cerevisiae*. J Cell Biol. 175(5):729–741. https://doi.org/10.1083/jcb.200605080

Gangadhar PL, Sandeep VH, Rajesh VB. 2020. Profile of plant growth regulator poisoning: A two-year study. Journal of the Indian Society of Toxicology. 16(2):19–22. DOI: 10.31736/2020v16i2/19-22.

Gong G, Kam H, Tse YC, Giesy JP, Seto SW, Lee SM. 2021. Forchlorfenuron (CPPU) causes disorganization of the cytoskeleton and dysfunction of human umbilical vein endothelial cells, and abnormal vascular development in zebrafish embryos. Environ Pollut. 271: 115791. https://doi.org/10.1016/j.envpol.2020.115791

Heasley LR, Garcia G 3rd, McMurray MA. 2014. Off-target effects of the septin drug forchlorfenuron on non-plant eukaryotes. Eukaryot Cell. 13(11):1411–1420. https://doi.org/10.1128/EC.00191-14

Heasley LR, McMurray MA. 2016. Small molecule perturbations of septins. Methods Cell Biol. 136:311–319. https://doi.org/10.1016/bs.mcb.2016.03.013

Henzi T, Lannes N, Filgueira L. 2021. Septins in Infections: Focus on Viruses. Pathogens. 10(3): 278. https://doi.org/10.3390/pathogens10030278

Hu Q, Nelson WJ, Spiliotis ET. 2008. Forchlorfenuron alters mammalian septin assembly, organization, and dynamics. J Biol Chem. 283(43):29563–29571. https://doi.org/10.1074/jbc.M804962200

Ivanov AI, Le HT, Naydenov NG, Rieder F. 2021. Novel Functions of the Septin Cytoskeleton: Shaping Up Tissue Inflammation and Fibrosis. Am J Pathol. 191(1):40–51. https://doi.org/10.1016/j.ajpath.2020.09.007

Iwase M, Okada S, Oguchi T, Toh-e A. 2004. Forchlorfenuron, a phenylurea cytokinin, disturbs septin organization in Saccharomyces cerevisiae. Genes Genet Syst. 79(4): 199–206. https://doi.org/10.1266/ggs.79.199

Keaton MA, Lew DJ. 2006. Eavesdropping on the cytoskeleton: progress and controversy in the yeast morphogenesis checkpoint. Curr Opin Microbiol. 9(6):540–546. https://doi.org/10.1016/j.mib.2006.10.004

Kim JG, Takami Y, Mizugami T, Beppu K, Fukuda T, Kataoka I. 2006. CPPU applicatio on size and quality of hardy kiwifruit. *Scientia Horticulturae*. 110(2):219–222. https://doi.org/10.1016/j.scienta.2006.06.017

Kinoshita M. 2006. Diversity of septin scaffolds. Curr Opin Cell Biol. 18(1):54–60. https://doi.org/10.1016/j.ceb.2005.12.005

Kocaman AY, Topaktaş M. 2007. In vitro evaluation of the genotoxicity of acetamiprid in human peripheral blood lymphocytes. Environ Mol Mutagen. 48(6):483–490. https://doi.org/10.1002/em.20309

Kocaman AY, Bucak S. 2016. Genotoxic and cytotoxic effects of flumetralin in human peripheral blood lymphocytes in vitro. Toxicol Ind Health. 32(12):1927–1934. https://doi.org/10.1177/0748233715595142

Kocaman AY, Güven B. 2016. In vitro genotoxicity assessment of the synthetic plant growth regulator, 1-naphthaleneacetamide. Cytotechnology. 68(4):947–956. https://doi.org/10.1007/s10616-015-9847-z

Kocaman AY, Kılıç, E. 2017. Evaluation of the genotoxicity of commercial formulations of ethephon and ethephon+cyclanilide on Allium cepa L. root meristematic cells. Caryologia. 70(3):229–237. https://doi.org/10.1080/00087114.2017.1329960

Kopecný D, Briozzo P, Popelková H, Sebela M, Koncitíková R, Spíchal L, Nisler J, Madzak C, Frébort I, Laloue M, Houba-Hérin N. 2010. Phenyl- and benzylurea cytokinins as competitive inhibitors of cytokinin oxidase/dehydrogenase: a structural study. Biochimie. 92(8):1052–1062. http://dx.doi.org/10.1016/j.biochi.2010.05.006.

Lew DJ. 2003. The morphogenesis checkpoint: how yeast cells watch their figures. Curr Opin Cell Biol. 15(6):648–653. https://doi.org/10.1016/j.ceb.2003.09.001

Lin LM, Huang DW, Guo JJ, Pan HY, Gong ZY. 2012. Study on toxicity test of forchlorfenuron in mice. Sci Technol Food Ind. 33:360–364. 2012年第14期360-362,共3页

Lu Y, Song S, Wang R, Liu Z, Meng J, Sweetman AJ, Jenkins A, Ferrier RC, Li H, Luo W, Wang T. 2015. Impacts of soil and water pollution on food safety and health risks in China. Environ Int. 77:5–15. https://doi.org/10.1016/j.envint.2014.12.010

Marcus EA, Tokhtaeva E, Turdikulova S, Capri J, Whitelegge JP, Scott DR, Sachs G, Berditchevski F, Vagin O. 2016. Septin oligomerization regulates persistent expression of ErbB2/HER2 in gastric cancer cells. Biochem J. 473(12):1703–1718. https://doi.org/10.1042/BCJ20160203

Meng X, Zhai Y, Yuan W, Lv Y, Lv Q, Bai H, Niu Z, Xu W, Ma Q. 2020. Ambient ionization coupled with

a miniature mass spectrometer for rapid identification of unauthorized adulterants in food. J Food Compos Anal. 85:103333. https://doi.org/10.1016/j.jfca.2019.103333

Mosesso P, Cinelli S, Natarajan AT, Palitti F. 2013. In Vitro Cytogenetic Assays: Chromosomal Aberrations and Micronucleus Tests. In: Dhawan A, Bajpayee M. (eds). Genotoxicity Assessment. Methods in Molecular Biology, Humana Press, Totowa, NJ. 1044, p. 123-146. https://doi.org/10.1007/978-1-62703-529-3_6

Murgia E, Ballardin M, Bonassi S, Rossi AM, Barale R. 2008. Validation of micronuclei frequency in peripheral blood lymphocytes as early cancer risk biomarker in a nested case-control study. Mutat Res. 639(1-2):27–34. https://doi.org/10.1016/j.mrfmmm.2007.10.010

Ozel CA, Unal F, Avuloglu-Yilmaz E, Erikel E, Mirici S, Yuzbasioglu D. 2022. Determination of genotoxic damages of picloram and dicamba with comet assay in Allium cepa rooted in tissue culture and distilled water. Mol Biol Rep. 49(12):11273-11280. https://doi.org/10.1007/s11033-022-07712-7

Özkul M, Özel ÇA, Yüzbaşıoğlu D, Ünal F. 2016. Does 2,4-dichlorophenoxyacetic acid (2,4-D) induce genotoxic effects in tissue cultured Allium roots? Cytotechnology. 68(6):2395–2405. https://doi.org/10.1007/s10616-016-9956-3

Phillips DH, Arlt VM. 2009. Genotoxicity: damage to DNA and its consequences. In: Luch A, Ed. Molecular, Clinical and Environmental Toxicology. Experientia Supplementum, 99, p. 87–110. Birkhäuser Basel. https://doi.org/10.1007/978-3-7643-8336-7_4

Rodrigues ET, Alpendurada MF, Ramos F, Pardal MÂ. 2018. Environmental and human health risk indicators for agricultural pesticides in estuaries. Ecotoxicol Environ Saf.150:224–231. https://doi.org/10.1016/j.ecoenv.2017.12.047

Sidhaye VK, Chau E, Breysse PN, King LS. 2011. Septin-2 mediates airway epithelial barrier function in physiologic and pathologic conditions. Am J Respir Cell Mol Biol. 45(1):120–126. https://doi.org/10.1165/rcmb.2010-0235OC

Smolka MB, Chen SH, Maddox PS, Enserink JM, Albuquerque CP, Wei XX, Desai A, Kolodner RD, Zhou H. 2006. An FHA domain-mediated protein interaction network of Rad53 reveals its role in polarized cell growth. J Cell Biol. 175(5):743–753. https://doi.org/10.1083/jcb.200605081

Sharma S, Quintana A, Findlay GM, Mettlen M, Baust B, Jain M, Nilsson R, Rao A, Hogan PG. 2013. An siRNA screen for NFAT activation identifies septins as coordinators of store-operated Ca2+ entry. Nature. 499(7457):238–242. https://doi.org/10.1038/nature12229

Shi X, Jin F, Huang Y, Du X, Li C, Wang M, Shao H, Jin M, Wang J. 2012. Simultaneous determination of five plant growth regulators in fruits by modified quick, easy, cheap, effective, rugged, and safe (QuEChERS) extraction and liquid chromatography-tandem mass spectrometry. J Agric Food Chem. 60(1):60–65. https://doi.org/10.1021/jf204183d

Shulewitz MJ, Inouye CJ, Thorner J. 1999. Hsl7 localizes to a septin ring and serves as an adapter in a regulatory pathway that relieves tyrosine phosphorylation of Cdc28 protein kinase in Saccharomyces cerevisiae. Mol Cell Biol. 19(10):7123–7137. https://doi.org/10.1128/MCB.19.10.7123

Su L, Wang M, Wang Y, Sharif R, Ren N, Qian C, Xu J, Chen X, Qi X. 2021. Forchlorfenuron Application Induced Parthenocarpic Fruit Formation without Affecting Fruit Quality of Cucumber. *Horticulturae*. 7(6):128. https://doi.org/10.3390/horticulturae7060128

Sun L, Cao X, Lechuga S, Feygin A, Naydenov NG, Ivanov AI. 2020. A Septin Cytoskeleton-Targeting Small Molecule, Forchlorfenuron, Inhibits Epithelial Migration via Septin-Independent Perturbation of Cellular Signaling. Cells. 9(1):84. https://doi.org/10.3390/cells9010084

Tixier C, Sancelme M, Bonnemoy F, Cuer A, Veschambre H. 2001. Degradation products of a phenylurea herbicide, diuron: synthesis, ecotoxicity, and biotransformation. Environ Toxicol Chem. 20(7):1381–1389. https://doi.org/10.1002/etc.5620200701

Tokhtaeva E, Capri J, Marcus EA, Whitelegge JP, Khuzakhmetova V, Bukharaeva E, Deiss-Yehiely N, Dada LA, Sachs G, Fernandez-Salas E, Vagin O. 2015. Septin dynamics are essential for exocytosis. J Biol Chem. 290(9):5280–5297. https://doi.org/10.1074/jbc.M114.616201

Toumi K, Joly L, Vleminckx C, Schiffers B. 2018. Assessment of Belgian florists exposure to pesticide residues. Comm Appl Biol Sci. Ghent University 83:355–368.

USEPA (U.S. Environmental Protection Agency). 2004. Pesticide Fact Sheet for new chemical: Forchlorfenuron; issued: September 2004. United States Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances (7501C). https://www3.epa.gov/pesticides/chem_search/reg_actions/registration/fs_PC-128819_01-Sep-04.pdf

Vardi-Oknin D, Golan M, Mabjeesh NJ. 2013. Forchlorfenuron disrupts SEPT9_i1 filaments and inhibits HIF-1. PLoS One. 8(8):e73179. https://doi.org/10.1371/journal.pone.0073179

Wang KS, Lu CY, Chang SH. 2011. Evaluation of acute toxicity and teratogenic effects of plant growth regulators by Daphnia magna embryo assay. J Hazard Mater. 190(1-3):520–528. https://doi.org/10.1016/j.jhazmat.2011.03.068

Wang Q, Yang Z. 2016.Industrial water pollution, water environment treatment, and health risks in China. Environ Pollut. 218:358-365. https://doi.org/10.1016/j.envpol.2016.07.011

Wang M, Yan H, Han Y, Qiao F. 2023. Miniaturized extraction and determination of swelling agents in fruits and vegetables based on deep eutectic solvent-molecularly imprinted hydrophilic resin. Food Chem. 398:133920. https://doi.org/10.1016/j.foodchem.2022.133920

Weirich CS, Erzberger JP, Barral Y. 2008. The septin family of GTPases: architecture and dynamics. Nat Rev Mol Cell Biol. 9(6):478–489. https://doi.org/10.1038/nrm2407

Zhang N, Liu L, Fan N, Zhang Q, Wang W, Zheng M, Ma L, Li Y, Shi L. 2016. The requirement of SEPT2 and SEPT7 for migration and invasion in human breast cancer via MEK/ERK activation. Oncotarget. 7(38):61587–61600. https://doi.org/10.18632/oncotarget.11402

Zhang W, Jiang F, Ou J. 2011. Global pesticide consumption and pollution: with China as a focus. Proc Int Acad Ecol Environ Sci. 1(2):125–144.

Zhang Z, Guo K, Bai Y, Dong J, Gao Z, Yuan Y, Wang Y, Liu L, Yue T. 2015. Identification, synthesis, and safety assessment of forchlorfenuron (1-(2-chloro-4-pyridyl)-3-phenylurea) and its metabolites in kiwifruits. J Agric Food Chem. 63(11):3059–3066. https://doi.org/10.1021/acs.jafc.5b01100

Caryologia
International Journal of Cytology, Cytosystematics and Cytogenetics

Firenze University Press
www.fupress.com/caryologia

OPEN ACCESS

Citation: Sazada Siddiqui (2023). Phenthoate toxicity evaluation in root meristem of *Pisum sativum* L. *Caryologia* 76(1): 57-66. doi: 10.36253/caryologia-2036

Received: February 5, 2023

Accepted: May 19, 2023

Published: September, 19, 2023

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

ORCID
SS: 0000-0001-5448-7617

Phenthoate toxicity evaluation in root meristem of *Pisum sativum* L.

Sazada Siddiqui

Department of Biology, College of Science, King Khalid University, Abha 61413, Saudi Arabia
E-mail: sasdeky@kku.edu.sa; kalasaz@yahoo.co.in

Abstract. Phenthoate is an organothiophosphate insecticide. Effect of phenthoate on the cytogenetic alterations in root tip cells of *Pisum sativum* L., a multiuse crop was investigated in this study. *Pisum sativum* L. seeds were exposed to different concentrations of phenthoate (0.1, 0.2, 0.3, 0.4, and 0.5%) and were germinated at 24°C for 72 hours and cytogenetic alterations were assessed. Analysis of mitotic index revealed that phenthoate has cytotoxic attributes, and cell proliferation kinetics frequencies showed alterations in the kinetics of the mitotic process. Phenthoate treatment of 0.1% to 0.5% resulted in an increase in the metaphases, and a reduction in prophases, anaphases, and telophases ratio, dose dependently. The findings of the study reveal that, phenthoate reduced the percentage of seed germination, mitotic index, radicle length and increased chromosomal abnormalities dose dependently. Root tip cells of *Pisum sativum* L. seeds treated with phenthoate showed an increased occurrence of single and double bridges, fragments, stickiness, laggard, and vagrants.

Keywords: phenthoate, seed germination, radicle length, Mitotic Index, genotoxicity, cell proliferation kinetics, *Pisum sativum* L.

INTRODUCTION

Insecticides are used to kill harmful pests, precisely the insects which are most frequent pests of economically significant plants. All chemicals used in controlling plant or animal pests like fungicides, insecticides, rodenticides, and weedicides (herbicides) comes under the term pesticide. Pesticides are chemicals which are used to inhibit the reproduction of pests or to kill them. They are designed to eliminate undesirable organisms while protecting those that are required, for example, a weed killer or weedicide will eliminate weeds while protecting the crops that is grown for food. Sumitomo Corporation developed phenthoate, a broad-spectrum chiral organophosphate, sold widely in 1972, and used as racemate. Due to its acute toxicity to humans and non-target creatures (bees), crops (celery and eggplant), as well as its residue in environment (soil and water), this pesticide is of specific concern (Esturk et al. 2014; Nara et al. 2018). Phenthoate is phytotoxic to some plants. It has fast knockdown action and penetration. Phenthoate, an organophosphorus compound, is used generally because of its efficiency, high

solubility in water, and quick biodegradation (Nelson et al.1990). Nevertheless, a lot of these compounds and the byproducts of their breakdown are DNA alkylating agents (Bedford and Robinson 1972). Therefore, it is essential to look for any prospective genetic harm caused by minimal exposure with organophosphorus pesticides (Degraeve et al. 1984).

Since pesticides can lower agricultural products loss, and increase affordable production and food quality, they play a significant role in agriculture (Aktar et al. 2009; Strassemeyer et al. 2017; Taufeeq et al. 2021). Pesticides use increased in World War II (1939–45) due to the pressing need to improve and increase production of food and regulate insect-borne illnesses. After 1940, increased usage of synthetic chemicals for protection of crops allowed for an even greater surge in food production (Carvalho 2017). Furthermore, global production of pesticides increased annually at 11 percent from 0.0002 billion tons in 1950 to greater than 0.005 billion tons by 2000 (Chang et al. 2017). Only 1% of pesticides were utilized to efficiently protect target plants from insect pests, even though 3 billion kgs of pesticides are consumed annually worldwide (Carvalho 2017) and hence huge amounts of remaining pesticides continue to enter or affect environment and non-target plants. Pesticide contamination as a result has greatly damaged the ecosystem and had a negative effect on the health of human beings (Hernández 2013; Tudi et al. 2022; Abdel-Halim et al. 2020). Equipment required for applying pesticides effectively is essential (Lozier et al. 2013) to reduce loss of spraying solution, eradicate residual pesticides in environment and avert detrimental effects on the health of human beings from residues and over spraying. In addition to indirect exposure from food, air, soil, and water adulterated with pesticides (Kim et al. 2017; Tudi et al. 2022), humans are also directly exposed at workplace to pesticides (Macfarlane et al. 2013). The most common routes for pesticides to enter a human's body are through their skin, oral, and respiratory system (Damalas et al. 2011; Anderson et al. 2014). Globally, 3 million people gets poisoned by pesticides and 200,000 of them die, as per UNEP and WHO report (Yadav et al. 2015).

Although certain pesticides are developed to attack a specific set of targets, their toxic components will strike the entire organism (Castellanos et al. 2022). As per a study, methomyl induces genotoxicity in fishes (Afaf et al. 2022). Aquatic organisms like water spinach, coastal creatures, fishes, and Danio rerio have demonstrated methomyl toxicity (Jablonski et al. 2022; Camilo-Cotrim et al. 2022). DNA damage is a primary biotic phenomenon that can harm biotic assemblies and procedures, as well as induce genotoxic disorders linked with the growth of carcinogenic developments (Acar et al. 2022; Siddiqui and Sulaiman 2022 a and b; El-Houseiny et al. 2022). According to a recent study (Pesavento et al. 2018; Velazquez et al. 2022; Liman et al. 2022), several causes, together with DNA damage instigated by pesticides, stimulate carcinogenic growth in a wide array of species. For economic reasons, *P. sativum* (Fabaceae) is a widely consumed legume in diet for protein source. Till now limited studies are conducted on the impact of phenthoate on pea plant despite it being a multiuse crop (Sandhu et al. 1987; Somaiah et al. 2014; Dong et al. 2022). In this study, an effort was undertaken to evaluate the noxious effects of phenthoate on Pea plant.

METHODOLOGY

Procuring seeds and chemicals

Phenthoate was purchased from Sigma Chemicals Ltd., United States (CAS No. 16752-77-5). *P. sativum* L. (pea) seeds were bought from a registered trader in Abha, Saudi Arabia.

Exposure settings

Pisum sativum L. seeds of uniform size were picked, pre-soaked in distilled water for 12 h and divided into several groups of 30 seeds each. Seeds were then soaked in 250 mL solutions of phenthoate for 1 hour to expose them to different concentrations (0.1, 0.2, 0.3, 0.4, and 0.5%) of phenthoate. Seeds were soaked in double distilled water in control. The containers were repetitively shaken during the treatment phase to provide seeds with air. After treatment, to remove any traces of adhering phenthoate, seeds were carefully rinsed with double distilled water and kept in Petri dishes on moistened Whatman Filter Paper. The Petri dishes were maintained at 25±2°C in dark for the next 72 hours in a plant growth cabinet. By inspecting the formation of the radicle, it was possible to estimate the seed germination time. A millimeter ruler was used to measure the radicle length of germinated seed every 24 hours till 72 hours. Newly growing roots between one to two centimeters in length were used in the experiment. The complete experiment was done thrice with the same settings.

Analyzing genotoxicity and kinetics of mitosis

In the morning from 8 to 10 am, 1 to 2 cm newly grown roots were taken, submerged in a fixation solution

(ethanol: glacial acetic acid, 3:1) for 24 hours, moved to 70% ethanol and kept at 5°C till microscopic inspection. Ten roots were hydrolyzed in 1N HCl solution for ten minutes for every sample and root tips were dyed with 2% acetocarmine for ten minutes for the preparation of each slide. From root tips, chromosome preparations were done as in Qian et al. (1998) with slight alterations (described in Siddiqui and Suleiman 2022 b). 1000 cells from all samples including control were examined to determine the mitotic index. Cell proliferation kinetics frequencies were analyzed using the number of cells in every division phase to total number of mitotic cells. In a light microscope (100 x) in oil immersion, all the mitotic cells were examined. All the slides were investigated blind and coded.

Cytogenetic analysis

Cytogenetic evaluation was done on root tips of germinated seeds exposed to various concentrations of phenthoate. Chromosome preparations were done from root tips by applying the technique described by Qian (1998) having slight modifications. Root tips were cut, fixed for 24 hours in Carnoy's fixative (anhydrous alcohol:glacial acetic acid, 3:1), passed to 70% alcohol, and then kept in the fridge until needed. For 1 hour, 2% acetocarmine solution was used to stain the root tips after they had been hydrolyzed in 5 N HCl at room temperature for 20 minutes. Chromosome spreads were made by squash technique as described by Savaskan and Toker (1991). All slides were coded and observed blind. For the purpose of analyzing the mitotic index and expressing the results in percentage, 500 cells from every preparation were scored. At least 100 metaphase- anaphase plates were used to study several kinds of chromosomal anomalies like fragments, single bridges, double bridges, stickiness, vagrants and laggards.

Statistical analysis

To determine the significance of differences amongst variables, a one-way ANOVA test was performed using the GPIS 1.13 program (GRAPHPAD, California, USA). All outcomes were reported as mean ± standard error.

RESULT

Effect of phenthoate on seed germination

In the control, after 1 hour and 3 hours, 77.40% of seed germinated at 24 hours, 85.54% at 48 hours and 96.11% at 72 hours (Figure 1 A and B). As compared to control, at 24 hours treatment of *P. sativum* seeds with phenthoate concentrations ranging from 0.1 to 0.5% for 1 hour and 3 hours caused very significant inhibition of seed germination rate (SG) ($p<0.01$). Analogous trend in seed germination was recorded at 48 and 72 hours. After 1 hour and 3 hours of phenthoate treatment, the highest seed germination rate was recorded at 0.1% concentration at 24 hours (74.76%), (70.14%); at 48 hours (81.43%), (80.99%); and 72 hours (90.12%), (89.44%) and minimal seed germination was found at a concentration of 0.5%

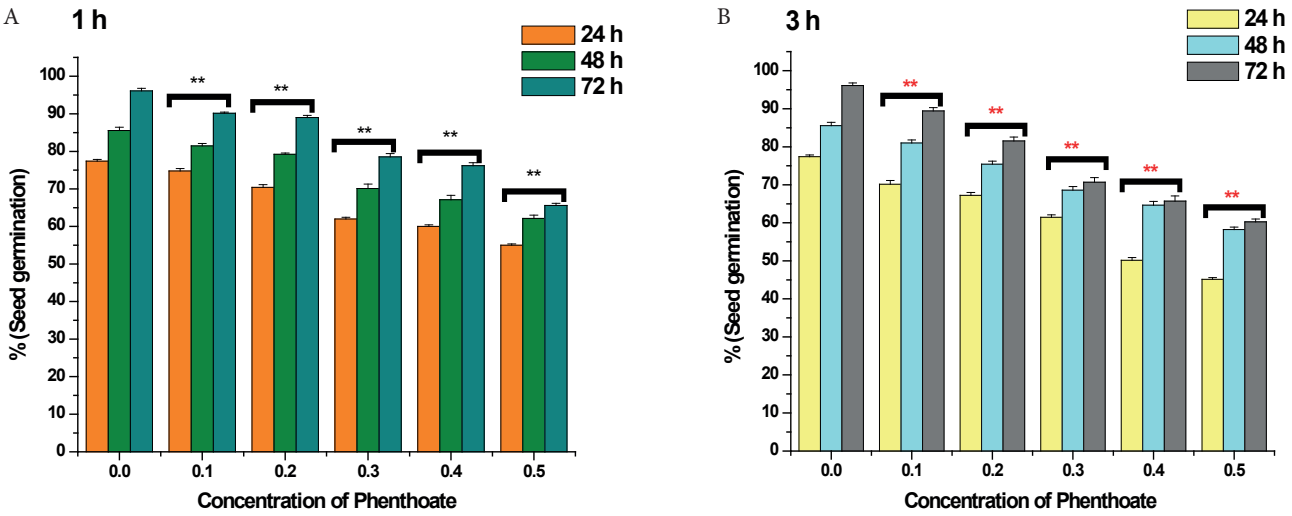


Figure 1. Effect of phenthoate on seed germination of *P. sativum* L. for 1 and 3 h. **p<0.01 compared to control group. Data are mean of three replicates ±SE, 0.0 = Control group.

Table 1. Effect of phenthoate on radicle length in *P. sativum* L for 1 h and 3 h.

Radicle length (cm)			
Conc. (%)	24 h	48 h	72 h
0.0	0.85 ± 0.03	1.67±0.09	2.20±0.09
1 h			
0.1	0.76 ± 0.02¥	1.25±0.07¥	1.98±0.07¥
0.2	0.54±0.02¥	0.98 ±0.02¥	1.69±0.07¥
0.3	0.51±0.04¥	0.78±0.04¥	1.32±0.06¥
0.4	0.46±0.01¥	0.84±0.05¥	1.10±0.12¥
0.5	0.39±0.02¥	0.50±0.04¥	0.64±0.02¥
3 h			
0.1	0.71±0.04$	1.26±0.07¥	1.95±0.05
0.2	0.62±0.03¥	1.12 ±0.14¥	1.50±0.06¥
0.3	0.54 ±0.05¥	0.65±0.04¥	1.32±0.05¥
0.4	0.46±0.06¥	0.58±0.05¥	0.98 ±0.12¥
0.5	0.35±0.03¥	0.53±0.04¥	0.78±0.05¥

$p<0.05; ¥p<0.01; compared to control group. Data are mean of three replicates ±SE, 0.0 = Control group, Conc. = concentration.

Table 2. Effect of phenthoate on cell proliferation kinetics in *P. sativum* L for 1 h and 3 h.

Conc. (%)	Prophases	Metaphase	Anaphases	Telophases
0.0	60.50±4.1	19.81±2.3	18.12±3.2	24.44±2.3
1 h				
0.1	52.00±3.6¥	22.51±1.5	16.23±2.1	21.56±3.0
0.2	50.70±2.2¥	24.31±3.2¥	14.11±1.4¥	19.34±1.3¥
0.3	48.20±2.0¥	26.23±3.5β	13.15± 1.6β	17.23±3.1¥
0.4	45.12±1.5¥	27.91±3.1β	12.45±1.4β	15.21±2.3¥
0.5	39.75±2.6¥	29.11±1.4β	00.23±1.3¥	13.43±3.2¥
3 h				
0.1	54.34±3.4¥	24.78±3.3¥	15.47±2.1	20.52±1.3$
0.2	52.12±2.7¥	27.34±4.5¥	12.28 ±3.2¥	18.22 ±2.7¥
0.3	50.43±3.2¥	29.33±3.5¥	10.78±2.2¥	16.32±3.2¥
0.4	43.42±1.2¥	25.91±2.2¥	9.12±2.15¥	13.42±3.4¥
0.5	35.75±2.8 ¥	23.12±2.2	8.23±2.20¥	10.57±3.5¥

$p<0.05; ¥p<0.01; βp<0.001 compared to control group. Data are mean of three replicates ±SE, 0.0 = Control group, Conc. = concentration.

at 24 hours (55%) (45.15%); 48 hours (62.15%), (58.22%); and 72 hours (65.6%), (60.27%) respectively in comparison to control (Figure 1A and B).

Effect of phenthoate on radicle length

The radicle length in the control group increased with time after treatment with double-distilled water for 1 h and 3 h: (0.85±0.03) at 24 h, (1.67±0.09) at 48 h, and (2.2±0.09) at 72 h (Table 1). In comparison to control, 0.1 to 0.5% phenthoate treatment for 1 hour and 3 hours caused a very significant reduction in radical length ($p<0.01$) and an analogous trend in the pattern of radical length was observed at 48 hours and 72 hours. Highest radicle length was found after treatment with phenthoate for 1 hour and 3 hours at 0.1% concentration at 24 hours (0.76±0.02), (0.71±0.04); 48 hours (1.25±0.07), (1.26±0.07); and 72 hours (1.98±0.07), (1.95±0.05) and the smallest radicle length was recorded at 0.5% concentration at 24 h (0.39±0.02), (0.35±0.03); 48 h (0.50±0.04), (0.48±0.04); and 72 h (0.64±0.02) (0.78±0.05) respectively, in comparison to control (Table 1).

Effect of phenthoate on cell proliferation kinetics

Cell proliferation kinetics, measured as the ratio of prophases, metaphases, anaphases, and telophases, demonstrated an increase in metaphase from 0.1 to 0.5% and a decrease in prophase, anaphase, and telophase as compared to the control (Table 2). At 1 hour, very significant reduction ($p<0.01$) was observed in prophase (0.1 to 0.5%); anaphase at 0.2% (14.11±1.4) and in telophase from 0.2 to 0.5%. Highly significant decrease ($p<0.001$) was reported in anaphase at 0.3% (13.15±1.6), 0.4% (12.45 ±1.4) and 0.5% (10.23±1.3). A very significant increase ($p<0.01$) was observed in metaphase at 0.2% (24.31±3.2) and highly significant increase ($p<0.001$) was reported at 0.3 to 0.5% in comparison to control. In case of 3 hours, very significant reduction ($p<0.01$) was observed in prophase; anaphase and telophase from 0.1 to 0.5% concentration and very significant increase ($p<0.01$) in metaphase was observed from 0.1% to 0.4% concentration in comparison to control (Table 2).

Effect of phenthoate on mitotic index

Figure 2 (A and B) depicts the effect of phenthoate on mitotic index of root tip cells in *P. sativum*. In the control, the mitotic index in case of seeds which were treated with double distilled water for 1 hour and 3 hours was 65.90%. Mitotic index decreased very significantly ($p<0.01$) in 0.1% to 0.5% phenthoate treated seeds for 1 hour and 3 hours, in a dose dependently as compared to control. Maximum mitotic index was reported at 0.1% for 1 hour and 3 hours (57.53%) (60.22%) and minimum mitotic index was reported at 0.5% (39.87%) (32%) respectively.

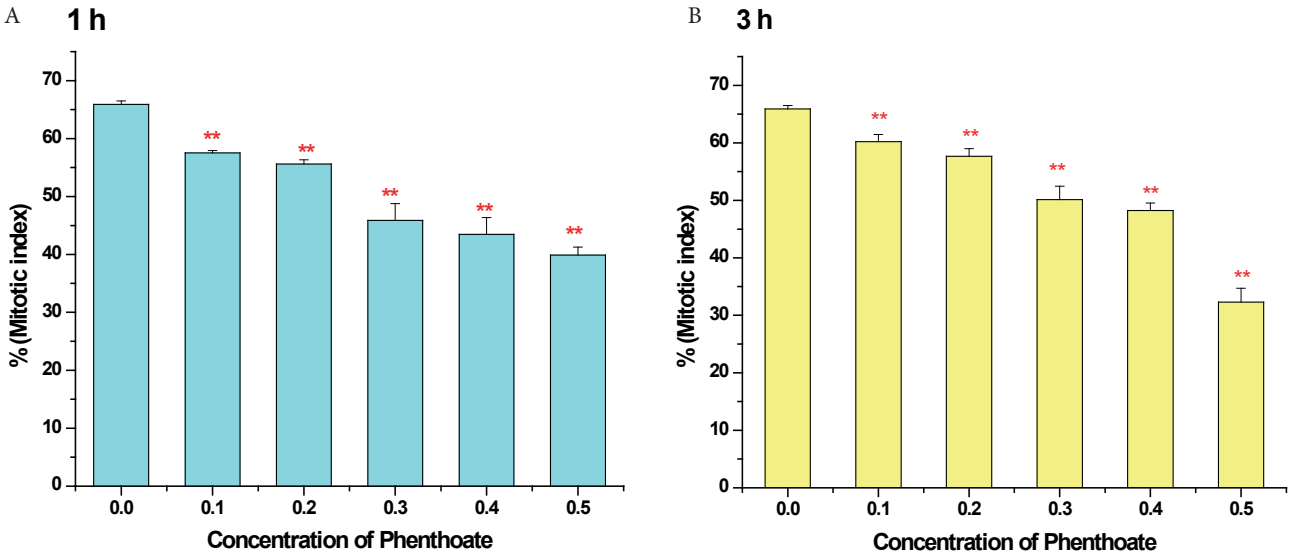


Figure 2. Effect of phenthoate on mitotic index of *P. sativum* L. for 1 h and 3 h. **p<0.01 compared to control group. Data are mean of three replicates ±SE, 0.0 = Control group.

Effect of phenthoate on chromosomal anomalies

In the control after 1 hour and 3 hours, the occurrence of chromosomal anomalies like fragment, single and double bridge, stickiness, vagrant and laggard in metaphase-anaphase plates was zero. Phenthoate treatment for 1 hour and 3 hours resulted in a dose dependent increase in chromosomal anomalies percentage like fragments, single and double bridges, stickiness, vagrants, and laggards, in metaphase-anaphase plates (Table 3, Figure 3). The treatment of seeds with 0.1% phenthoate for 1 and 3 hours caused 0% chromosomal anomalies like fragment, single and double bridge, stickiness, and vagrants. Laggards were found at 1 h (0.15%) and at 3 hours (0.3%).

Seeds treated with 0.2% phenthoate for 1 hour and 3 hours showed 0% chromosomal anomalies like fragment, stickiness, and vagrant for 1 hour and stickiness for 3 hours. However, single bridges (0.38%); double bridges (0.34%); and laggard (0.26%) were reported for 1 h at 0.2 % which were very significant (p<0.01) and fragments (0.12%); single bridges (0.42%); double bridges (0.55%); vagrant (0.25%) and laggards (0.29%) were

Table 3. Effect of phenthoate on chromosomal aberrations in *P. sativum* L root tip cells for 1 h and 3 h.

Conc. (%)	Fragment	Single bridge	Double Bridge	Stickiness	Vagrant	Laggard
0.0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
1 h						
0.1	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.15±0.003¥
0.2	0.00±0.00	0.38±0.01¥	0.34±0.01¥	0.00±0.00	0.00±0.00	0.26±0.09¥
0.3	0.65±0.06¥	0.55±0.05¥	0.96 ± 0.05¥	0.25±0.03¥	0.35±0.03 ¥	0.46±0.03¥
0.4	0.97±0.05¥	0.76±0.02¥	1.66±0.63¥	0.98±0.10¥	0.68±0.05¥	0.66±0.05¥
0.5	1.24±0.20¥	0.98±0.10¥	1.50±0.99¥	0.97±0.23¥	0.91±0.23¥	0.86±0.11¥
3 h						
0.1	0.00±0.00	0.00 ± 0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.30±0.003¥
0.2	0.12±0.001	0.42±0.02¥	0.55±0.21¥	0.00±0.00	0.25±0.01	0.29±0.070¥
0.3	0.78±0.03¥	0.76±0.05¥	1.55±0.73¥	0.73±0.13¥	0.68±0.06¥	0.68±0.10¥
0.4	1.01±0.21¥	1.25±0.34¥	2.53±0.81¥	1.98±0.17¥	0.75±0.12¥	0.75±0.05¥
0.5	1.78±0.35¥	2.79±0.43¥	2.79±0.72¥	1.97±0.45¥	1.98 ±0.34¥	0.97±0.22¥

¥p<0.01 compared to control group. Data are mean of three replicates ±SE, 0.0 = Control group, Conc. = concentration.

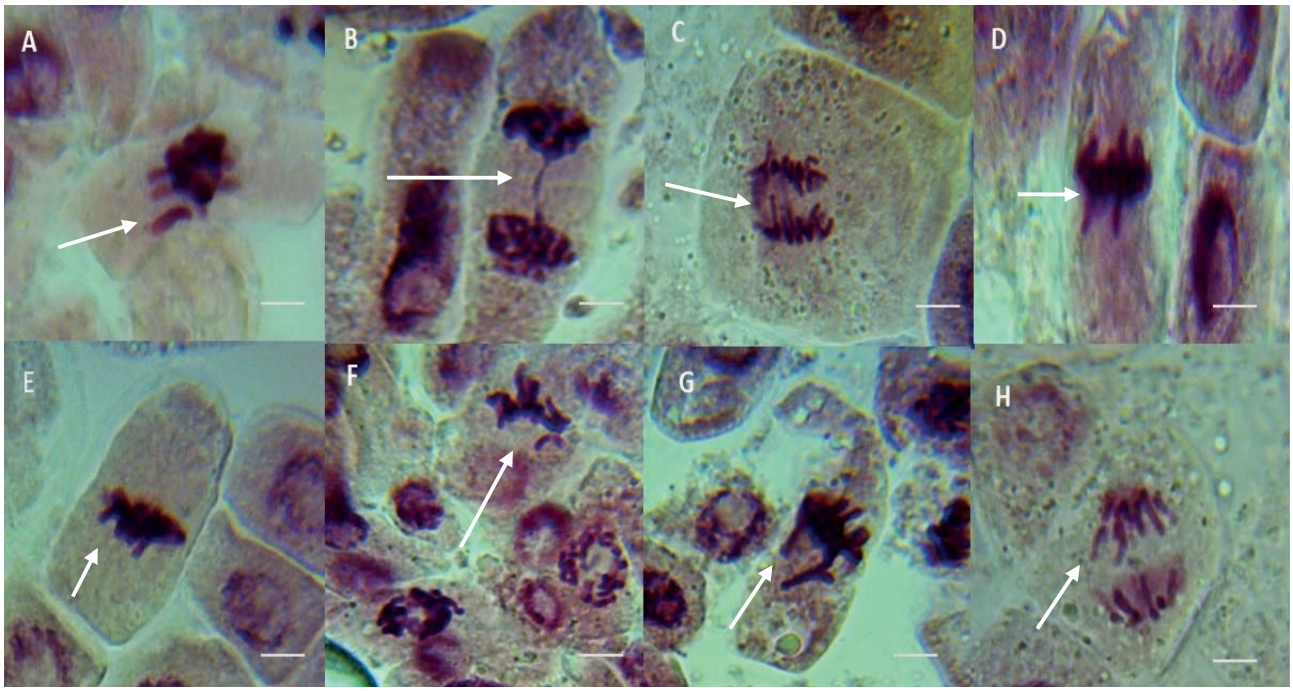


Figure 3. Chromosomal aberrations in phenthoate treated *P. sativum* L. root tip cells for 1 h and 3 h. A- Fragment. B- Single bridge., C- Double bridge., D-E- Stickiness., F-G-Vagrant., H- Laggard. Bar – 10 µm.

reported at 0.2 % for 3 h when compared to control. Seeds treated with 0.3% phenthoate for 1 hour and 3 hours caused very significant increase ($p<0.01$) in occurrence of chromosomal anomalies like fragments, single and double bridges, stickiness, vagrants, and laggards for 1 hour and fragments, single bridges, vagrants, and laggards for 3 hours as compared to control. Seeds treated with 0.4 to 0.5 % phenthoate for 1 and 3 hours caused very significant increase ($p<0.01$) in occurrence of chromosomal anomalies like fragments, single and double bridges, stickiness, vagrants, and laggards for 1 h and 3 h as compared to control.

DISCUSSION

The outcomes of the present study reveal that exposing seeds to increasing concentrations of phenthoate delays seed germination in *P. sativum*. The possibility of seeds to germinate is highly sensitive to environmental influences. Studies revealed that pesticide exposure considerably lowers seed germination rate (Siddiqui et al. 2008; Mahapatra et al. 2019; Bano et al. 2022). Endosulfan and Kitazin at higher doses inhibited seed germination in brinjal (*Solanum melongena* L.) (Sammaiah et al. 2011), *Capsicum annuum*, *Solanum lycopersicum, Solanum melongena, P. sativum, Zea mays, Brassica nigra* and *Typha latifolia* (Khan et al. 2021; Das et al. 2021). Pesticides in soil can prevent plant roots from absorbing essential nutrients, causing nutrient deficiency and retardation in growth (Sharma et al. 2019). Radicle lengthening is related to multiplication of cells. Nevertheless, phenthoate inhibited cell proliferation in this research study, as indicated by the mitotic index results. It could be due to variations in expression of certain genes that regulate the cell cycle. Methomyl and imbraclaobrid have previously been shown to be mutagenic in *P. sativum* and *Allium cepa* (Ozel et al. 2022; Sengupta et al. 2022; Siddiqui and Alrumman 2022 a and b). In our analysis, we found that phenthoate had an analogous effect on seeds of *P. sativum*. Significant inhibitory effect is shown by phenthoate on mitosis in *P. sativum* root tips, which could be attributed to its repressive action on spindle fibers (Barbara et al. 1991), DNA, RNA, and protein synthesis (Ogut et al. 2019; Kalefetoglu et al. 2021; Gogoi et al. 2021). Glyphosate halts cell cycle at G2-M stage by inhibiting activation of CDK1/cyclin (Marc et al. 2002; Das et al. 2021). Similar effects of organophosphates have been indicated by previous research on biological organisms (Ismail et al. 2009; Abdelsalam et al. 2022).

According to the results of the proportions of distribution of precise mitotic phases, phenthoate decreased the percentages of anaphase, prophase and telophase and augmented the metaphase percentage at all concen-

trations dose-dependently. These findings are consistent with the results of (Liman et al. 2010; Priya et al. 2014; Ozkul et al. 2016). Moreover, telophase percentage decreased as compared to the control. This suggests that decrease in telophase and hence mitotic index, could be caused by the arrest of one or more mitotic phases, or by a slowdown in the pace of cell development in mitosis (Ping et al. 2012).

Cytological anomalies in plants can be utilized to detect environmental pollutants that pose serious genetic concerns. Several types of chromosomal anomalies were observed in *P. sativum* after treatment with phenthoate comprising of single and double bridges, fragments, stickiness, laggards, and vagrants. The results prove that these substances, as previously described by other researchers (Siddiqui et al. 2012; Siddiqui and Al-Rumman 2020 a and b; Rahman et al. 2022; Siddiqui and Al-Rumman 2022 c), could produce mitotic anomalies. These pesticides have been linked to chromosomal abnormalities by blocking spindle proteins and inducing exchange of sister chromatids (Lukaszewicz et al. 2019; Khan et al. 2021; Siddiqui et al. 2021). Genetic instability is caused by free radicles in cells. Reactive oxygen is extremely unstable, causing cytoskeleton disruption, imbalance in energy metabolism, and DNA harm, ensuing chromosomal anomalies (Acar et al. 2021; Sengupta et al. 2022). DNA damage is a primary biotic incidence which can harm biotic assemblies and procedures, as well as induce genotoxic disorders linked with the growth of carcinogenic developments (Kaur et al. 2022; Ajermoun et al. 2022; Zhang et al. 2022). According to a recent study (Pesavento et al. 2018), several causes, together with DNA damage instigated by pesticides, stimulate carcinogenic growth in a wide array of species. The genotoxic influence of phenthoate observed in this research work might have been partly induced by the oxidative stress instigated by these substances. Various investigations have shown that these compounds alter redox status in plant cells, lending credence to this theory (Bonciu et al. 2018; Acar 2021; Acar et al. 2022). Phenthoate demonstrated a strong genotoxic effect on *P. sativum* plant in experimental conditions used in this investigation. Additional research on quality of crops derived from plants treated with phenthoate is required in relation to disease vulnerability, dietary value, and vulnerability to acclimatized stress.

CONCLUSION

The outcomes of the present study indicate that insecticides can be genotoxic to nontarget species like plants. Higher concentrations of phenthoate demonstrated detrimental effects on germination of seeds, radical length, frequency of cell kinetics, mitotic index, and chromosomal anomalies in *P. sativum* plant. Farmers are normally guided by dealers to use insecticides at twice the permitted level, which might have unfavorable cytogenetic consequences and limit plant growth. Hence, exceeding the recommended amount of insecticides should be prevented. Farmers and insecticide vendors should be educated regarding proper and optimum use of insecticides. The effect of insecticides on non-target host plants must be investigated further at the level of gene expression to identify the mechanism through which they cause harm to non-target plants.

FUNDING

We are grateful to King Abdul Aziz City for Science and Technology (KACST), Riyadh, Saudi Arabia for funding this project under grant number: 13-AGR2119-07.

ACKNOWLEDGMENTS

We are grateful to King Abdul Aziz City for Science and Technology (KACST), Riyadh, Saudi Arabia for funding this project under grant number: 13-AGR2119-07. We also express our gratitude to King Khalid University, Saudi Arabia for providing administrative and technical support.

REFERENCES

Abdel-Halim KY, Osman SR. 2020. Cytotoxicity and oxidative stress responses of imidacloprid and glyphosate in human prostate epithelial wpm-y. 1 cell line. J. Toxicol. 4364650.

Abdelsalam NR, Abdel-Megeed A, Ghareeb RY, Ali HM, Salem MZ, Akrami M, Al-Hayalif, MF, Desoky ESM. 2022. Genotoxicity assessment of amino zinc nanoparticles in wheat (*Triticum aestivum* L.) as cytogenetical perspective. Saudi J. Biol. Sci. 29: 2306–2313.

Acar A, Singh D, Srivastava AK. 2022. Assessment of the ameliorative effect of curcumin on pendimethalin-induced genetic and biochemical toxicity. Sci. Rep. 12: 1–16.

Acar A. 2021. In vivo toxicological assessment of diquat dibromide: cytotoxic, genotoxic, and biochemical approach. Environ. Sci and Pollut Res. 28(34): 47550–61.

Afaf N, Abdel R, Amany AR, Mohamed ND, Mohamed FM, Farag LS, Alqahtani MA, Nassan Saed AAl Thobaiti, Nesma IEl-Naseery. 2022. Appraisal of sub-chronic exposure to lambada-cyhalothrin and/or methomyl on the behavior and hepato-renal functioning in *Oreochromis niloticus*: Supportive role of taurine-supplemented feed. Aquat. Toxicol. 250:106257.

Ajermoun N, Aghris S, Ettadili F, Alaoui OT, Laghrib F, Farahi A, Lahrich S, Bakasse M, Saqrane S, El Mhammedi MA. 2022. Phytotoxic effect of the insecticide imidacloprid in *Phaseolus vulgaris* L. plant and evaluation of its bioaccumulation and translocation by electrochemical methods. Environ. Res. 214: 113794.

Aktar MW, Sengupta D, Chowdhury A. 2009. Impact of pesticides use in agriculture: Their benefits and hazards. Interdiscip. Toxicol. 2: 1–12.

Anderson SE, Meade BJ. 2014. Potential health effects associated with dermal exposure to occupational chemicals. Environ. Health Insights. 8: EHI-S15258

Bano A, Gupta A, Rai S, Fatima T, Sharma S, Pathak N. 2022. Mechanistic role of reactive oxygen species and its regulation via the antioxidant system under environmental stress. In Plant Stress Physiology—Perspectives in Agriculture; IntechOpen: London,UK, 2022.

Barbara LA, William TM, Bugg MW. 1991. Effects of herbicide dithiopyr on cell division in wheat root tips. Pest. Biochem. Physiol. 39:110–120.

Bedford C. Robinson J. 1972. The alkylating properties of organophosphates. Xenobiotica. 4: 307–337

Bonciu E, Firbas P, Fontanetti CS, Wusheng J, Karaismailoglu MC, Donghua Liu, Menicucci F, Pesnya DS, Popescu A, Romanovsky AV, Schiff S, Ślusarczyk J, de Souza CP, Srivastava A, Sutan A, Papini A. 2018. An evaluation for the standardization of the *Allium cepa* test as cytotoxicity and genotoxicity assay. Caryologia, 71(3): 191–209.

Camilo-Cotrim CF, Bailao EFLC, Ondei LS, Carneiro MF, Almeida LM. 2022. What can the *Allium cepa* test say about pesticide safety? A review. Environ. Sci. Pollut. Res. 29: 48088–48104.

Carvalho FP. 2017. Pesticides, environment, and food safety. Food Energy Secur. 6: 48–60.

Castellanos NL, Smagghe G, Taning CNT, Oliveira EE and Christiaens O. 2022. Risk assessment of RNAi-based pesticides to non-target organisms: Evaluating the effects of sequence similarity in the parasitoid wasp *Telenomus podisi*. Sci. Total Environ. 832:154746.

Chang C, Chen M, Gao J, Luo J, Wu K, Dong T, Zhou K, He X, Hu W, Wu W. 2017. Current pesticide profiles in blood serum of adults in *jiangsu province* of china and a comparison with other countries. Environ. Int. 102: 213–222.

Damalas CA, Eleftherohorinos IG. 2011. Pesticide exposure, safety issues, and risk assessment indicators. Int. J. Environ. Res. Public Health. 8: 1402–1419.

Das T, Hazra S, Sengupta S, Hazra P, Chattopadhyay D. 2021. Genotoxic effect of saccharin on *Allium cepa* root tips. Biologia. 76: 3191–3199.

Degraeve N, Chollet M, Moutschen J. 1984. Cytogenetic and genetic effects of sub-chronic treatments with organophosphorus insecticides. Arch. Toxicol. 55: 66–67.

Dong C, Zhou J, Zuo W, Li Z, Li J. 2022. Enantioselective determination of phenthoate enantiomers in plant-origin matrices using reversed-phase high performance liquid chromatography–tandem mass spectrometry. Biomed. Chromatogr. 36 (1): e5229.

El-Houseiny, Algharib W, Mohamed SA, Metwally EA, Mahmoud MM, Alghamdi YK, Soliman YS, Abd-Elhakim MM, El-Murr AE. 2022. Dietary parsley seed mitigates methomyl-induced impaired growth performance, hemato-immune suppression, oxidative stress, hepato-renal damage, and pseudomonas aeruginosa susceptibility in Oreochromis niloticus. Antioxidants. 11(6):1185.

Esturk O, Yakar Y, Ayhan Z. 2014. Pesticide residue analysis in parsley, lettuce and spinach by LC-MS/MS. J. Food Sci. Technol. 51: 458–466.

Gogoi JY, Karabi D, Dutta PA. 2021. Effect of preservatives and pesticides on mitotic index of *Allium cepa* roots-biological model experiment for genotoxicity. Poll. Res. 40: 777–781.

Hernández AF, Parrón T, Tsatsakis AM, Requena M, Alarcón R, López-Guarnido O. 2013. Toxic effects of pesticide mixtures at a molecular level: their relevance to human health. Toxicology. 307:136–145.

Ismail C, Atilla Y, Yusuf T, Levent O. 2009. Glyphosate reduced seed and leaf concentrations of calcium, manganese, magnesium and iron in non-glyphosate resistant soybean. Eur. J. Agron. 31: 114–119.

Jablonski CA, Pereira TCB, Teodoro LDS, Altenhofen S, Rübensam G, Bonan CD, Bogo MR. 2022. Acute toxicity of methomyl commercial formulation induces morphological and behavioral changes in larval zebrafish (*Danio rerio*). Neurotoxicol Teratol. 89:107058

Kalefetoglu Macar T. 2021. Investigation of cytotoxicity and genotoxicity of abamectin pesticide in *Allium cepa* L. Environ. Sci. Pollut. Res. 28: 2391–2399.

Kaur H, Hundal SS, Singh J. 2022. Imidacloprid affects the reproductive performance and genotoxicity in

Eudrilus eugeniae and *Metaphire posthuma*. Turk. J. Agric.-Food Sci. Technol. 1–10.

Khan Z, Ansari MYK, Shahwar D. (Eds.) Induced genotoxicity and oxidative stress in plants; Springer: Singapore, 2021; ISBN 978-981-16-2074-4.

Kim KH, Kabir E, Jahan SA. 2017. Exposure to pesticides and the associated human health effects. Sci. Total Environ. 575: 525–535.

Liman R, Akyıl D, Eren Y, Konuk M. 2010. Testing of the mutagenicity and genotoxicity of metolcarb by using both Ames/Salmonella and *Allium test*. Chemosphere. 80(9):1056–1061.

Liman R, Ali MM, Istifli ES, Cigerci IH, Bonciu E. 2022. Genotoxic and cytotoxic effects of pethoxamid herbicide on *Allium cepa* cells and its molecular docking studies to unravel genotoxicity mechanism. Environ. Sci. Pollut. Res. 29: 63127–63140.

Lozier MJ, Montoya JFL, Del Rosario A, Martinez EP, Fuortes L, Cook TM, Sanderson, WT. 2013. Personal air sampling and risks of inhalation exposure during atrazine application in honduras. Int. Arch. Occup. Environ. Health. 86: 479–488.

Lukaszewicz G, Fernando G, Iturburu DSG, Mirta, LM, Stephan P. 2019. Imidacloprid modifies the mitotic kinetics and causes both aneugenic and clastogenic effects in the macrophyte Bidens laevis L. Heliyon. 5: e02118.

Macfarlane E, Carey R, Keegel T, El-Zaemay S, Fritschi L. 2013. Dermal exposure associated with occupational end use of pesticides and the role of protective measures. Saf. Health Work. 4:136–141.

Mahapatra K, De S, Banerjee S, Roy S. 2019. Pesticide mediated oxidative stress induces genotoxicity and disrupts chromatin structure in fenugreek (*Trigonella foenum-graecum* L.) seedlings. J. Hazard. Mater. 369: 362–374. [CrossRef]

Marc J, Mulner-Lorillon O, Boulben S, Hureau D, Durand G, Belle R. 2002. Pesticide roundup provokes cell division dysfunction at the level of CDK1/Cyclin B activation. Chem. Res. Toxicol. 15: 326–331.

Nara A, Yamada C, Kodama T, Saka K, Takagi T. 2018. Fatal poisoning with both dichlorvos and phenthoate. J. Forensic Sci. 63:1928-1931.

Nelson CM, Jalal SM, Larson OR. 1990. Genotoxicity of organophosphorus insecticide chlorpyrifos based on human lkymphocyte culture. Cytologia 55: 589-592.

Ogut S. 2019. Genotoxic effects of pesticides. J. Environ. Prot. Ecol. 20: 224–229.

Ozel CA, Unal F, Avuloglu-Yilmaz E, Erikel E, Mirici S, Yuzbasioglu D. 2022. Determination of genotoxic damages of picloram and dicamba with comet assay in *Allium cepa* rooted in tissue culture and distilled water. Molecular Biology Reports. 49(12):11273-80.

Ozkul M, Ozel CA, Yuzbaşıoglu D, Unal F. 2016. Does 2,4-dichlorophenoxyacetic acid (2,4-D) induce genotoxic effects in tissue cultured *Allium* roots? Cytotechnology. 68(6):2395–2405.

Pesavento PA, Agnew D, Keel MK, Woolard KD. 2018. Cancer in wildlife: Patterns of emergence. Nat. Rev. Cancer.18: 646–661.

Ping KY, Darah I, Yusuf UK, Yeng C, Sasidharan S. 2012. Genotoxicity of *Euphorbia hirta*: an *Allium cepa* assay. Molecules.17(7):7782–7791.

Priya JS, Purushothaman P, Hannah C, Matthew S, Thomas S. 2014. Genotoxic effect of ethephon on the root meristems of *Allium cepa* L. Commun Plant Sci. 4:19–22.

Qian XW. 1998. Improvement on experiment method of micronucleus in root tip cell of *Vicia faba*. J Wenzhou Norm Coll. 19: 64–65.

Rahman ANA, Mansour DA, Abd El-Rahman GI, Elseddawy NM, Zaglool AW, Khamis T, Mahmoud SF, Mahboub HH. 2022. Imidacloprid toxicity in *Clarias gariepinus*: Protective role of dietary *Hyphaene thebaica* against biochemical and histopathological disruption, oxidative stress, immune genes expressions, and Aeromonas sobria infection. Aquaculture. 555: 738170.

Sammaiah D, Shekar CC, Prasad VR, Reddy KJ. 2011. Pesticides induced alterations in physiological responses in Solanum melongena L. Int. J. Pharma Bio Sci. 2: 383.

Sandhu SS, Chander P, Singh J, Sidhu AS. 1987. Effect of insecticidal sprays on the plant and secondary pest inductions in *Hirsutum* cotton in Punjab. Agric. Ecosyst. Environ. 19(2):169–176.

Savaskan C, Toker MC. 1991. The effects of various doses gamma irradiation on the seed germination and root tips chromosomes of rye (*Secale cereale* L.). Turk. J. Bot. 15: 349–359.

Sengupta A, Sengupta RK. 2022. Genotoxic effects of profenophos on *Pisum sativum*. J. Agric. Appl. Biol. 3: 1–7.

Sharma A, Kumar V, Shahzad B, Tanveer M, Sidhu GPS, Handa N, Kohli SK, Yadav P, Bali AS, Parihar RD. et al. 2019. et al. Worldwide pesticide usage and its impacts on ecosystem. SN Appl. Sci. 1: 1446.

Siddiqui S, Al Amri SAM, Al Ghamdy HA, Alqahtani WSS, Alquyr SM, Yassin HM. 2021. Impact of Bisphenol A on seed germination, radicle length and cytogenetic alterations in *Pisum sativum* L. Caryologia. 74(2): 103-109.

Siddiqui S, Al-Rumman S. 2020 a. Clethodim induced pollen sterility and meiotic abnormalities in vegetable crop *Pisum sativum* L. Caryologia. 73: 37–44.

Siddiqui S, Al-Rumman S. 2020 b. Cytological changes induced by clethodim in *Pisum sativum* plant. Bangladesh J. Bot. 49(2):367–374.

Siddiqui S, Al-Rumman S. 2022 a. Methomyl, imbraclaobrid and clethodim induced cytomixis and syncytes behaviors in PMCs of *Pisum sativum* L: Causes and outcomes. Saudi J Biol Sci. 29(9):103390. doi: 10.1016/j.sjbs.2022.103390.

Siddiqui S, Al-Rumman S. 2022 b. Exposure of *Pisum sativum* L. seeds to methomyl and imidacloprid cause genotoxic effects in pollen-mother cells. Biology. 11: 1549. https://doi.org/10.3390/ biology11111549

Siddiqui S, Al-Rumman S. 2022 c. Methomyl has clastogenic and aneugenic effects and alters the mitotic kinetics in *Pisum sativum* L. Caryologia. 75(3): 91-99.

Siddiqui S, Khan SS, Meghvansi MK, Nazoora SA. 2008. Mutagenic effect of herbicide maleic hydrazide on seed germination and radicle length on Trigonella foenum-graecum. Indian J. Appl. Pure Biol. 23: 103–106.

Siddiqui S, Meghvansi MK, Khan SS. 2012. Glyphosate, alachor and maleic hydrazide have genotoxic effect on *Trigonella foenum graecum L*. Bull. Environ. Contam. Toxicol. 88: 659–665.

Somaiah K, Satish PVV, Sunita K, Nagaraju B, Oyebola OO. 2014. Toxic impact of phenthoate on protein and glycogen levels in certain tissues of Indian major carp Labeo rohita (Hamilton). J. Environ. Sci. Toxicol. Food Technol. 8(9): 65–73.

Strassemeyer J, Daehmlow D, Dominic A, Lorenz S, Golla B. 2017. Synops-Web, an online tool for environmental risk assessment to evaluate pesticide strategies on field level. Crop Prot. 97: 28–44.

Taufeeq A, Baqar M, Sharif F, Mumtaz M, Ullah S, Aslam S, Qadir A, Majid, M.; Jun, H. 2021. Assessment of organochlorine pesticides and health risk in tobacco farming associated with river Barandu of Pakistan. Environ. Sci. Pollut. Res. 28: 38774–38791.

Tudi M, Wang L, Ruan HD, Tong S, Atabila A, Sadler R, Yu QJ, Connell D, Phung DT. 2022. environmental monitoring and potential health risk assessment from pymetrozine exposure among communities in typical rice-growing areas of China.Environ. Sci. Pollut. Res.1–14.

Velazquez G, Pérez B, Rodríguez V, Handal A. 2022. Genotoxicity and cytotoxicity of *Sambucus canadensis* ethanol extract in meristem cells of *Allium sativum*. Caryologia.75(1): 99–107.

Yadav IC, Devi NL, Syed JH, Cheng Z, Li J, Zhang G, Jones KC. 2015. Current status of persistent organic pesticides residues in air, water, and soil, and their possible effect on neighboring countries: a comprehensive review of India. Sci. Total Environ. 511:123–137.

Zhang Y, Huang L, Liu L, Cao X, Sun C, Lin X. 2022. Metabolic disturbance in lettuce (*Lactuca sativa*) plants triggered by imidacloprid and fenvalerate. Sci. Total Environ. 802:149764.

Caryologia
International Journal of Cytology, Cytosystematics and Cytogenetics

Firenze University Press
www.fupress.com/caryologia

OPEN ACCESS

Citation: Meryem Nassar, Nora Sakhraoui, Gianniantonio Domina (2023). Karyomorphology of two subspecies of *Anthemis maritima* (Asteraceae) from Algeria. *Caryologia* 76(1): 67-75. doi: 10.36253/caryologia-2084

Received: March 10, 2023

Accepted: July 4, 2023

Published: September, 19, 2023

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

ORCID
MN: 0000-0002-1161-6957
NS: 0000-0002-9853-5702
GD: 0000-0002-9125-764X

Karyomorphology of two subspecies of *Anthemis maritima* (Asteraceae) from Algeria

Meryem Nassar[1,4,*], Nora Sakhraoui[2,4], Gianniantonio Domina[3]

[1]*Department of Natural Sciences and Life BP 26, University of 20 August 1955, Route d'El Hadaiek-Skikda 21000, Algeria*
[2]*Department of Ecology and Environment BP 26, University of 20 August 1955, Route d'El Hadaiek-Skikda 21000, Algeria*
[3]*Department of Agricultural, Food and Forest Sciences University of Palermo, Viale delle Scienze, Bldg 4. 90128, Palermo, Italy*
[4]*Laboratory of Research in Biodiversity Interaction, Ecosystem and Biotechnology 'LRIBEB', University of 20 August 1955 BP 26, Route d'El Hadaiek-Skikda 21000, Algeria*
*Corresponding author. E-mail: meryem4321@yahoo.fr; m.nassar@univ-skikda.dz

Abstract. The chromosome number and karyomorphology of two subspecies of the *Anthemis maritima* complex collected from two different coastal localities in the Skikda region (northeastern Algeria) are reported in this study: *Anthemis maritima* subsp. *maritima*, which is common throughout the Mediterranean, and *A. maritima* subsp. *bolosii*, a strict Algerian endemic recently rediscovered after 100 years of disappearance. The Feulgen staining method indicated that *Anthemis maritima* subsp. *maritima* is a tetraploid with $2n=4x=36$ chromosomes (2*n*=11m+3sm+4st), and *Anthemis maritima* subsp. *bolosii* is a diploid with $2n=2x=18$ chromosomes (2*n*=4m+3sm+2st). Both taxa have symmetrical karyotypes, 2A and 1A, respectively, according to Stebbins classification. These findings are novel for both the subspecies *Anthemis maritima* subsp. *bolosii* and the Algerian population of *Anthemis maritima* subsp. *maritima*.

Keywords: *Anthemis maritima* subsp. *bolosii*, *Anthemis maritima* subsp. *maritima*, karyotype, chromosome, symmetrical, Skikda.

INTRODUCTION

The Asteraceae is an extremely diverse family of flowering plants, representing a large part of the world's flora with approximately 1,900 genera and 32,000 species (Mandela *et al.* 2019) distributed across 40 tribes (Funk *et al.* 2009). The tribe Anthemideae, which is a member of this family, stands out with 111 genera and nearly 1,800 species, making it one of the most important tribes of the Asteraceae family (Oberprieler *et al.* 2006; Oberprieler *et al.* 2007). Following a thorough revision based on molecular phylogenetic analyses, six new subtribes of the Asteraceae -Anthemideae family were identified, which are: the Anthemidinae, Artemisiinae, Glebionidinae, Handeliinae, Leucantheminae, and Leucanthemopsidinae. Five other monotypic sub-

tribes were also added. As a consequence, this important tribe currently contains 19 subtribes (Oberprieler *et al.* 2022a). One of the most important genera in the Anthemideae tribe and a member of the Anthemidinae subtribe is *Anthemis*, which has 175 species distributed throughout the Mediterranean and Southwest Asia. However, due to the huge micromorphological differentiation of its species and those of related genera, the taxonomy of this genus is often challenging. Hybridization and polyploidy may have played crucial roles in the evolution of this genus, further complicating its taxonomy (Oberprieler 1998; Oberprieler 2001; Lo Presti *et al.* 2010). Phylogenetic analyses based on ITS sequences have improved our understanding of the evolutionary relationships among *Anthemis* species, revealing that some species previously included in the subgenus *Anthemis* were actually more closely related to species in adjacent genera (*Tripleurospermum* and *Nananthea*) than to those in the subgenus *Cota*. This finding resulted to the reclassification of several species, as well as the exclusion of the subgenus *Cota* as a distinct genus (Greuter *et al.* 2003; Oberprieler 2001; Oberprieler *et al.* 2006; Oberprieler *et al.* 2009).

Anthemis appears to be one of the few genera with an ancestral base number of x=9, evolved from x=10 fairly early in the evolutionary process (Bremer and Humphries 1993). In spite the fact that cytogenetic events such as ascending dysploidy (*Leptinella*, *Artemisia*) and descending dysploidy (*Cotula*, *Artemisia*, *Athanasia*, and *Ursinia*) are common in the Anthemideae tribe, resulting in base number alterations ranging from x=5 to x=11, 13, and 17, and driving their evolution.

Indeed, all chromosomal counts in *Anthemis* sections indicated a majority of diploid species ($2n$=$2x$=18) with an x=9 base number. Thus, polyploidization is primarily responsible for the evolution in some of its species (Oberprieler 1998; Inceer and Hayirlioglu-Ayaz 2007; Pellicer *et al.* 2007; Javadi *et al.* 2013; Shariat *et al.* 2021). Hiorthia is regarded as one of the six sections of the genus that have undergone true evolution due to the occurrence of polyploid species ($2n$=$4x$=36) (Oberprieler 2001). One of these is the North African *A. maritima*, specifically the subspecies *A. maritima* subsp. *maritima* (Oberprieler 1998).

Despite the revision of (Oberprieler 1998) several taxa of the genus in North Africa are still poorly understood. This concerns not only the morphological aspects but above all the karyological and molecular ones. The study of ploidy levels within the various subspecies allows us to better clarify the phylogenetic relationships between the investigated taxa. Although Algeria holds 20 taxa (12 species and 8 subspecies) among North African species (Dobignard and Chatelain 2011), but unfortunately, karyological information on these taxa is scarce.

The *Anthemis maritima* complex is present with three subspecies: *A. maritima* subsp. *maritima* and two strictly endemic subspecies to Algeria, *A. maritima* subsp. *bolosii* Benedí et Molero and *A. maritima* subsp. *Pseudopunctata* Oberpr. (Dobignard and Chatelain 2011; Tison and De Foucaul 2014). *A. maritima* subsp. *bolosii* was first distinguished by Benedi Gonzalez and Molero Briones (1990) from *A. maritima* subsp. *maritima* by its more erect stem. Oberprieler (1998) reported the total absence of hairs on the peduncle and involucre of *A. maritima* subsp. *bolosii,* and Sakhraoui *et al.* (2021) added other salient features, namely color and leaf appearance, mode of reproduction, habitat, and aromaticity.

A. maritima subsp. *maritima* occurs on coastal dunes throughout the Mediterranean region (Dobignard and Chatelain 2010–2011; Tison and De Foucaul 2014). In Algeria, the subspecies is given as quite common on the littoral of the small and great Kabylia (Quézel and Santa 1963; Boulemtafes *et al.* 2018). *A. maritima* subsp. *bolosii* is considered rare, particularly since its distribution is very restricted. According to Sakhraoui *et al.* (2021), the plant has been reported only at two coastal localities (Annaba and Skikda) in northeastern Algeria, and the presence of the subspecies has not been recorded for more than a century. The oldest record dates back to 6/23/1920 (specimen: P 03697947). The same author reported the rediscovery of the subspecies in the Stora locality (Skikda region).

A. maritima subsp. *bolosii* is a perennial plant with a woody stump, a hairless stem and pinnate leaves that are sessile, or petiolate. The flowers with ligules and yellow centers are arranged into capitula that range in diameter from 14 to 35 mm and are carried by glabrous peduncles, the plant grows on cliffs and sea rocks (Sakhraoui *et al.* 2021) unlike *A. maritima* subsp. *maritima*, which prefers coastal dunes and sandy beachs (Boulemtafes *et al.* 2018; Sakhraoui *et al.* 2021). This taxon includes aromatic plants of 20 to 70 cm in size with creeping stems rising during flowering and achenes with smooth ribs. The spangles of the receptacle are oblong-lanceolate, and the pinnate leaves are more or less fleshy, weakly to strongly puberulent (Quézel and Santa 1963; Tison and De Foucaul 2014; Sakhraoui *et al.* 2021).

The aim of this contribution is to investigate and characterize, from a karyological point of view, *A. maritima* subsp. *bolosii* in comparison with the geographically closest populations of *A. maritima* subsp. *maritima.* In fact, no cytogenetic investigation of these subspecies from Algeria has been conducted. This study is therefore

Table 1. The location of the two *A. maritima* subspecies.

Subspecies	Locality	Latitudes	Longitudes	Altitude	Month of collection
A. maritima subsp. *maritima*	Larbi ben M'Hidi	36°53'33"N	7°00'25"E	10 m	November 2022
A. maritima subsp. *bolosii*	Stora	36°53'11"N	6°53'55"E	4 m	August 2021

the first to report on the chromosome number and karyological features of *A. maritima* subsp. *bolosii* as well.

MATERIALS AND METHODS

The seeds used in this study were collected from wild plants growing in two separate locations in Skikda's region (Table 1). Chromosome observation and detection were achieved by the standard Feulgen staining. Roots of 0.5-1.5 cm in length were obtained from the germinated seeds, then pretreated with 0.03% 8-hydroxyquinoline for 4 h at 4°C before being fixed in a 3 ethanol/1 acetic acid solution for 24 h. Hydrolysis was performed in 1M hydrochloric acid for 5 min at 60°C. Root tips were stained with Schiff's reagent for 2 h before being crushed in a drop of 45% acetic acid between the slide and coverslip. Five metaphase plates for each subspecies were analyzed to obtain the different measurements indicated in this work. The IdeoKar software was used for calculating karyotype parameters (Mirzaghaderia and Marzangib 2015).

RESULTS AND DISCUSSION

The karyomorphological results for *A. maritima* subsp. *maritima* and *A. maritima* subsp. *bolosii* are presented in Tables 2 and 3, respectively. The studied population of *A. maritima* subsp. *maritima* was identified as tetraploid, with a chromosomal number of $2n$=36. The subspecies has a total haploid length of 67.54 μm. The total length of chromosomes ranges from 2.50 μm to 5.14 μm, and the ratio between the long arm and short arm varies from 1.12 to 3.31. The karyotype consists of 18 pairs of chromosomes, of which eleven (1, 2, 3, 4, 5, 6, 8, 10, 12, 15, 16) are metacentric, three (9, 14, 17) are submetacentric, and four (7, 11, 13, 18) are subtelocentric. The chromosomal formula of *A. maritima* subsp. *maritima* is $2n=4x=36$=11m+3sm+4st (Figure 1).

A. maritima subsp. *bolosii* has nine pairs of chromosomes, four pairs (1, 3, 6, 9) are metacentric, three pairs (2, 4, 7) are submetacentric, and two pairs (5, 8) are subtelocentric. Based on these data, it was determined that this subspecies is diploid, with x=9 basic

Table 2. Karyomorphological analysis of *A. maritima* subsp. *maritima.*

N	L (μm)	S (μm)	LT (μm)	AR	RL%	CI	CT
1	3.02±0.11	2.12±0.71	5.14±0.80	1.42	7.59	40.30	m
2	2.90±0.28	1.88±0.16	4.78±0.44	1.54	7.03	39.44	m
3	2.60±0.07	1.98±0.08	4.58±0.14	1.31	6.74	43.32	m
4	2.60±0.10	1.88±0.06	4.48±0.16	1.38	6.59	42.33	m
5	2.18±0.04	1.94±0.08	4.12±0.11	1.12	6.09	47.05	m
6	2.32±0.21	1.74±0.50	4.06±0.70	1.33	6.00	40.33	m
7	2.96±0.16	0.98±0.08	3.94±0.20	3.02	5.83	23.18	st
8	2.42±0.06	1.48±0.08	3.90±0.20	1.63	5.77	37.80	m
9	2.50±0.10	1.34±0.08	3.84±0.12	1.86	5.68	35.22	sm
10	2.28±0.06	1.56±0.10	3.84±0.16	1.46	5.48	40.50	m
11	2.84±0.08	0.88±0.08	3.72±0.15	3.22	5.47	23.86	st
12	2.06±0.13	1.50±0.08	3.56±0.15	1.37	5.24	40.20	m
13	2.50±0.24	0.82±0.08	3.32±0.34	3.04	4.91	24.22	st
14	2.00±0.17	1.18±0.40	3.18±0.57	1.69	4.71	37.30	sm
15	1.80±0.17	1.18±0.36	2.98±0.50	1.52	4.41	39.87	m
16	1.74±0.21	1.08±0.32	2.82±0.52	1.61	4.18	38.57	m
17	1.88±0.11	0.90±0.40	2.78±0.50	2.08	4.12	32.55	sm
18	1.92±0.11	0.58±0.20	2.50±0.30	3.31	3.69	23.36	st

Length of chromosome (L: long arm, S: short arm, LT: total length), AR: Arm ratio, RL: Relative length, CI: Centromeric index, CT: Chromosome type.

Table 3. Karyomorphological analysis of *A. maritima* subsp. *bolosii.*

N	L (μm)	S (μm)	LT (μm)	AR	RL%	CI	CT
1	2.79±0.06	2.39±0.11	5.07±0.17	1.16	14.1	46.07	m
2	2.96±0.16	1.56±0.08	4.52±0.24	1.89	13.0	34.61	sm
3	2.40±0.18	1.64±0.20	4.04±0.39	1.46	11.6	40.67	m
4	2.55±0.18	1.37±0.12	3.92±0.31	1.86	11.2	35.03	sm
5	2.98±0.25	0.82±0.07	3.80±0.33	3.63	10.9	21.65	st
6	2.14±0.21	1.58±0.11	3.72±0.32	1.35	10.7	42.49	m
7	2.28±0.27	1.36±0.08	3.64±0.35	1.67	10.4	37.38	sm
8	2.52±0.10	0.64±0.06	3.16±0.16	3.93	9.11	20.61	st
9	1.80±0.23	1.22±0.13	3.02±0.37	1.47	8.65	40.55	m

Length of chromosome (L: long arm. S: short arm. LT: total length), AR: Arm ratio, RL: Relative length,CI: Centromeric index, CT: Chromosome type.

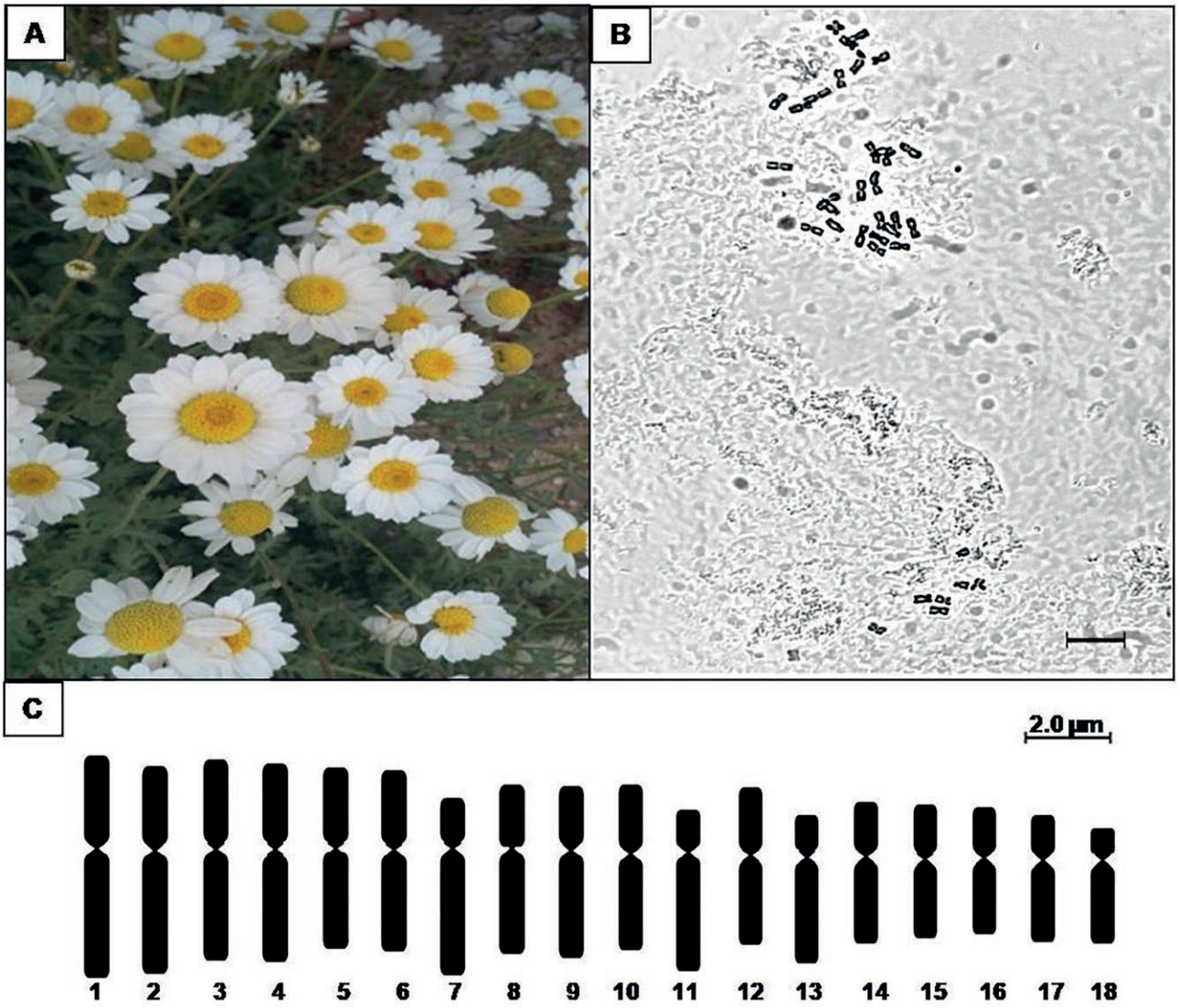


Figure 1. Karyotype of *Anthemis maritima* subsp. *maritima*. A: The plant, B: Metaphase plate, scale bars= 10 μm, C: Idiogram.

chromosomes number and a karyotype formula of $2n=2x=18=4m+3sm+2st$ (Figure 2). The chromosomal lengths range from 3.02 μm to 5.07 μm, the haploid genome is 34.87 μm long, and the long arm to short arm ratio ranges from 1.16 to 3.93. These findings agree with prior research on the basic number $x=9$ and the degree of ploidy.

Among the 28 species of *Anthemis* studied in North Africa (from Morocco and Tunisia), 23 were diploid, and the remaining species were tetraploid ($2n=4x=36$), including *A. maritima* subsp. *maritima* (Tunisian population) (Oberprieler 1998).

All karyotypes were symmetrical, with metacentric, submetacentric, and subtelocentric chromosomes and at least two satellites. In our case, for both subspecies, all chromosomes were metacentric, submetacentric, and subtelocentric without satellites or secondary constrictions. Generally, species in the genus *Anthemis* contain at least one chromosomal pair bearing a satellite (Oberprieler 1998; Goula *et al.* 2022). The absence of satellites for the two studied taxa could be due to strong chromosomal condensation that prevented their appearance.

The asymmetry index (AsI) for *A. maritima* subsp. *maritima* and *A. maritima* subsp. *bolosii* is 63.40% and 64.02%, respectively. According to Stebbins' classification (1971), both karyotypes are symmetrical 2A and 1A, types respectively. Other research on the karyology of the genus *Anthemis* has already confirmed the dominance of the base $x=9$ (Inceer and Hayirlioglu-Ayaz 2007; Chehregani and Mehanfar 2008; Javadi *et al.* 2013; Qari

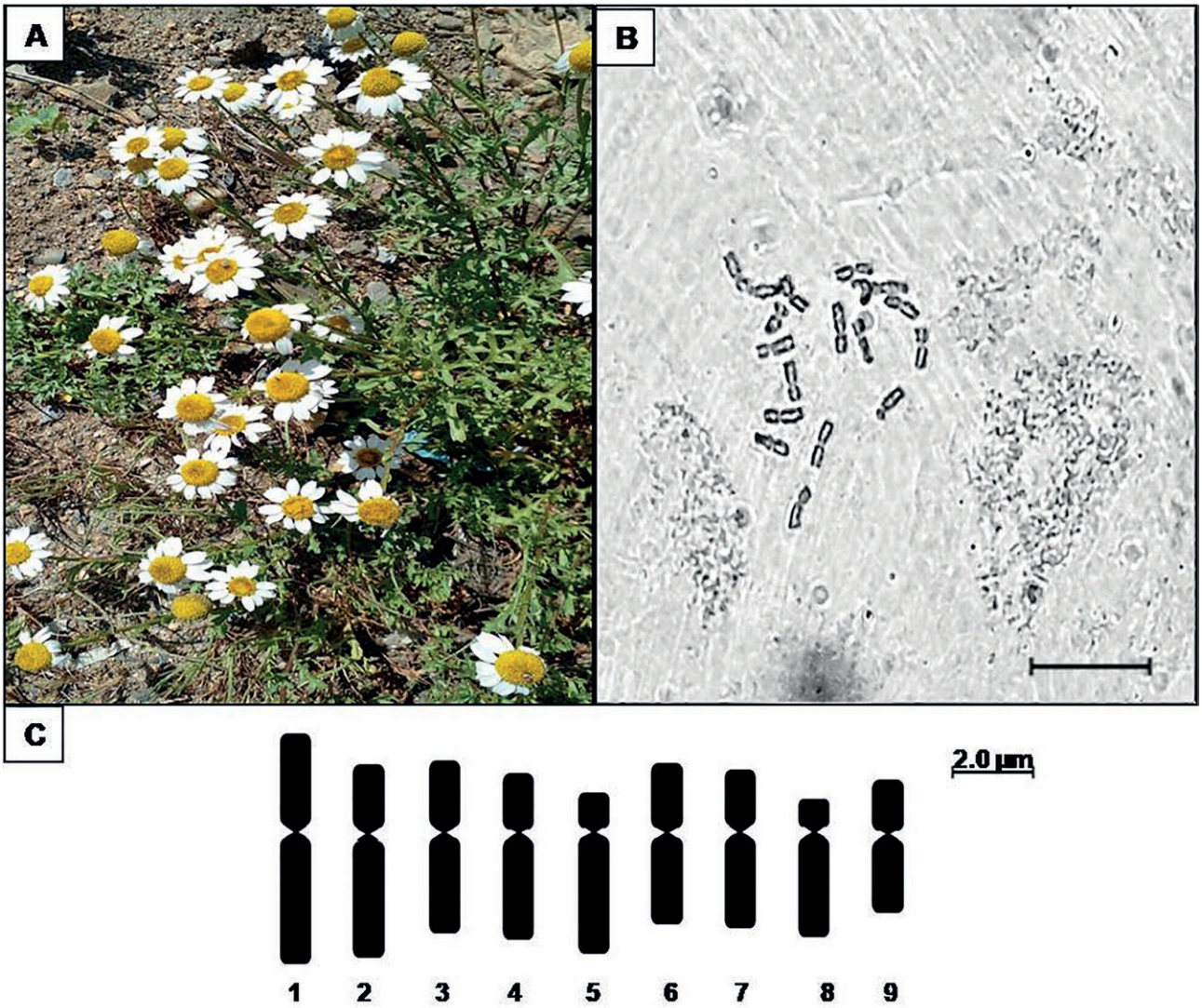


Figure 2. Karyotype of *Anthemis maritima* subsp. *bolosii*. A: The plant, B: Metaphase plate, scale bars= 10 μm, C: Idiogram.

et al. 2016; Shariat *et al.* 2021; Goula *et al.* 2022). The base *x*=9 appears to be constant in the genus *Anthemis*, implying that disploidy, which is often the origin of chromosomal number changes, remains very low.

Several asymmetry indices were calculated to assess the evolutionary linkages between the two subspecies, with values presented in Table 4 for intrachromosomal asymmetry indices (A_1, TF%, CV_{CI}, and M_{CA}) and interchromosomal indices (A_2 and CV_{CL}). These criteria allowed for a more accurate description of their distinct karyotypes. The intrachromosomal asymmetry indices A_1, CV_{CI}, and M_{CA} are higher in the diploid subspecies (*A. maritima* subsp. *bolosii*) than in the tetraploid subspecies (*A. maritima* subsp. *maritima*). In contrast, the tetraploid subspecies (*A. maritima* subsp. *maritima*) possess higher interchromosomal indices A_2 and CV_{CL} than the diploid subspecies (Table 4).

The M_{CA} and CV_{CL} indices are considered reliable quantitative parameters capable of estimating even minor intrachromosomal and interchromosomal variations in the chromosomal complement (Peruzzi *et al.* 2009; Peruzzi and Eroglu 2013; Baeza *et al.* 2015; Genc and Firat 2016; Phukan and Saha 2019; Eroglu and Budak 2020). CV_{CL} and M_{CA} values for *A. maritima* subsp. *maritima* and *A. maritima* subsp. *bolosii* are 19.65, 17.36, 24.3, and 28, respectively. When compared to its tetraploid relative, the diploid subspecies had higher CV_{CI} and M_{CA} values, indicating more variability in centromere location. The tetraploid subspecies, on the other hand, exhibits higher CV_{CL} and A_2 values,

Table 4. Asymmetry indices of *A. maritima* subsp. *maritima and A. maritima* subsp. *bolosii.*

Subspecies	HCL	TF%	AsI%	CV_{CL}	CV_{CI}	AI	A_1	A_2	M_{CA}	S Cl
A. maritima subsp. *maritima*	67,54	36,5	63.4	19,65	25,18	4,94	0.41	0.19	24,3	2A
A. maritima subsp. *bolosii*	34.87	35.97	64.02	17.36	26.56	4,61	0.42	0.17	28	1A

HCL: Haploid total length, TF: Total form percentage (Huziwara 1962), AsI: Karyotype asymmtry index (Arano and Saito 1980), CV_{CL}: Coefficient of variation of chromosome length, CV_{CI}: Relative variation in centromeric index, AI: Asymmetry index (Paszko 2006), A_1: Intrachromosomal asymmetry, A_2: Interchromosomal asymmetry (Romero Zarco 1986), M_{CA}: Mean centromeric asymmetry (Peruzzi and Eroğlu 2013), S Cl: Stebbins classification (Stebbins 1971).

indicating more variation in chromosomal size. Furthermore, the IA index (Paszko 2006), another statistic parameter used to assess karyotype coherence, is higher in the tetraploid subspecies (4.94) than in the diploid subspecies (4.61). A higher IA index value reflects increased chromosomal variability. As a result, it appears that the karyotype of *A. maritima* subsp. *maritima* has evolved more than that of *A. maritima* subsp. *bolosii*. Additionally, Stebbins' (1971) classification classified the karyotypes of the subspecies *A. maritima* subsp. *bolosii* and *A. maritima* subsp. *maritima* in the symmetrical classes 1A and 2A, respectively, indicating some similarity between the two taxa but also a more primitive karyotype of the diploid subspecies compared to the tetraploid one. According to Oberprieler (1998), the karyotypes of Hiorthia section species are consistently of type A2, with less variation in intrachromosomal symmetry but higher variation in interchromosomal symmetry. As a consequence, our findings are consistent with previous research, particularly for the tetraploid subspecies. The differences in interchromosomal indices between the two subspecies are quite considerable, as are the differences in intrachromosomal indices, especially M_{CA}, which can be induced by chromosomal mutations (translocations and inversions) resulting in changes between the two taxa. Despite the fact that 82.14% of North African species are diploid (Oberprieler, 1998), practically all species of the section Hiorthia, to which the *A. maritima* complex belongs, have been found to be tetraploid (Oberprieler, 1998; Presti *et al*. 2010). Mitsuoka and Ehrendorfer (1972) raised doubts about limiting the *A. maritima* complex to its tetraploid cytotype, suggesting that this plant may also possess a diploid cytotype, like its Moroccan counterpart, the *Anthemis pedunculata* complex.

Thus, our data show unequivocally that the subspecies *A. maritima* subsp. *bolosii* represents the diploid cytotype for the *A. maritima* complex. Furthermore, *A. pedunculata* and *A. maritima*, are the only species that exhibit both diploid and tetraploid cytotypes. Phylogenetic investigations have also shown that the two complexes are closely related (Oberprieler 2001; Presti *et al*. 2010; Oberprieler *et al.* 2022b). The similarity in terms of chromosome size and type between the karyotypes of both subspecies leads us to propose the following hypothesis: the tetraploid cytotype of *A. maritima* may have resulted from either duplication (autopolyploidy) of the diploid cytotype of *A. maritima* subsp. *bolosii* or potentially from an allopolyploidy event involving *A. maritima* subsp. *bolosii* and another related species. Knowing that hybridization between related *Anthemis* species is not new as evidenced by various studies (Uitz 1970; Mitsuoka and Ehrendorfer 1972; Nagl and Ehrendorfer 1974; Oberprieler 1998; Oberprieler 2001; Tison and De Foucaul 2014; Oberprieler *et al*. 2022b). This is also the case for the tetraploid *A. cupaniana*, which is native to Sicily, and has been shown to be the result of allopolyploidization (during the pleistocene). Specifically, this involved a maternal parent from the *A. cretica* group and a paternal parent from the North African complex *A. pedunculata* (Horthia section), namely the diploid Sicilian *A. ismelia* (Oberprieler *et al*. 2022b).

The comparative palynological study conducted by Oberprieler (1998) suggested that the subspecies *A. maritima* subsp. *bolosii* is the only diploid subspecies (based on pollen size) among the three subspecies in the *A. maritima* complex, and *A. maritima* subsp. *maritima* and *A. maritima* subsp. *pseudopunctata* were identified as tetraploid.

Furthermore, Algeria is the only territory where all three representatives of the *A. maritima* complex have been found. As mentioned above, *A. maritima* subsp. *bolosii* is interesting due to its rarity resulting from its limited distribution along coastal rocks and cliffs. All these information support the hypothesis that *A. maritima* subsp. *bolosii* is the ancestor of the *A. maritima* complex and that Algeria is the center of its diversification, especially since the Algerian population ($2n=4x=36$=11m+3sm+4st) and the Tunisian population ($2n=4x=36$=11m+3sm+4st^{sat}) (Oberprieler 1998) of *A. maritima* subsp. *maritima* appear to be identical from a cytological point of view. According to Greuter (1979),

populations of the same taxon that are geographically separated but have karyotype stability are remnants of an ancient Mediterranean flora present during the Messinian period. Therefore, *A. maritima* complex might represent one of the groups that are ancient polyploids. In contrast to the diploid subspecies, the tetraploid *A. maritima* subsp. *maritima* is found in a number of coastal locations in Algeria (Quézel and Santa 1963; Boulemtafes *et al.* 2018) and across the Mediterranean area (Oberprieler 1998, 2001; Oberprieler *et al.* 2009). According to Winter *et al.* (1999), polyploid species spread more easily than their diploid ancestors, which grow rare and eventually survive only in isolated places shielded from hybridization. Stebbins (1971) asserted that polyploid plants are more adapted to changing environmental circumstances than their diploid ancestors. This adaptation has evolved over millions of years as a result of genetic changes such as mutations and genetic recombination (Adams and Wendel 2005; Alix *et al.* 2017).

Indeed, such a hypothesis requires strong molecular evidence to be confirmed, especially given that the data presented in this study is preliminary and insufficient to prove it.

CONCLUSION

The karyomorphological data of the Algerian population of *A. maritima* subsp. *maritima* and the subspecies *A. maritima* subsp. *bolosii* are reported for the first time and are the only data for the Algerian *Anthemis* taxa. Morphological differences between the two species previously documented by numerous authors are closely related to karyological differences, namely the ploidy level, proportions of each chromosomal type, and chromosomal formula. However, a few similarities between the two subspecies have been identified, including the degree of asymmetry and chromosomal size. As a result, *A. maritima* subsp. *bolosii* might be the origin of the *A. maritima* complex.

Molecular phylogenetic studies need to be considered to confirm this hypothesis and establish the evolutionary links between the subspecies of the *Anthemis maritima* complex in Algeria, and effectively contribute to the understanding of the cytotaxonomy of the *Anthemis* genus in the Mediterranean region. We underline the importance of enacting actual conservation actions to protect the taxa in issue in order to avoid extinction, given their extremely low germinative power, as found during this study.

ACKNOWLEDGMENTS

The authors are grateful to the MESRS, Algeria.

REFERENCES

Adams KL, Wendel JF. 2005. Polyploidy and genome evolution in plants. Curr Opin Plant Biol. 8: 135–141.

Alix K, Gerard RP, Trude Schwarzacher T, Heslop-Harrison JS. 2017. Polyploidy and interspecific hybridization: partners for adaptation, speciation and evolution in plants. Ann. Bot. 120: 183–194.

Arano H, Saito H. 1980. Cytological studies in family Umbelliferae. V. Karyotypes of seven species in the subtribe Seselinae. La Kromosomo. 2: 471–480.

Baeza C, Finot VL, Ruiz E. 2015. Comparative karyotype analysis of populations in the *Alstroemeria presliana* Herbert (Alstroemeriaceae) complex in Chile. Genet. Mol. Biol. 38: 199–204.

Benedi Gonzalez C, Molero Briones J. 1990. Une nouvelle sous-espèce d'*Anthemis maritima* L. Fontqueria. 28: 1–3.

Boulemtafes A, Hamel T, de Bélair G, Véla E. 2018. Nouvelles données sur la distribution et l'écologie de seize taxons végétaux du littoral de la péninsule de l'Edough (Nord-Est algérien). Bull. Soc. Linn. Provence. 69: 1–17.

Bremer K, Humphries CJ. 1993. Generic monograph of the Asteraceae-Anthemideae. Bulletin of the Natural History Museum London, Botany Series. 23: 71–177.

Chehregani A, Mehanfar N. 2008. New chromosome counts in the tribe Anthemideae (Astraceae) from Iran. Cytologia. 73: 189–196.

Dobignard A, Chatelain C. 2011. Index synonymique de la flore d'Afrique du Nord. 2nd Ed. Cons. Jard. Bot., Genève.

Dobignard A, Chatelain C. 2010. Index synonymique de la flore d'Afrique du Nord. Pteridophyta, Gymnospermae, Monocotyledonae. Cons. Jard. Bot., Genève.

Dupont F, Guignard JL. 2012. Botanique, Les familles de plantes. Elsevier Masson, SAS.

Eroğlu HE, Budak U. 2020. Karyotype Analysis of *Paronychia anatolica* subsp. *Anatolica* (Caryophyllaceae, Paronychioideae). Biology. 10: 2424–2430.

Funk VA, Susanna A, Stuessy TF, Bayer RJ, 2009. Systematics, Evolution, and Biogeography of Compositae. Washington: IAPT.

Genc I, Firat M. 2016. Karyological studies of six taxa of the genus *Allium* L. (Amaryllidaceae) from Turkey. Caryologia. 69: 187–90.

Goula K, Touloumis K, Dimopoulos P, Constantinidis T. 2022. A Morphometric and Karyological Study

of the *Anthemis macedonica* Group (Asteraceae, Anthemideae) Reveals a New Species from Greece. Plants. 11: 1–22.

Greuter W, Oberprieler C, Vogt R. 2003. The Euro+Med treatment of Anthemideae (Compositae)-generic concepts and required new names. Willdenowia. 33: 37–43.

Greuter W. 1979. The origin and evolution of the Island floras as exemplified by the Aegean Archipelago. 87-106. In: Bramweel D. (ed.) Plants and Islands. Academic Press, London and New York.

Huziwara Y. 1962. Karyotype analysis in some genera of Compositae. VIII. Further studies on the chromosomes of Aster. Am. J. Bot. 49: 116–119.

Inceer H, Hayirlioglu-Ayaz S. 2007. Chromosome numbers in the tribe Anthemideae (Asteraceae) from north-east Anatolia. Bot. J. Linn. Soc. 153: 203–211.

Javadi H, Alizadeh MA, Emami H, Shanjani PS. 2013. Cytogenetics investigation in different populations of *Anthemis tinctoria* L. (yellow chamomile) accessions from Iran. Chromosome Sci. 16: 39–46.

Lo Presti RM, Oppolzer S, Oberprieler C. 2010. A molecular phylogeny and a revised classification of the Mediterranean genus *Anthemis* (Compositae, Anthemideae) based on three molecular markers and micromorphological characters. Taxon. 59: 1441–1456.

Mandela JB, Dikowb RB, Siniscalchia CM, Thapaa R, Watsonc LE, Funkd VA. 2019. A fully resolved backbone phylogeny reveals numerous dispersals and explosive diversifications throughout the history of Asteraceae. PNAS. 116: 14083–14088.

Mirzaghaderia G, Marzangib K. 2015. IdeoKar: an ideogram constructing and karyotype analyzing software. Caryologia. 68: 31–35.

Mitsuoka S, Ehrendorfer F. 1972. Cytogenetics and evolution of *Matricaria* and related genera (Asteraceae-Anthemideae). Oesterr. Bot. Z. 120: 155-200.

Nagl W, Ehrendorfer F. 1974. DNA content, heterochromatin, mitotic index, and growth in perennial and annual Anthemideae (Asteraceae). Plant Syst. Evol. 123: 35–54.

Oberprieler C, Töpfer A, Dorfner M, Stock M, Vogt R. 2022a. An updated subtribal classification of *Compositae* tribe *Anthemideae* based on extended phylogenetic reconstructions. Willdenowia. 52: 118–149.

Oberprieler C, Verkhoturova E, Domina G. 2022b. Allopolyploidisation in a geological collision zone: on the origin of the tetraploid *Anthemis cupaniana* Nyman (Compositae, Anthemideae) in Sicily. Plant Syst. Evol. 308: 1–9.

Oberprieler C, Himmelreich S, Källersjö M, Vallès J, Watson LE, Vogt R. 2009. Tribe *Anthemideae* Cass. pp. 631–666 in: Funk VA, Susanna A, Stuessy TF, Bayer RJ. (ed). Systematics, Evolution, and Biogeography of the *Compositae*. Washington: IAPT.

Oberprieler C, Himmelreich S, Vogt R. 2007. A new subtribal classification of the tribe Anthemideae (Compositae). Willdenowia. 37: 89–114.

Oberprieler C, Vogt R. 2006. The taxonomic position of *Matricaria macrotis* (Compositae-Anthemideae). Willdenowia. 36: 329–338.

Oberprieler C. 2001. Phylogenetic relationships in *Anthemis* L. (Compositae, Anthemideae). Taxon. 50: 745–762.

Oberprieler C. 1998. The systematics of *Anthemis* L. (Compositae, Anthemideae) in W and C North Africa. Bocconea. 9: 1–103.

Paszko B. 2006. A critical review and a new proposal of karyotype asymmetry indices. Plant Syst. Evol. 258: 39–48.

Pellicer J, Garcia S, Garnatje T, Hidalgo O, Korobkov AA, Dariimaa S, Vallès J. 2007. Chromosome counts in Asian *Artemisia* L. (Asteraceae) species: from diploid to the first report of the highest polyploidy in the genus. Bot. J. Linn. 153: 301–310.

Peruzzi L, Eroğlu HE. 2013. Karyotype asymmetry: again, how to measure and what to measure? Comp Cytogenet. **7**: 1–9.

Peruzzi L, Leitch IJ, Caparelli KF. 2009. Chromosome diversity and evolution in Liliaceae. Ann. Bot. 103: 459–475.

Petrova A, Vladimirov V. 2020. Chromosome atlas of the Bulgarian vascular plants. Phytol. Balc. 26: 217– 427.

Phukan HD, Saha K. 2019. A study on genetic relationship between *Allium sativum* L. and *Scadoxus multiflorus* (Martyn) Raf. of Amaryllidaceae. Plant Sci. Today. 6: 491–494.

Qari SH, Kamel EA, Ali KS. 2016. karyotype criteria of some taxa of Asteraceae (Compositae) from Saudi arabia. Int. J. Biol. Pharm. Allied Sci. 5: 2956–2966.

Quézel P, Santa S. 1963. Nouvelle flore de l'Algérie et des régions désertiques méridionales. CNRS, Paris.

Romero zarco C. 1986. A new method for estimating karyotype asymmetry. Taxon. 35: 526–530.

Sakhraoui N, Boussouak R, Chefrour A. 2021. Redécouverte d'une endémique algérienne méconnue, *Anthemis maritima* subsp. *bolosii* Benedí et Molero, et mise en évidence de nouvelles caractéristiques d'identification. Bull. Soc. Linn. Provence. 72: 75–80.

Shariat A, Jalili A, MirzadehVaghefi SS, Khanhasani M. 2021. Chromosome counts of twelve vascular plant species from Iran. Iran. J. Bot. 27: 183–190.

Stebbins GL. 1971. Chromosomal evaluation in higher plants. London: Edward Arnold.

Tison JM, De Foucaul TB. 2014. Flora Gallica. Flore de France. Biotope, Mèze.

Uitz H. 1970. Cytologische und bestaubungs experimentelle Beitrage zur Verwandtschaftu und Evolution der Anthemideae (Asteraceae). Ph.D. Thesis, Univ. Graz.

Véla E, Benhouhou S. 2007. Évaluation d'un nouveau point chaud de biodiversité végétale, dans le Bassin méditerranéen (Afrique du Nord). C. R. Bio. 330: 589–605.

Winter PC, Hickey GI, Fletcher HL. 1999. Instant notes in genetics. Bios Scientific publishers limited. Oxford. BERTI Editions London.

Caryologia
International Journal of Cytology, Cytosystematics and Cytogenetics

Firenze University Press
www.fupress.com/caryologia

OPEN ACCESS

Citation: Ali Bouzekri, Meryem Nassar, Souheila Slimani, Zohra Chekroud (2023). Cytogenetic effects of *Tribulus terrestris* L. on meristematic cells of *Allium cepa* L. and Vicia faba L.. *Caryologia* 76(1): 77-86. doi: 10.36253/caryologia-2092

Received: March 22, 2023

Accepted: June 17, 2023

Published: September, 19, 2023

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

ORCID
AB: 0000-0002-1161-6957
MN: 0000-0002-1161-6957
SS: 0000-0002-2771-3727
ZC: 0000-0002-9845-6027

Cytogenetic effects of *Tribulus terrestris* L. on meristematic cells of *Allium cepa* L. and *Vicia faba* L.

Ali Bouzekri[1,2,*], Meryem Nassar[1,2], Souheila Slimani[1,2], Zohra Chekroud[1,2]

[1] *Department of Biology, BP 26, University of 20 August 1955, El Hadaiek-Skikda route 21000, Algeria*
[2] *Laboratory of Research in Biodiversity Interaction, Ecosystem and Biotechnology "LRIBEB", University of 20 August 1955 BP 26, El Hadaiek-Skikda route 21000, Algeria*
*Corresponding author. E-mail: alibouzekri21@gmail.com

Abstract. *Tribulus terrestris* is a plant of the Zygophyllaceae family frequently used worldwide to treat various diseases due to the therapeutic effects of its pharmacological components. This study examines the cytotoxic and genotoxic effects of *T. terrestris* using two plant models, *Allium cepa* and *Vicia faba*. Extracts of 0.00625, 0.0125, 0.025, 0.05 and 0.1mg/mL were tested on meristematic cells of *A. cepa* and *V. faba* roots. This assessment includes the study of root growth, structure and coloration, as well as the determination of the mitotic index (MI) and chromosomal aberrations (CAs) as accurate indicators of toxicity. Our results showed a significant decrease in the mean length of roots treated with 0.025, 0.05 and 0.1 mg/ml for *A. cepa* and 0.1 mg/ml for *V. faba*. Cytotoxicity and genotoxicity results showed a significant decrease in MI from 0.025 mg/ml in *A. cepa* and from 0.05 mg/ml in *V. faba*, and this decrease in MI is linked to the increase in concentration and treatment time with *T. terrestris*. Furthermore, a significant increase in CAs was observed in A. cepa and *V. faba* from the 0.025 mg/ml concentration. The significant reduction in MI and CAs abundance suggests the genotoxicity of *T. terrestris*. Therefore, *T. terrestris* is a medicinal plant that should be used with caution, appropriately and based on essential therapeutic needs.

Keywords: *Tribulus terrestris*, *Allium cepa*, *Vicia faba*, cytotoxicity, genotoxicity.

INTRODUCTION

Recently, there has been a rapid increase in the use of dietary supplements derived from bioactive compounds of plant origin (Izzo *et al*. 2016). Herbal medicines are often used because many people believe that all that is natural is not toxic or harmful to health. This is a mistaken belief, as many therapeutic plants have high toxicity and harmful effects on human health (Proença da Cunha *et al*. 2012). Many studies examine the biological effects of extracts from different plants for their potential therapeutic use. However, there is little data available on the cyto-genotoxic effects of most plants.

Therefore, there is a need for research on these plants to assess their potential cytotoxic and genotoxic effects (Chukwujekwu and Van Staden 2014). Previous studies using different bioassays have revealed significant cytotoxic and genotoxic effects in different plants. Abudayyak *et al*. (2015) reported that *T. terrestris* L. had estrogenic and genotoxic activities in rat kidney cell lines exposed to this plant. Another study by Kumar *et al*. (2013) on the effect of *Tinospora cordifolia* in *A. cepa* meristematic cells showed a significant cytotoxic and genotoxic at high concentrations. In addition, Results obtained from the genotoxic study of Ayubi *et al*. (2021) showed that the hydro-alcoholic extract of *Z. multiflora* had no genotoxic effect. Bocayuva Tavares *et al*. (2021) reported that Seed proteins extract of *S. saponaria* causes cytotoxic and genotoxic effects in the human liver cancer cell line. In another study to evaluate the genotoxic effects of the plant *Angelica keiskei,* Maronpot (2015) demonstrated that it's not genotoxic in Chinese hamster ovary cells.

Researchers have long been interested in the genotoxic and cytotoxic effects of natural substances such as plant extracts. Higher plants, including *A. cepa* and *V. faba*, are used frequently to assess the genotoxicity of these environmental pollutants (Leme and Marin-Morales 2009). Furthermore, *A. cepa* and *V. faba* tests were ideal for evaluating chromosome damage and mitotic cycle disruptions due to their excellent chromosome characteristics, including large and fewer in number with a stable karyotype (Fiskesjo 1985). Moreover, this test method has demonstrated high sensitivity, which depends on a quick response in root development dynamics and simple detection of the endpoints associated with genotoxicity (Firbas and Amon 2014). Genotoxic endpoints include changes in MI values compared to controls (Akgündüz et al. 2020), morphological and number chromosome modifications expressed as CAs (Bonciu et al. 2018), and the frequency of Micronuclei as a simple quantitative characteristic (Bonciu et al. 2018; Younis et al. 2019).

T. terrestris is an annual plant from the Zygophyllaceae family. It has mainly cultivated in the Mediterranean and subtropical areas (Zhu *et al*. 2017). The *T. terrestris* extract is one of the natural therapeutic products that is used most frequently. This extract has shown several pharmacological activities, most of which are linked to diverse flavonoid and terpenoid components. These activities include antioxidants, antimicrobial, antibacterial, antitoxic, antiapoptotic molecules, platelet aggregation inhibitors, and anti-inflammation (Almasi *et al*. 2017). Also, these activities treat cardiovascular diseases, tumors, diabetes, respiratory diseases, and reproductive dysfunction (Qureshi *et al*. 2014). The widespread distribution of *T. terrestris*, its high content of active compounds (especially sterol saponins, as well as flavonoids, terpenoids, tannins, phenol, alkaloids and carboxylic acids), and the prevalence with which it is used in traditional medicine, all highlight the importance of analysing the phytopharmacological characteristics of the plant (Stefănescu *et al*. 2020), and due to the potential toxic effect of its active compounds, an assessment of a potential cytotoxic and genotoxic effect is essential for its use is safe and effective (Celik 2012). Although the therapeutic effects of this plant have been studied by several researchers, but the evaluation of the potential cytogenotoxic effects of *T. terrestris* on meristematic cells has not yet been studied.

Thus, the present work aimed to evaluate the cytotoxic and genotoxic effects of *T. terrestris* methanolic extract on *A. cepa* and *V. faba* roots by assessing the root growth, structure and color as well as the MI and CAs.

MATERIALS AND METHODS

Plant methanolic extract preparation

600 g of *T. terrestris* aerial parts (Trunk, branches and leaves) were obtained from a local medicinal plant market (Setif-Algeria). The plant material was identified by Dr. Sakhraoui Nora (a botanist) and then dried in the dark and powdered with a domestic mixer. 500 g of *T. terrestris* powder was dissolved in 4L of 96% hydro-methanolic solution (80%) for 24 hours to obtain a methanolic extract. The solution obtained was then double filtered using a Whatman No.1 paper filter. The filtrate obtained was then evaporated in a rotavapor (RE-100 pros) at 45°C to obtain a final dry residue. During processing, the dry residue was dissolved in distilled water to prepare the different concentrations of 0.00625, 0.0125, 0.025, 0.05 and 0.1 mg/mL for the different treatments.

Plants assay and application concentrations

Healthy onion bulbs (*A. cepa*, 2n = 16) and *V. faba* seeds were obtained from a local market. Both species were kept in tap water until their roots reached 1.5-2 cm. Then they were divided into six groups, one serving as a control, while the other five were given different concentrations (0.00625, 0.0125, 0.025, 0.05 and 0.1 mg/mL) of *T. terrestris* at 24°C. Each concentration was tested on a minimum of three bulbs, and seven seeds with solutions changed daily. Using a ruler, root length was measured

at 24 h, 48 h, 72 h, 96 h, and 120 h. Other indicators of toxicity, including root structure and color changes, are also assessed.

Genotoxicity assessment

To investigate the potential effect of genotoxicity, *A. cepa* bulbs and *V. faba* seeds with a root length of 1.5-2 cm were exposed to *T. terrestris* at different concentrations (0.00625, 0.0125, 0.025, 0.05 and 0.1mg/mL) for 12 h and 24 h. After treatment, the bulbs and seeds were washed thoroughly and then placed in an ethanol/acetic acid solution (3v/1v) for 24 h before storage in 70% ethanol at 4°C. After a brief wash with distilled water, the root tips were hydrolysed in 1 N HCl solution for 5 min at 60°C and stained with Schiff's reagent for 20 min. The slides were prepared following the method of Sharma and Sharma (2014). After the first pre-treatment, the root tips were carefully washed in distilled water several times. Then they were subjected to hydrolysis in a 1 N HCl solution for five minutes at a temperature between 60°C and 70°C. Then the apical 2 mm were crushed in a 45% acetic acid solution. The apical meristems were analysed at 40x magnification after being crushed in a drop of 45% acetic acid. The mitotic index and chromosome aberration index were calculated according to the methods of Fiskesjo (1985). using at least 1000 cells per slide and five slides for each concentration.

The following formulae (Akwu *et al*. 2019) were used to determine the proportion of chromosomal aberrations (CAs), the mitotic index (MI), and the chromosomal aberration frequency (AF) in the cells:

$$\text{Mitotic index (MI) (\%)} = \frac{Total number of dividing cells}{Total number of cells observed} \text{x } 100$$

$$\text{Chromosome Aberrations (CAs) (\%)} = \frac{Total number of abnomal cells}{Total number of cells observed} \text{x } 100$$

$$\text{Aberrations type frequency (AF) (\%)} = \frac{Number of a type of aberrant cell}{Total number of aberrant cells} \text{ x } 100$$

Statistical data analysis

Graph pad prism 9.2.0 (Graph Pad Software, LLC, CA, USA) was used for statistical analysis of root length, MI (%), and ACs (%). Data Results were compared statistically using one-way ANOVA and Dunnett's multiple comparison test. All values were expressed as mean ± SD and were determined statistically significant when $P<0.05$.

RESULTS

The changes in root length, form and color of roots are illustrated in Table 1 (*A. cep*a) and Table 2 (*V. faba*) following different treatment with *T. terrestris* compared to the control. It was observed that the inhibition effect of *T. terrestris* increased with the increase in concentration and duration of treatment. Therefore, control showed highest root elongation, with mean lengths of 10.77 ± 0.06 cm and 10.48 ± 0.05 cm after 120 h in *A. cepa* and *V. faba*, respectively.

In other hand, throughout the five-day treatment, the mean root lengths of *A. cepa* were unaffected by the 0.00625 mg/mL and 0.0125 mg/mL concentrations and 0.00625, 0.0125, 0.025, and 0.05 mg/mL concentrations in *V. faba*.

However, *A. cepa* roots exposed to the 0.025, 0.05, and 0.1 mg/mL concentrations range showed a significant decrease in root growth with values of 4.71, 3.92, and 3.16 cm, respectively. Moreover, a significant decrease ($P<0.05$) in root length was seen in *V. faba* roots treated with the 0.1 mg/mL concentration (3.33 cm) compared to the control. Simultaneously, the inhibitory effect of *T. terrestris* increased positively with concentration and time of exposure, whose value increased from 26.84% (0.006125 mg/mL) to 62.77% (0.1 mg/mL) after 120 h of exposure. Furthermore, it was remarked that *A. cepa* roots were more sensitive to *T. terrestris* than *V. faba* roots (62.77% and 55.92% inhibition, respectively).

Concerning the morphology of the roots, structural and color modifications were observed, particularly in *V. faba* roots treated from the 0.05 mg/mL concentration, which appeared slimy to slimy dark brown compared to the control. However, after 120 h of treatment, roots treated to the 0.05 and 0.1 mg/mL concentrations showed necrosis.

Figure 1 shows the effect of different *T. terrestris* concentrations on *A. cepa* and *V. faba* mitotic index. Meristematic cells of these two plants that are treated with different concentrations of *T. terrestris* showed a significant decrease in MI compared to the control. Our result showed that the control has the highest MI in both *A. cepa* and *V. faba* (59.26 ± 0.88% and 59.90 ± 0.40%, respectively) (12 h), and (60.74 ± 0.45% and 60.14 ± 0.48%, respectively) (24 h). In addition, Cell division was unaffected by the concentrations of 0.00625, 0.0125, and 0.025 mg/mL in *A. cepa* and 0.00625 and 0.0125 mg/mL in *V. faba*. In contrast, the values were as high as the control (12 h and 24 h) in *A. cepa* (60.80 ± 1.12%, and 60.24 ± 1.00%, respectively) and *V. faba* (59.88 ± 0.32% and 58.14 ± 0.35%, respectively). However, cytotoxic

Table 1. The change in mean root length (cm) of *A. cepa* after application of different concentrations (%) of *T. terrestris* at different exposure times .

Treatments (mg/ml)	Nr	Mean root length (cm) and inhibition (%) affected by *T. terrestris* treatment in *A. cepa.*											
		00 h	24 h		48 h		72 h		96 h		120 h		Mean of longth ± SD	Form and color
		Mean ± SD	Mean ± SD	In %	Mean ± SD	In %	Mean ± SD	In %	Mean ± SD	In %	Mean ± SD	In %		
Control	15	1.78 ± 0.08	4.18 ± 0.03	-	5.65 ± 0.02	-	7.32 ± 0.05	-	9.57 ± 0.09	-	10.77 ± 0.06	-	7.49 ± 2.71	Straight, white
0.00625	15	1.87 ± 0.07	4.01 ± 0.04	4.07	5.02 ± 0.03	11.16	5.84 ± 0.04	20.22	7.03 ± 0.07	26.55	6.91 ± 0.03	26.84	5,95± 1,54	Straight, white
0.0125	15	1.87 ± 0.07	3.83 ± 0.07	8.38	4.55 ± 0.06	19.47	5.09 ± 0.04	30.47	6.66 ± 0.07	30.41	5.09 ± 0.03	31.30	5.50 ± 1.48	Straight, white
0.025	15	1.71 ± 0.07	3.56 ± 0.04	14.84	4.20 ± 0.11	25.67	4.93 ± 0.06	32.66	5.35 ± 0.04	44.10	4.71 ± 0.03	48.57	4.71 ± 0.82*	Straight, white
0.05	15	1.86 ± 0.05	2.98 ± 0.03	28.71	3.3 ± 0.04	41.60	3.97 ± 0.03	45.77	4.45 ± 0.09	53.51	4.30 ± 0.04	54.14	3.92 ± 0.80**	Slimy, white
0.1	15	1.69 ± 0.02	2.24 ± 0.06	46.42	2.95 ± 0.05	47.79	3.01 ± 0.08	58.88	3.59 ± 0.05	62.49	4.19 ± 0.04	62.77	3.16 ± .67***	Slimy, white

Data are shown as mean ± SD, Nr: Number of roots, In (%): the inhibition percentage, * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ versus control group using one-way ANOVA; Dunnett's test.

Table 2. The change in mean root length (cm) of *V. faba* after application of different concentrations (%) of *T. terrestris* at different exposure times.

Treatments (mg/ml)	Nr	Mean root length (cm) and inhibition (%) affected by *T. terrestris* treatment in *V. faba*											
		00 h	24 h		48 h		72 h		96 h		120 h		Mean of longth ± SD	Form and color
		Mean ± SD	Mean ± SD	In %	Mean ± SD	In %	Mean ± SD	In %	Mean ± SD	In %	Mean ± SD	In %		
Control	7	1.78 ± 0.08	3.38 ± 0.04	-	4.78 ± 0.06	-	6.24 ± 0.04	-	8.55 ± 0.06	-	10.48 ± 0.04	-	6.68 ± 2.85	Straight, white
0.00625	7	1.79 ± 0.07	3.20 ± 0.04	5.53	4.27 ± 0.05	10.67	5.01 ± 0.04	19.72	6.77 ± 0.07	20.82	8.21 ± 0.06	21.67	5.49 ± 2.00	Straight, white
0.0125	7	1.77 ± 0.02	3.01 ± 0.06	10.95	4.17 ± 0.02	12.77	4.77 ± 0.03	23.56	6.02 ± 0.05	29.60	7.32 ± 0.06	30.16	5.05 ± 1.66	Straight, white
0.025	7	1.74 ± 0.06	2.89 ± 0.07	14.50	3.68 ± 0.04	23.02	4.04 ± 0.05	35.26	5.22 ± 0.08	38.95	6.01 ± 0.03	44.56	4.36 ± 1.24	Slimy, white
0.05	7	1.80 ± 0.03	2.32 ± 0.06	31.37	2.98 ± 0.03	37.66	3.67 ± 0.06	41.19	4.52 ± 0.05	47.14	4.99 ± 0.02	52.39	3.69 ± 1.09	Slimy, dark brown
0.1	7	1.81 ± 0.06	2.22 ± 0.07	34.32	2.58 ± 0.10	46.03	3.24 ± 0.06	48.08	3.99 ± 0.09	50.34	4.62 ± 0.05	55.92	3.33 ± 0.98 *	Slimy, dark brown

Data are shown as mean ± SD, Nr: Number of roots, In (%): The inhibition percentage, * $p < 0.05$ versus control, used one-way ANOVA; Dunnett test.

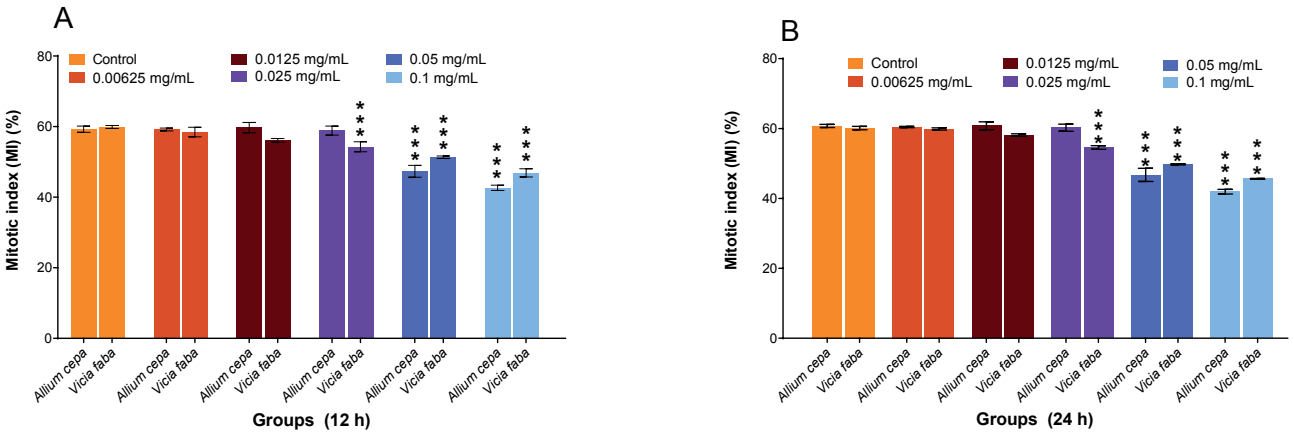


Figure 1. The effect of different *T. terrestris* concentrations (mg/mL) and exposure times on the MI (%) in *A. cepa* cells and *V. faba* cells after 12 h (A) and 24 h (B). Three bulbs and seven seeds were treated in each concentration, including the control. Five roots for each concentration were used. At least 1000 cells on each slide and five slides for each concentration were examined.

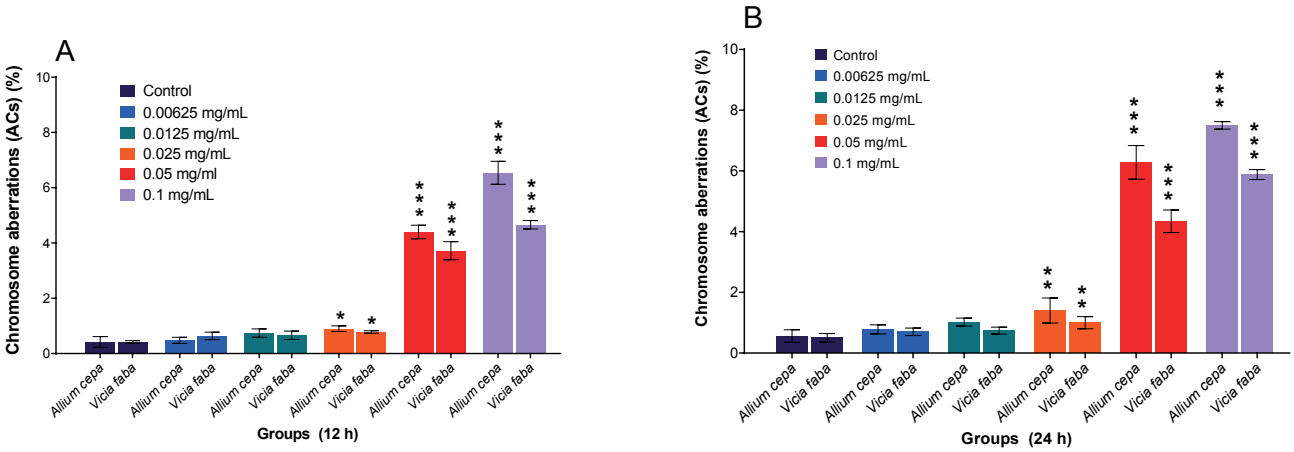


Figure 2. Percentage of CAs in *A. cepa* and *V. faba* cells induced by different concentrations of *T. terrestris* (mg/mL) after 12 h (A) and 24 h (B). Three bulbs and seven seeds were treated in each concentration, including the control. Five roots for each concentration were used. At least 1000 cells on each slide and five slides for each concentration were examined.

effects were observed from the 0.025 mg/ml concentration in *V. faba* and 0.05 mg/mL in *A. cepa* accompanied by a significant decrease in MI ($P<0.001$) (12 h and 24 h).

Figures 2, 3, 4, and 5 show *T. terrestris*-induced aberration percentages and different chromosomal abnormalities. It was showed that the increase in CAs (%) depends on *T. terrestris* concentration and treatment duration.

Compared to the control, no significant effect on CAs (%) was noted after treatment with the concentrations 0.00625 and 0.05 mg/mL (12 h and 24 h) in *A. cepa* and *V. faba*. While a significant increase ($P<0.001$) in ACs (%) was observed in cells treated with the concentrations 0.025, 0.05, and 0.1 mg/mL (12 h and 24 h) compared to the control.

The highest CAs (%) in *A. cepa* and *V. faba* were 7.5 ± 0.129% and 5.88 ± 0.16%, respectively, after 24 h treatment with *T. terrestris* at the 0.1 mg/mL concentration, and the lowest was 0.48 ± 0.10% and 0.64 ± 0.13%, respectively at the 0.00625 mg/mL concentration (12 h).

The most frequent types of CAs were multipolar in *A. cepa* (34.89 ± 2.30%) (12 h) and (33.97 ± 4.36%) (24 h) and in *V. faba* (34.08 ± 2.23%) (12 h) and (30.96 ± 4.13%) (24 h), followed by break in *A. cepa* (19.89% ± 1.75%) (12 h) and (26.07 ± 3.79%) (24 h) and in *V. faba* (24.20 ± 0.79%) (12h) and (24.19 ± 1.35%) (24 h), stickiness in *A. cepa* (17.49 ± 1.25%) (12 h) and (20.24 ± 3.58%) (24 h) and in *V. faba* (20.05 ± 3.79%) (12 h) and (19.18 ± 1.77%) (24 h), vagrant in *A. cepa* (18.94 ± 2.27%)

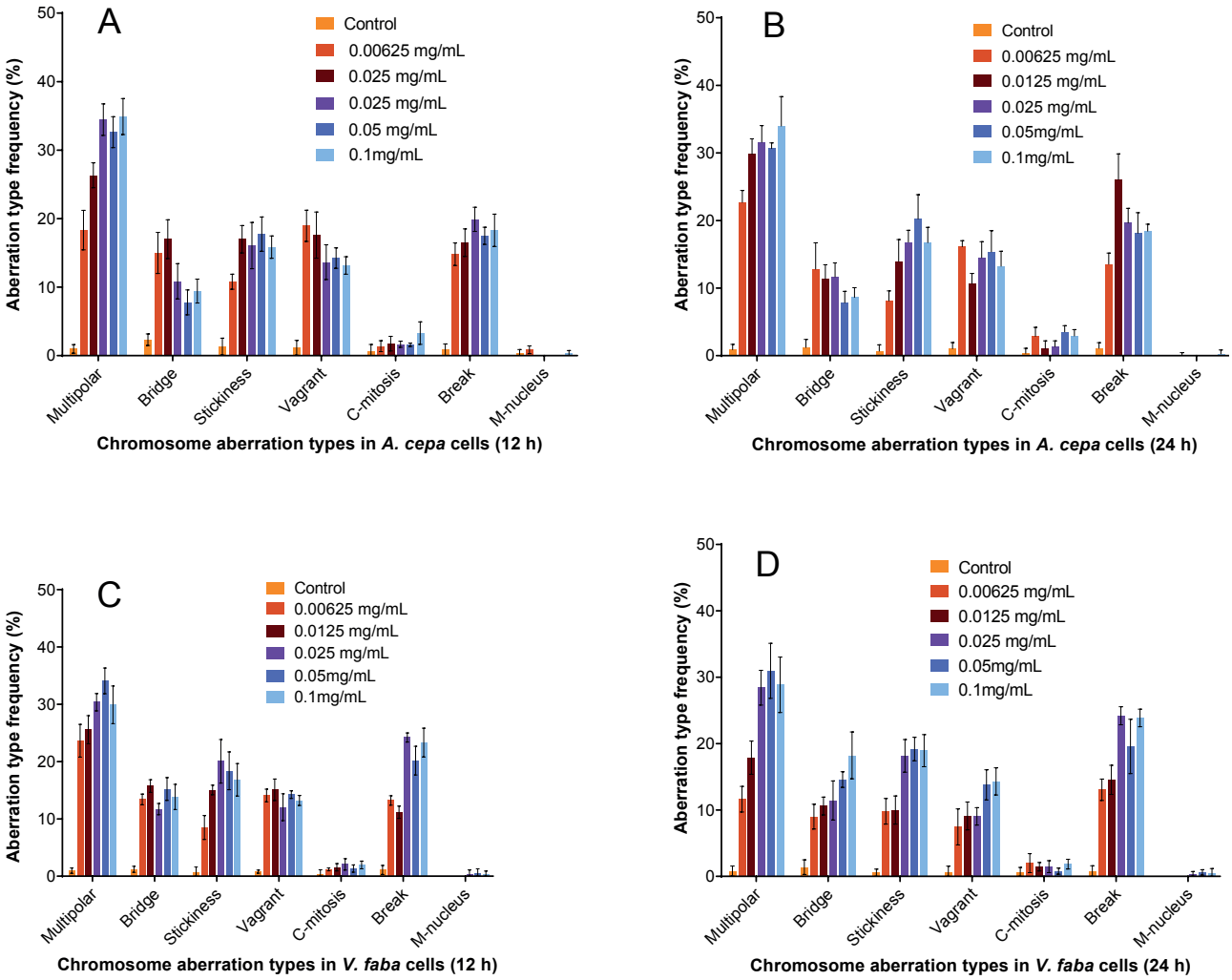


Figure 3. Frequency of different types of CAs induced by different concentrations of *T. terrestris* (mg/mL) in *A. cepa* cells after 12 h (A) and after 24 h (B) and in *V. faba* cells after 12 h (C) and after 24 h (D). Three bulbs and seven seeds were treated in each concentration, including the control. Five roots for each concentration were used. At least 1000 cells on each slide and five slides for each concentration were examined.

(12 h) and (15.38 ± 3.11%) (24 h) and in *V. faba* (15.07 ± 1.88%) (12 h) and (14.31 ± 2.05%) (24h), bridge in *A. cepa* (17.00 ± 2.82%) (12 h) and (12.80 ± 3.89%) (24 h) and in *V. faba* (15.73 ± 1.10%) (12 h) and (18.22 ± 3.52%) (24 h), C-mitosis in *A. cepa* (3.29 ± 1.63%) (12 h) and (3.52 ± 0.91%) (24 h) and in *V. faba* (2.06 ± 0.98%) (12 h) and (1.89 ± 1.34%) (24 h), and M-nucleus in *A. cepa* (0.35 ± 0.39%) (12 h) and (0.52 ± 0.75%) (24 h) and in *V. faba* (0.54 ± 0.74%) (12 h) and (0.59 ± 0.49%) (24 h) (Fig. 3A, 3B, 3C, 3D).

DISCUSSION

This study was conducted to assess the cytotoxic and genotoxic effects of *T. terrestris* methanolic extract by analysing the change in root growth, morphology, and color of *A. cepa* and *V. faba*. as well as the determination of MI and different types of CAs. Our results revealed that *T. terrestris* treatment induced cytotoxic and genotoxic effects manifested by inhibition of root growth, modification of root colour and structure, decrease in MI (%), and increase in CAs (%) with the appearance of various types of chromosomal aberrations. These changes are dependent on the different concentrations and duration of exposure to *T. terrestris*. Furthermore,

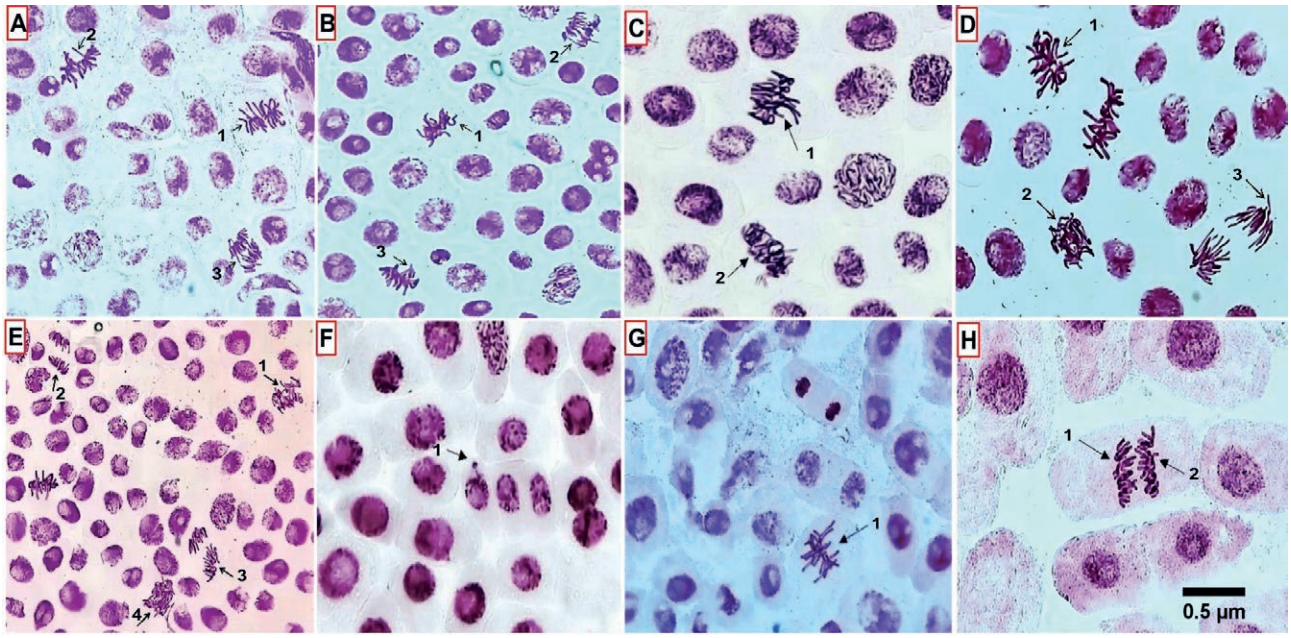


Figure 4. Photomicrographs showing different CAs induced by different concentrations of *T. terrestris* in *A. cepa* root tip cells: vagrant (A1) with break (A2), and bridge (A3), multipolar and bridge (B2), break (B1) and stickiness (B3), stickiness (C1, C2), c-mitosis (D1), stickiness (D2) and break (D3), stickiness (E1, E4) and multipolar (E2, E3), micro-nucleus (F1), c-mitosis (G1), stickiness (H1, H2). After 12 h and 24 h of treatment. scale bar = 0.5 μm.

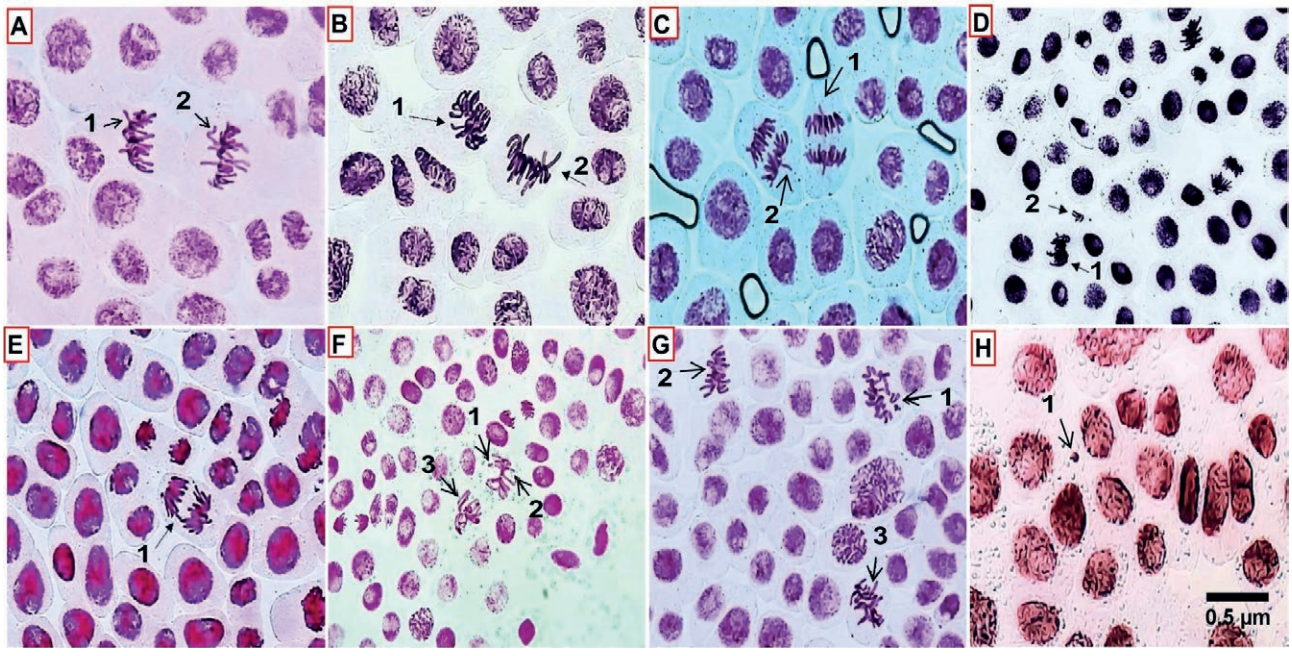


Figure 5. Photomicrographs showing different CAs induced by different concentrations of *T. terrestris* in *V. faba* root tip cells: stickiness (A1) and vagrant (A2), stickiness (B1, B2), multipolar (C1) and multipolar with break (C2), stickiness (D1) and vagrant (D2), multipolar with bridge (E1), break (F1, F2, F3), c-mitosis (G1) and stickiness (G2, G3), micro-nicleus (H1). After 12 h and 24 h of treatment. scale bar = 0.5 μm.

the results obtained on root growth and structure are in agreement with the results of Basu and Tripura (2021) on *Cascabela thevetia* who found a decrease in root growth, turgescence, and color change in *A. cepa* and *V. faba* treated with a high concentration of *Cascabela thevetia* extract. Similar results were approved by the studies of Issa *et al*. (2020) on the roots of *Avena fatua* and *Echinochloa crus-galli* exposed to a high concentration of *Vitex negundo*. According to Wierzbicka (1988), root growth is directly related to the enzymatic activity and cell elongation of the meristematic zone. This activity promotes cell elongation and membrane release during cell differentiation (Silveira *et al*. 2017). Thus, the slowing of root growth may be due to the inhibitory effect of *T. terrestris* on the enzymatic activity that promotes the elongation of the meristematic region.

The mitotic index (MI) is an indicator to determine the cytotoxicity induced by toxic substances (Leme and Marin-Morales 2009). The MI is also used to measure the portion of dividing and arrested cells during the cell cycle (Rojas *et al*. 1993). In this study, the decrease in MI after 12 h and 24 h of treatment with *T. terrestris* suggested the significant cytotoxic effect of this plant. Furthermore, our results indicate that *T. terrestris* inhibits cell division in *A. cepa* and *V. faba*, significantly reducing MI at high concentrations. The cytotoxic and genotoxic effects of plants are evaluated by several studies, including *Vitex negundo* (Issa *et al*. 2020), *Citrus aurantiifolia* (Fagodia *et al*. 2017) and *Plantago major* (Ždralović *et al*. 2019). However, research investigating the genotoxicity of *T. terrestris* on meristematic cells is scarce.

The current study showed a correlation between the increase in *T. terrestris* concentration and the reduction of MI. According to the results of Qari and El-Assouli (2019), the aqueous extract of *T. terrestris* fruit can inhibit the proliferation of human lymphocytes in culture. This decrease in IM could be caused by the arrest of mitotic phases or by decelerating the cytokinesis process (Kundu and Ray 2017). However, our results suggest that this inhibition is caused by the genotoxic effects of one or more components of the *T. terrestris* extract that can damage DNA strands in a specific way (Qari and El-Assouli 2019). In addition, Kundu and Ray (2017) found that *T. terrestris* fruit extract can inhibit cell division due to a DNA defect, suggesting that it could be used as an anticancer agent based on its ability to inhibit cell proliferation, its safety on the DNA molecule at lower doses, and its antioxidant component.

Different types of chromosomal aberrations (CAs) were observed at all concentrations applied. There is a significant increase in CAs (%) from the concentration of 0.025 mg/mL. It also produced aberrant chromosome segregation and caused the formation of various anomalies such as multipolar, chromosome bridge, stickiness, vagrant, c-mitosis and micro-nucleus. Similar results were obtained by *Anita Sharma et al*. (2019) in *A. cepa* root cells treated with *H. suaveolens* extract. Furthermore, Sabeen *et al*. (2020) reported that CAs are produced by proteolysis and by blocking DNA synthesis. Data analysis showed that multipolar anaphases were the most common CAs. Khallef *et al*. (2019) suggests that mitotic spindle instabilities may produce anaphase multipolarity. In addition, Sabeen *et al*. (2020) suggests that CAs, like chromosomal breaks and bridges, indicate clastogenic activity. However, stickiness, which causes cell death, may be caused by excess chromosomal condensation or inappropriate nucleoprotein biosynthesis (Sabeen *et al*. 2020). According to Mercykutty and Stephen (1980), stickiness may be caused by the depolymerization of DNA, the partial dissolution of nucleoproteins, the breakage and exchanges of the basic folded fiber units of chromatids, and the stripping of the protein covering of DNA in chromosomes.

This study typically found chromosome break formation and stickiness. Kuchy *et al*. (2015) suggests that chromosomal condensation or excessive nucleoprotein production can form stickiness. Another notable abnormality was the chromosomal vagrant. Due to spindle abnormality, vagrant chromosomes are induced, resulting in the dissociation of an unequal distribution of chromosomes in the daughter nuclei and the generation of daughter cells with abnormally small or sized nuclei during interphase (El-Ghamery *et al*. 2003). C-mitosis and micro-nucleus were rare in our results and their presence may be due to the spindle apparatus' incapacity to arrange and function appropriately (Rosculete *et al*. 2019).

T. terrestris has been traditionally used for medicinal purposes throughout history, addressing various health issues such as impotence, rheumatism, edema, hypertension, and kidney stones (Chhatre *et al*. 2014). Pharmacological studies conducted on *T. terrestris* have demonstrated its aphrodisiac, analgesic, antibiotic, antihyperglycemic, antihyperlipidemic, larvicidal, repellent, antioxidant, cytotoxic, immunomodulatory, hypolipidemic, anticancer, antibacterial, and antifungal properties (Chhatre et al., 2014). Furthermore, in order to ensure safe therapy, it is important to test the cytogenotoxicity of *T. terrestris*. Our current study on the cytogenetic impact of *T. terrestris* revealed cytotoxic and genotoxic effects depending on the concentrations applied and the duration of treatment. It observed that signs of toxicity appeared from a concentration of

0.05 mg/mL, resulting in a significant reduction in the mitotic index and a significant increase in chromosomal aberrations, as well as a change in the shape and colour of the roots of *A. cepa* and *V. faba*, indicating necrosis. Our results underline the interest in using *T. terrestris* as an effective medicinal plant for various diseases, but its use must respect appropriate therapeutic doses and not be anarchic.

CONCLUSION

This study is the first to investigate the cytotoxic and genotoxic effects of *T. terrestris* on meristematic cells of *A. cepa* and *V. faba*. Decreased root growth with decreased mitotic index and increased chromosomal abnormalities resulting from treatment with *T. terrestris* are signs of cytotoxicity and genotoxicity. Therefore, this plant should be used with caution in traditional medicine.

ACKNOWLEDGEMENTS

The authors would like to thank all those who contributed to the completion of this work. Special thanks go to the Laboratory of Research in Biodiversity Interaction, Ecosystem and Biotechnology "LRIBEB", where this research was conducted.

REFERENCES

Abudayyak M, Jannuzzi AT, Özhan G, Alpertunga B. 2015. Investigation on the toxic potential of Tribulus terrestris in vitro. Pharm Biol 53(4):469-476.

Akgündüz MÇ, Çavuşoğlu K. and Yalçın E. 2020. The Potential Risk Assessment of Phenoxyethanol with a Versatile Model System. Sci Rep 10: 1-10.

Akwu NA, Naidoo Y, Singh M. 2019. Cytogenotoxic and biological evaluation of the aqueous extracts of Grewia lasiocarpa: An Allium cepa assay. S Afr J Bot 125: 371-380

Almasi F, Khazaei M, Chehrei S, Ghanbari A. 2017. Hepatoprotective Effects of Tribulus terrestris Hydro-Alcholic Extract on Non-Alcoholic Fatty Liver-Induced Rats Int J Morphol 35(1):345-350.

Ayubi Z, Jafari AA, Ayatollahi-Mousavi SA, Jafari H, Panddeh M. 2021. Using Comet Assay for Genotoxic Evaluation of Zataria multiflora Boiss., An Iranian Endemic Plant. J Food Qual Hazards Control 8(1):45-49.

Basu S, Tripura K. 2021. Differential sensitivity of Allium cepa L. and Vicia faba L. to aqueous extracts of Cascabela thevetia (L.) Lippold. S Afr J Bot 139: 67-78.

Bonciu E, Firbas P, Fontanetti CS, Wusheng J, Karaismailoğlu MC, Liu D, Menicucci F, Pesnya DS, Popescu A, Romanovsky AV, Schiff S, Ślusarczyk J, de Souza CP, Srivastava A, Sutan A and Papini A. 2018. An evaluation for the standardization of the Allium cepa test as cytotoxicity and genotoxicity assay. Caryologia 71(3):191-209.

Bocayuva Tavares GD, Fortes Aiub CA, Felzenszwalb I, Carrão Dantas EK, Araújo-Lima CF, Siqueira Júnior CL. 2021. In vitro biochemical characterization and genotoxicity assessment of Sapindus saponaria seed extract. J Ethnopharmacol 276:114170.

Celik TA. 2012. Potential Genotoxic and Cytotoxic Effects of Plant Extracts. A Com- pendium of Essays on Alternative Therapy, Dr. Arup Bhattacharya ed. 233-250

Chhatre S, Nesari, T, Somani G, Kanchan D and Sathaye S. (2014). Phytopharmacological overview of Tribulus terrestris. Pharmacog Rev 8(15): 45–51.

Chukwujekwu JC, Van Staden J. 2014. Cytotoxic and genotoxic effects of water extract of Distephanus angulifolius on Allium cepa Linn. S Afr J Bot 92:147-150.

Eck-Varanka B, Kováts N, Hubai K, Paulovits G, Ferincz Á, Horváth E. 2015. Genotoxic effect of Lythrum salicaria extract determined by the mussel micronucleus test short communication. Acta. Biol. Hung 66(4):460–463.

El-Ghamery AA, El-Kholy MA, Abou El-Yousser MA. 2003. Evaluation of cytological effects of Zn2+ in relation to germination and root growth of Nigella sativa L. and Triticum aestivum L. Mutat Res Genet Toxicol Environ Mutagen 537(1):29-41.

Fagodia SK, Singh HP, Batish DR, Kohli RK. 2017. Phytotoxicity and cytotoxicity of Citrus aurantiifolia essential oil and its major constituents: Limonene and citral. Ind Crops Prod 108:708-715.

Firbas P and Amon T. 2014. Chromosome damage studies in the onion plant Allium cepa L. Caryologia 67(1): 25-35.

Fiskesjo Geirid. 1985. The Allium test as a standard in environmental monitoring. Hereditas 12(102):345-361.

Fiskesjo Geirid. 1985. The Allium test as a standard in environmental monitoring. Hereditas 102(1):99-112.

Issa M, Chandel S, Pal Singh H, Rani Batish D, Kumar Kohli R, Singh Yadav S, Kumari A. 2020. Appraisal of phytotoxic, cytotoxic and genotoxic potential of essential oil of a medicinal plant Vitex negundo. Ind Crops Prod 145:112083.

Izzo AA, Hoon-Kim S, Radhakrishnan R, Williamson EM. 2016. A Critical Approach to Evaluating Clinical Efficacy, Adverse Events and Drug Interactions of Herbal Remedies. Phytother Res 30(5):691-700.

Khallef M, Benouareth DE, Konuk M, Liman R, Bouchelaghem S, Hazzem S, Kerdouci K. 2019. The effect of silver nanoparticles on the mutagenic and the genotoxic properties of the urban wastewater liquid sludges. Sci Pollut Res 26(18):18403-18410.

Kuchy AH, Wani AA, Kamili AN. 2015. Cytogenetic effects of three commercially formulated pesticides on somatic and germ cells of Allium cepa. Sci Pollut Res 23(7):6895–6906.

Kumar A, Dandapat S, Kumar M, Sinha MP. 2013. Evaluation of Genotoxicity and Cytotoxicity of Tinospora Cordifolia. Bioscan 8(3):1083-1088.

Kundu LM, Ray S. 2017. Mitotic abnormalities and micronuclei inducing potentials of colchicine and leaf aqueous extracts of Clerodendrum viscosum Vent. in Allium cepa root apical meristem cells. Caryologia 70(1):7-14.

Leme DM, Marin-Morales MA. 2009. Allium cepa test in environmental monitoring: A review on its application. Mutat Res Rev Mutat Res 682(1):71-81.

Maronpot RR. 2015. Toxicological assessment of Ashitaba Chalcone. Food Chem Toxicol 77:111-119.

Mercykutty VC, Stephen J. 1980. Adriamycin Induced Genetic Toxicity as Demonstrated by the Allium Test. Cytologia 45(4):769-777.

Proença da Cunha A, da Silva AP and Roque OR. 2012. Plantas e Produtos Vegetais em Fitoterapia, 4a Edição. Fundação Calouste Gulbenkian: Lisbon.

Qari SH, El-Assouli SM. 2019. Evaluation of cytological and genetic effects of Tribulus terrestris fruit aqueous extract on cultured human lymphocytes. Saudi J Biol Sci 26(1):91–95.

Qureshi A, Naughton DP, Petroczi A. 2014. A systematic review on the herbal extract tribulus terrestris and the roots of its putative aphrodisiac and performance enhancing effect. J Diet Suppl 11(1):64-79).

Rojas E, Herrera LA, Sordo M, Gonsebatt ME, Montero R, Rodriguez R, Ostrosky-Wegman P. 1993. Mitotic index and cell proliferation kinetics for identification of antineoplastic activity. Anti Cancer Drugs 4(6):637-640.

Rosculete CA, Bonciu E, Rosculete E, Olaru LA. 2018. Determination of the environmental pollution potential of some herbicides by the assessment of cytotoxic and genotoxic effects on allium cepa. Int J Environ Res Public Health 16(1):1-10

Sabeen M, Mahmood Q, Ahmad Bhatti Z, Faridullah Irshad M, Bilal M, Hayat MT, Irshad U, Ali Akbar T, Arslan M, Shahid N. 2020. Allium cepa assay based comparative study of selected vegetables and the chromosomal aberrations due to heavy metal accumulation. Saudi J Biol Sci 27(5):1368-1374.

Sharma A, Sharma A. 2014. Chromosome techniques: theory and practice. Butterworth-Heinemann.

Sharma A, Singh HP, Batish DR, Kohli RK. 2019. Chemical profiling, cytotoxicity and phytotoxicity of foliar volatiles of Hyptis suaveolens. Ecotoxicol Environ Saf 171:863-870.

Silveira GL, Lima MGF, Reis GB, Palmieri MJ, Andrade-Vieria LF. 2017. Toxic effects of environmental pollutants: Comparative investigation using Allium cepa L. and Lactuca sativa L. Chemosphere 178:359-367.

Stefănescu R, Tero-Vescan A, Negroiu A, Aurică E, And Camil-Eugen Vari. 2020. A Comprehensive Review of the Phytochemical , Pharmacological , and Toxicological Properties of. Biomolecules 10(752):1-32.

Younis ME, Abdel-Aziz HMM and Heikal YM. 2019. Nanopriming technology enhances vigor and mitotic index of aged Vicia faba seeds using chemically synthesized silver nanoparticles. S. Afr. J. Bot 125:393-401.

Wierzbicka M. 1988. Mitotic disturbances induced by low doses of inorganic lead. Caryologia, 41(2):143-160.

Ždralović A, Mesic A, Eminović I, Parić A. 2019. Cytotoxic and genotoxic activity of Plantago major l. Extracts. Caryologia 72(3):35-40.

Zhu W, Du Y, Meng H, Dong Y, Li L. 2017. A review of traditional pharmacological uses , phytochemistry , and pharmacological activities of Tribulus terrestris. Chem Cent J 11(60):1-16.

Caryologia
International Journal of Cytology, Cytosystematics and Cytogenetics

Firenze University Press
www.fupress.com/caryologia

OPEN ACCESS

Citation: Esra Martin, Metin Armağan, Halil Erhan Eroğlu, Aslı Doğru-Koca, Osman Tugay, Golshan Zare, Osman Kola, Mahmut Miski, Nur Tan, Ernst Vitek (2023). New chromosomal data, karyotype asymmetry and polyploid variations of some *Gundelia* (Asteraceae) species from Turkey. *Caryologia* 76(1): 87-95. doi: 10.36253/caryologia-2103

Received: April 8, 2023

Accepted: July 10, 2023

Published: September, 19, 2023

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

ORCID
EM: 0000-0002-5484-0676
MA: 0000-0002-3913-954X
HEE: 0000-0002-4509-4712
AD-K: 0000-0001-7477-0225
OT: 0000-0003-3980-7648
GZ: 0000-0002-5972-5191
OK: 0000-0003-0000-248X
MM: 0000-0003-2653-0563
NT: 0000-0001-7958-1917
EV: 0000-0002-8977-1754

New chromosomal data, karyotype asymmetry and polyploid variations of some *Gundelia* (Asteraceae) species from Turkey

Esra Martin[1], Metin Armağan[2], Halil Erhan Eroğlu[3,*], Aslı Doğru-Koca[4], Osman Tugay[5], Golshan Zare[6], Osman Kola[7], Mahmut Miski[8], Nur Tan[9], Ernst Vitek[10]

[1] *Necmettin Erbakan University, Faculty of Science, Department of Biotechnology, Konya, Turkey*
[2] *Necmettin Erbakan University, Ereğli Faculty of Agriculture, Department of Field Crops, Konya, Turkey*
[3] *Yozgat Bozok University, Faculty of Science and Arts, Department of Biology, Yozgat, Turkey*
[4] *Hacettepe University, Faculty of Science, Department of Biology, Ankara, Turkey*
[5] *Selçuk University, Faculty of Pharmacy, Pharmaceutical Botany, Konya, Turkey*
[6] *Hacettepe University, Faculty of Pharmacy, Pharmaceutical Botany, Ankara, Turkey*
[7] *Adana Alparslan Türkeş Science and Technology University, Faculty of Engineering, Department of Food Engineering, Adana, Turkey*
[8] *İstanbul University, Faculty of Pharmacy, Department of Pharmacognosy, Istanbul, Turkey*
[9] *İstanbul University, Center for Research and Practice in Drug Development from Natural Sources, Istanbul, Turkey*
[10] *Naturhistorisches Museum Wien, Department of Botany, Wien, Austria*
*Corresponding author. E-mail: herhan.eroglu@bozok.edu.tr

Abstract. The genus *Gundelia* is currently represented with 18 species, of which 16 are in Turkey. In genus *Gundelia*, the chromosomal data were reported from 12 species. In the present study, it is aimed to eliminate the deficiencies in the knowledge about chromosomal data of *Gundelia* species. In Genus *Gundelia*, only a single chromosome number had been detected as $2n$=18 so far. The chromosome numbers of four species were reported here for the first time: *G. armeniaca*, *G. cappadocica*, *G. siirtica*, and *G. tehranica*. In addition, the polyploidy in the genus was rare and *G. anatolica* was identified as the first polyploid species. All karyotypes except *G. tehranica* were symmetrical, consisting of metacentric and submetacentric chromosomes. Secondary constrictions were observed in the distal regions of the long arms of the longest metacentric and submetacentric chromosomes. Thus, the chromosomal data of all Turkish *Gundelia* species were completed. In conclusion, the present study presented new data into the karyological records relating the karyotype evolution and interspecific relations of genus *Gundelia*.

Keywords: *Gundelia*, karyology, polyploidy, dysploidy, Anatolia.

INTRODUCTION

Gundelia was first collected by Leonhard Rauwolf during his travel to the Levant between 1573 and 1575 for his herbal medicine and botanical collections. Rauwolf and some have confused it with Dioscorides' Slybum, which today is considered to be *Silybum marianum* (L.) Gaertn. (Asteraceae). Based on Rauwolf's description and the similarity of the synflorescens of several *Eryngium* L. (Apiaceae) species, Morison recommended the name - *Eryngium Surgerycum foliis Chamaeleontis longis & spinosis* - at 17th century (Hind 2013). Linnaeus described *G. tournefortii* in 1753, after which many authors accepted *Gundelia tournefortii* monospecifically and the described taxa thereafter synonymous of it (Linnaeus 1753; Komarov 1961; Kupicha 1975; Feinbrun Dothan 1978; Meikle 1985; Rechinger 1989). The genus is currently represented with 18 species, of which 16 (12 endemic) are in Turkey (Vitek et al. 2010, 2014, 2017; Nersesyan 2014; Armağan 2016; Fırat 2016, 2017a, 2017b, 2017c; Vitek and Noroozii 2017; Vitek 2018; Vitek and Armağan 2023).

Due to the monotypic genus of *Gundelia*, not many investigations have been studied with molecular techniques. In the first studies, the phylogenetic position of the genus *Gundelia* in the family was determined (Karis et al. 2001, Funk et al. 2004, Panero and Croizer 2016). According to Panero and Croizer (2016), the genus is a member of the subfamily Cichorioideae. After the new species started to be published, whether the species were phylogenetically supported became a matter of curiosity. Firstly, with limited species and samples, Vitek et al. (2010) and Tarıkahya-Hacıoğlu and Fırat (2017) were studied using the internal transcribed spacer marker. Finally, in 2021, an updated and expanded phylogeny based on DNA sequences of both ITS and ndhF genes was published by Ateş et al. (2021).

Gundelia genus has special inflorescence different from other genus in the Asteraceae family. The synflorescens of *Gundelia* as a whole in inflorescence is a composition of partial synflorescens (disseminules) in the axils of bracts. These partial synflorescens (disseminules) consist of 3-9 flowers and this number is an important characteristic structure for each species (Classen-Bockhoff et al. 1989).

Gundelia grows in the semi-humid to dry meadows, mountain (steppe) meadows, dry slope areas. The evidence showed that the center of diversity of *Gundelia* is Turkey, even if the genus is distributed in the area from Armenia to Egypt and from Turkey to Afghanistan (Figure 1). *Gundelia* is present everywhere in Turkey except the Black Sea, Marmara and coastal Aegean regions. The

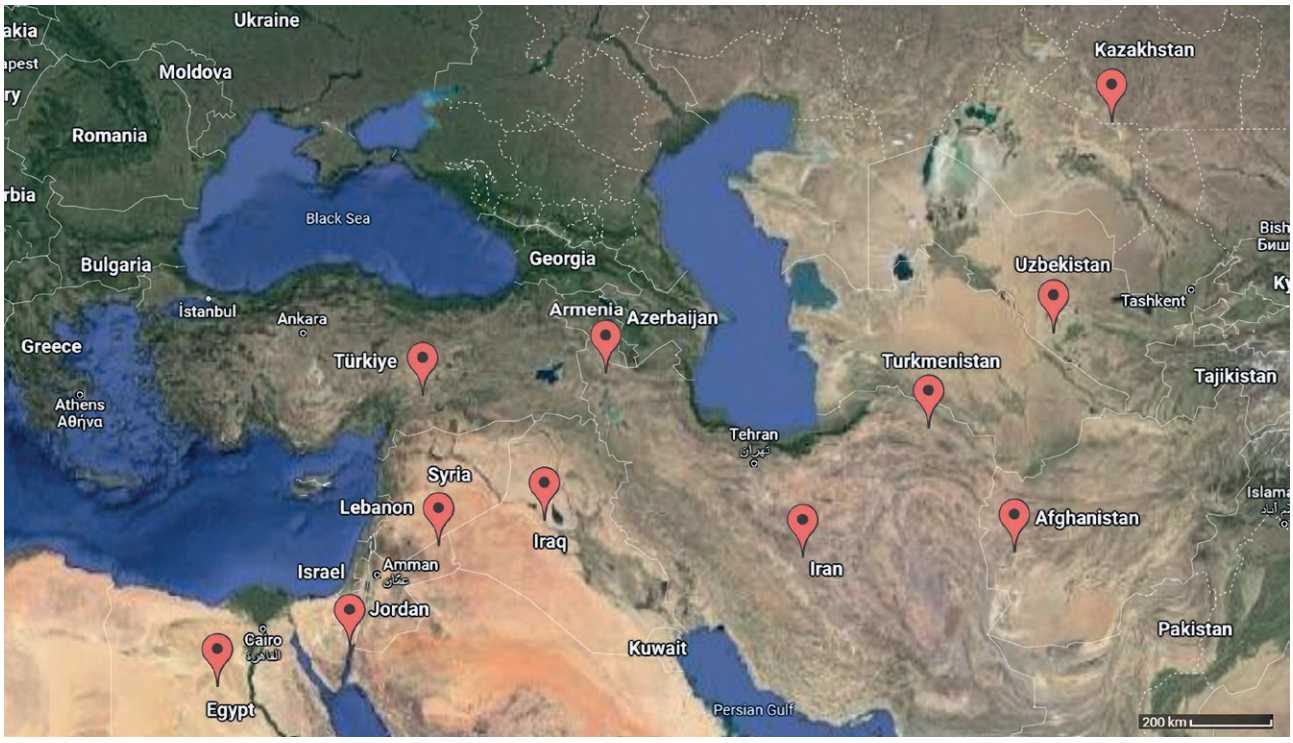


Figure 1. The distribution map of genus *Gundelia*.

geographical boundaries play a decisive role in their distribution (Karis et al. 2001; Vitek 2018).

The species belonging to the genus *Gundelia* are called "Kenger" in Turkish, "Akub" in Arabic and "Kuub" in Persian, and there are some local dialectical differences. Young shoots are used as vegetables in dishes and pickles. The latex obtained from the cut shoots is dried and used as gum. Roasted fruits are consumed like coffee. It is also used as animal food in some regions.

Cytotaxonomy, one of the sub-branches of taxonomy, uses karyological parameters in the classification of organisms. In this context, chromosomal configurations are used to understand the relationships between species and based on the assumption that "closely related species show similar chromosomal configurations". Thus, karyotype evolution or interspecies relationships can be reconstructed by exploiting karyological similarities or variations. The chromosome number, chromosome structure and chromosomal behaviors stand out as important parameters of cytotaxonomy, and especially basic chromosome number (*x*), diploid chromosome number (2*n*) and karyotype asymmetry are the most preferred parameters (Eroğlu et al. 2020; Martin et al. 2020; Eroğlu et al. 2021; Kavcı et al. 2022). In genus *Gundelia*, the chromosomal data were reported from 12 species. All species were represented by only one base number ($x = 9$) and there were no reports of polyploidy (Al-Taey and Hossain 1984; Genç and Fırat 2019).

In Turkish species of *Gundelia*, the chromosomal data were reported from 12 species, which were *G. anatolica*, *G. asperrima*, *G. cilicica*, *G. colemerikensis*, *G. dersim*, *G. glabra*, *G. komagenensis*, *G. mesopotamica*, *G. munzuriensis*, *G. rosea*, *G. tournefortii*, and *G. vitekii* (Al-Taey and Hossain 1984; Genç and Fırat 2019). There was no record of the chromosome number of four species, which were *G. armeniaca*, *G. cappadocica*, *G. siirtica*, and *G. tehranica*. Due to the lack of some chromosome reports from Turkey, where there are many species of the genus, some cytotaxonomic knowledge is lacking. In this study, it is aimed to complete the missing chromosomal data in Turkish *Gundelia* species.

MATERIALS AND METHODS

Plant material

Within the scope of this study, sixteen *Gundelia* taxa distributed in different localities of Turkey were evaluated karyologically. The evaluated samples were collected from natural habitats by Dr. Metin Armağan et al. Table 1 represents the collection information and distribution regions. Turkish *Gundelia* species whose chromosomal data were reported in previous studies were collected from different localities to investigate chromosomal variations.

Chromosome preparation

The plant seeds were germinated between moist Whatman papers and pretreated by α-monobromonaphthalene at 4°C for 16 h. Then, root tips were fixed by fixative solution (3 absolute alcohol and 1 glacial acetic acid - v:v) at 4°C for 24 h. The fixed root tips were stored in ethanol (70%) at 4°C. Then, root tips were hydrolyzed in 1 N hydrochloric acid at room temperature for 10 min and stained in aceto-orcein (2%). Then, squash preparations were prepared by acetic acid (45%). The preparations were frozen in liquid nitrogen, dried at room temperature, and stabilized with Depex medium (Eroğlu et al. 2020; Martin et al. 2020; Eroğlu et al. 2021; Kavcı et al. 2022).

Karyotype analysis

Well-spread ten metaphase plates were used to detect for the chromosome numbers of all species. Detailed chromosomal measurements of four species whose chromosome number was investigated for the first time were made by Karyotype software. The following parameters and formulae were used to chromosome characterizations karyotype analysis: short arm length of chromosome (p), long arm length of chromosome (q), total chromosome length (p + q), total haploid length (THL), mean haploid length (MHL), relative length (RL) = [(p + q) / THL] × 100, and centromeric index (CI) = [(p) / (p + q)] × 100. The karyotype formulae were detected based on centromere position (Levan et al. 1964) and the monoploid ideograms were drawn.

The following formulae were used to determine the intrachromosomal asymmetry (M_{CA}) and interchromosomal asymmetry (CV_{CL}): M_{CA} = [mean ($q_t - p_t$) / (qt + pt)] × 100; q_t, total length of long arms and p_t, total length of short arms (Peruzzi and Eroğlu 2013). CV_{CL} = (S_{CL} / X_{CL}) × 100; S_{CL}, standard deviation in a chromosome set and X_{CL}, mean chromosome length in a chromosome set (Paszko 2006).

RESULTS

Chromosome records of 16 species are herein provided (Figure 2), four of which are reported for the first time (*G. armeniaca*, *G. cappadocica*, *G. siirtica*, and *G.*

Table 1. Collection information and voucher specimens of *Gundelia* taxa.

Species (alphabetically)	Locality	Voucher
G. anatolica Fırat	Konya: Karapınar, S of Meke Crater Lake, 1080 m, volcanic dune (steppe), 37 40 22.3 N 33 38 32.4 E, 16 May 2016	M.Armağan 6734
G. armeniaca Nersesian	Muş: Karabey, 1400 m, steppe, 38 55 50.2 N 41 10 40.0 E, 01 June 2015	M.Armağan 6552
G. asperrima (Trautv.) Fırat	Bingöl: between Elazığ and Bingöl, Kuruca Pass, 1720 m, meadows, 38 57 22,0 N 40 14 50,6 E, 21 June 2017	M.Armağan 7544
G. cappadocica Fırat	Nevşehir: Avanos, Bozca, 1070 m, steppe (on calcareous soils), 38 46 06.2 N 34 59 56.9 E, 30 May 2021	O.Tugay 18.345
G. cilicica Fırat	Mersin: Erdemli, Tozlu, 1565 m, on degraded fields of *Juniperus sp*. forest (steppe), 36 49 14.4 N 34 08 42.1 E, 25 June 2020	M.Armağan Obs-MTN55, O.Tugay, E.Karahisar
G. colemerikensis Fırat	Van: Başkale, between Kovalıpınar and Ömerabat, 1960 m, dry slopes, 37 49 35.8 N 44 04 20.0 E, 24 June 2020	M.Armağan 8335
G. dersim Vitek, Yüce & Ergin	Tunceli: Ovacık, Adaköy, 1285 m, meadows, 39 20 45.2 N 39 06 53.6 E, 29 May 2020	M.Armağan 8308, M.Özel, R.Karapınar
G. glabra Mill.	Elazığ: Yukarıbağ, 856 m, steppe, 38 38 01.8 N 39 34 54.6 E, 28 May 2020	M.Armağan 8306
G. komagenensis Fırat	Malatya: Battalgazi, NE of Beydağı, 1080 m, 38 18 10.0 N 38 27 23.6 E, 18 July 2020	M.Armağan Obs-MTN58, O.Tugay, E.Karahisar
G. mesopotamica Fırat	Mardin: Artuklu, Avcılar, 715 m, steppe on limestone bedrock, 37 17 53.8 N 40 42 12.9 E, 26 May 2020	M.Armağan 8293
G. munzuriensis Vitek, Yüce & Ergin	Tunceli: Ovacık, above Gözeler, Munzur Mountains, 2500 m, mountain steppe, 39 26 35,9 N 39 14 22,4 E, 15 July 2020	M.Armağan 8358, M.Özel, R.Karapınar, K.Es.
G. rosea M.Hossain & Al-Taey	Şırnak: Uludere, Güzelyazı, Hakkari-Şırnak roadside, 1380 m, dry slopes, 37 22 28.3 N 42 59 47.6 E, 23 June 2020	M.Armağan 8328, O.Tugay
G. siirtica Fırat	Siirt: Kurtalan, Erdurağı, mountain (steppe) meadows, 926 m, 37 53 59.1 N 41 35 32.2 E, 30 July 2020	M.Armağan 8300
G. tehranica Vitek & Noroozi	Şırnak: Silopi, Dedeler740 m, dry slopes, 37 19 18.5 N 42 25 32.8 E, 23 June 2020	M.Armağan 8325, O.Tugay
G. tournefortii L.	Karaman: Ermenek, Aşağıakın, 1110 m, steppe, 36 53 47.8 N 33 01 06.2 E, 22 August 2020	O.Tugay 17786, E.Karahisar
G. vitekii Armağan	Tunceli: Ovacık, Şahverdi, Mercan Valley, 1623 m, steppe, 39 27 10.8 N 39 23 51.9 E, 14 July 2020	M.Armağan 8309, M.Özel, R.Karapınar, K.Es.

tehranica), one presents polyploidy for the first time (*G. anatolica*), and twelve agree previous reports. Table 2 shows the chromosome numbers of present and previous reports. Except for polyploidy (Figure 3), the only one chromosome number detected was $2n = 18$.

Detailed chromosomal data and monoploid ideograms of the four species, whose chromosome numbers were reported for the first time were given in Table 3 and Figure 4. The smallest chromosome length among the species was 3.94 µm, in *G. siirtica*. The largest chromosome length was detected in *G. tehranica*, with 8.65 µm. The smallest total haploid length was 44.37 µm, in *G. siirtica*, and the highest value was 51.71 µm, in *G. armeniaca* and *G. cappadocia*.

Genus *Gundelia* was a monobasic genus by $x = 9$ with ploidy levels of $2x$ and $4x$. Fifteen species were diploid with $2n = 2x = 18$. *G. anatolica* was diploid and polyploid, which revealed only one polyploidy level of tetraploidy ($2n = 4x = 36$).

All species except *G. tehranica* had median (m) and submedian (sm) chromosomes, but not subtelocentric (st) and telocentric (t) chromosomes. Two different karyotype formulae were observed, which were 14m + 4sm and 12m + 2sm + 2st. Secondary constrictions were observed in the distal regions of the long arms of the longest metacentric and submetacentric chromosomes (Figure 4).

In intrachromosomal asymmetry, M_{CA} value ranged from 9.98 (*G. siirtica*) to 10.63 (*G. tehranica*), which referred to symmetric karyotypes. In interchromosomal asymmetry, CV_{CL} value ranged from 13.82 (*G. armeniaca*) to 23.75 (*G. tehranica*), which referred to karyotype heterogeneity (Table 3).

DISCUSSION

Only one chromosome number excluding polyploidy was detected as $2n = 18$, which was the only diploid

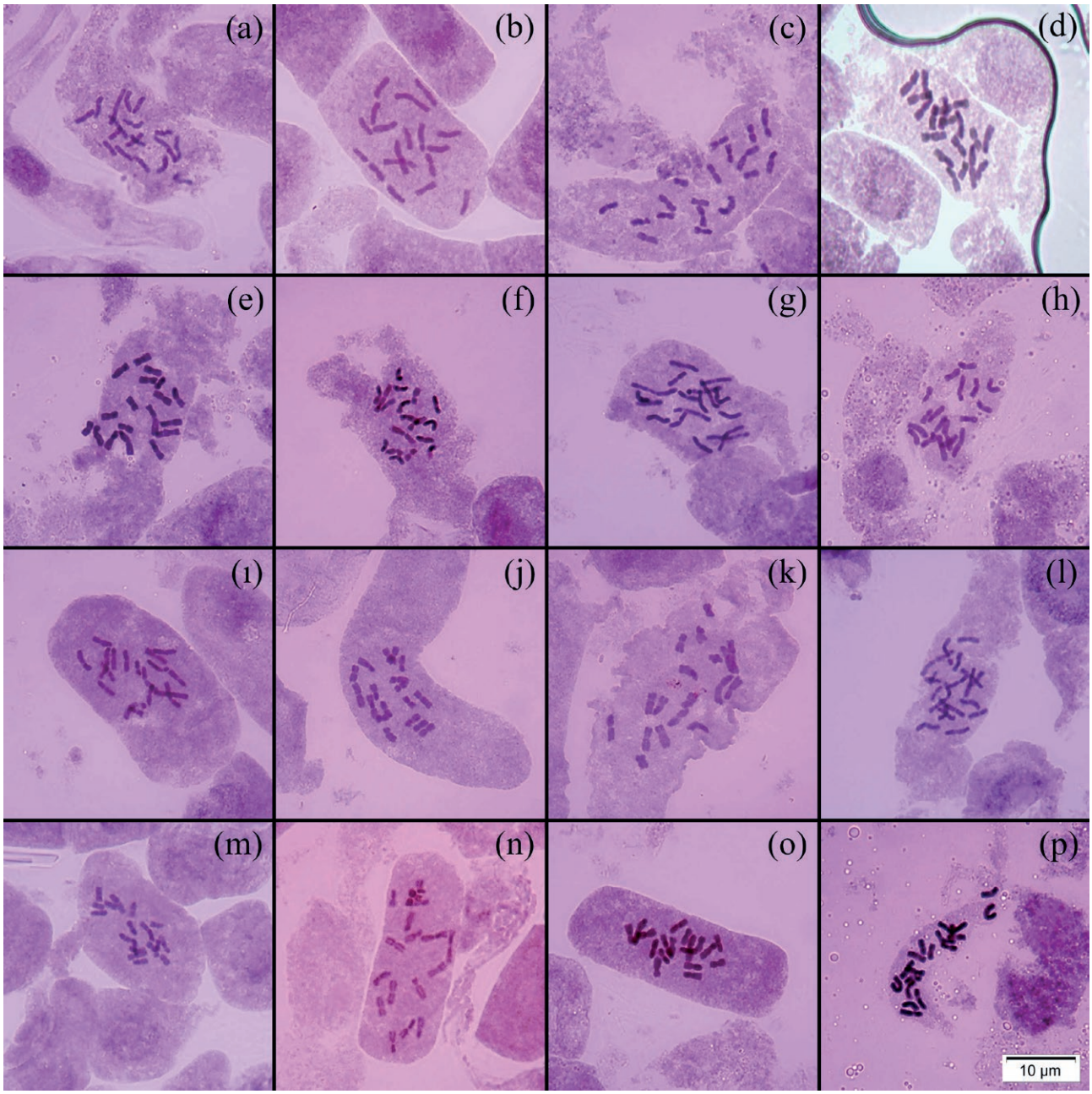


Figure 2. Somatic metaphase chromosomes of Turkish *Gundelia* species. (a) *G. armeniaca*; (b) *G. cappadocica*; (c) *G. siirtica*; (d) *G. tehranica*; (e) *G. anatolica*; (e) *G. asperrima*; (e) *G. cilicica*; (e) *G. colemerikensis*; (e) *G. dersim*; (e) *G. glabra*; (e) *G. komagenensis*; (e) *G. mesopotamica*; (e) *G. munzuriensis*; (e) *G. rosea*; (e) *G. tournefortii*; and (e) *G. vitekii*.

number ever reported in the genus. The chromosome numbers of four species were reported here for the first time: *G. armeniaca*, *G. cappadocica*, *G. siirtica*, and *G. tehranica*. The chromosome numbers were the same as in previous reports in 12 species, which were *G. anatolica*, *G. asperrima*, *G. cilicica*, *G. colemerikensis*, *G. dersim*, *G. glabra*, *G. komagenensis*, *G. mesopotamica*, *G. munzuriensis*, *G. rosea*, *G. tournefortii*, and *G. vitekii* (Al-Taey and Hossain 1984; Genç and Fırat 2019).

A basic chromosome number of $x = 9$ dominates in genus *Gundelia* and the genus is monobasic. The absence of basic number variations in genus *Gundelia* indicated that the mechanism of dysploidy probably did not occur in the karyotype evolution of the genus. Because

Table 2. The chromosome numbers of Turkish *Gundelia* in present and previous studies. All species were studied in this study. Turkish *Gundelia* species whose chromosomal data were reported in previous studies were collected from different localities to investigate chromosomal variations.

Species (alphabetically)	Previous results x = basic number, $2n$ (ploidy level)	References	Presents results x = basic number, $2n$ (ploidy level)	Observation
G. anatolica	$x = 9$, $2n = 18$ (diploid)	Genç and Fırat 2019	$x = 9$, $2n = 18$ (diploid) $x = 9$, $2n = 36$ (polyploid)	Equal count First report
G. armeniaca			$x = 9$, $2n = 18$ (diploid)	First report
G. asperrima	$x = 9$, $2n = 18$ (diploid)	Genç and Fırat 2019	$x = 9$, $2n = 18$ (diploid)	Equal count
G. cappadocica			$x = 9$, $2n = 18$ (diploid)	First report
G. cilicica	$x = 9$, $2n = 18$ (diploid)	Genç and Fırat 2019	$x = 9$, $2n = 18$ (diploid)	Equal count
G. colemerikensis	$x = 9$, $2n = 18$ (diploid)	Genç and Fırat 2019	$x = 9$, $2n = 18$ (diploid)	Equal count
G. dersim	$x = 9$, $2n = 18$ (diploid)	Genç and Fırat 2019	$x = 9$, $2n = 18$ (diploid)	Equal count
G. glabra	$x = 9$, $2n = 18$ (diploid)	Genç and Fırat 2019	$x = 9$, $2n = 18$ (diploid)	Equal count
G. komagenensis	$x = 9$, $2n = 18$ (diploid)	Genç and Fırat 2019	$x = 9$, $2n = 18$ (diploid)	Equal count
G. mesopotamica	$x = 9$, $2n = 18$ (diploid)	Genç and Fırat 2019	$x = 9$, $2n = 18$ (diploid)	Equal count
G. munzuriensis	$x = 9$, $2n = 18$ (diploid)	Genç and Fırat 2019	$x = 9$, $2n = 18$ (diploid)	Equal count
G. rosea	$x = 9$, $2n = 18$ (diploid)	Al-Taey and Hossain 1984 Genç and Fırat 2019	$x = 9$, $2n = 18$ (diploid)	Equal count
G. siirtica			$x = 9$, $2n = 18$ (diploid)	First report
G. tehranica			$x = 9$, $2n = 18$ (diploid)	First report
G. tournefortii	$x = 9$, $2n = 18$ (diploid)	Al-Taey and Hossain 1984 Genç and Fırat 2019	$x = 9$, $2n = 18$ (diploid)	Equal count
G. vitekii	$x = 9$, $2n = 18$ (diploid)	Genç and Fırat 2019	$x = 9$, $2n = 18$ (diploid)	Equal count

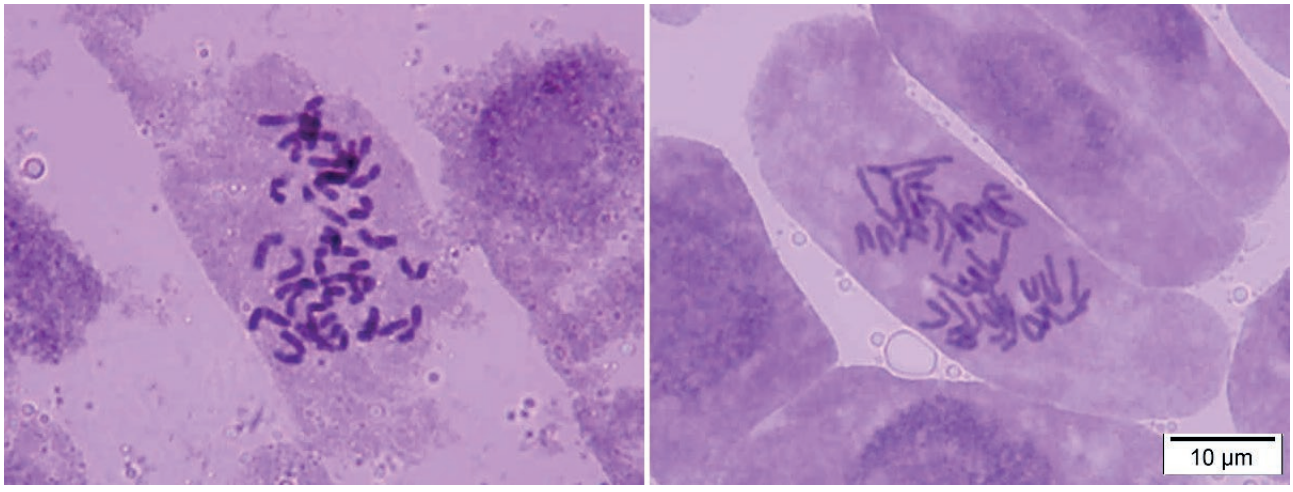


Figure 3. The polyploidy of *Gundelia anatolica*.

dysploidy causes basic number variations by fusion of metacentric chromosomes or reciprocal translocations (Eroğlu et al. 2020; Martin et al. 2022). In addition, the polyploidy in the genus was rare and *G. anatolica* was identified as the first polyploid species.

Fifteen species had metacentric and submetacentric chromosomes and only one species had subtelocentric chromosomes, whereas no telocentric (t) chromosomes were observed. Two different karyotype samples were observed, which were m-sm and m-sm-st including secondary constrictions. Thus, five chromosome types were determined according to the positions of the primary and secondary constrictions: (i) metacentric (ii) metacentric with secondary constriction in the distal region

Table 3. The detailed chromosomal data and asymmetry indices of species whose chromosome number was reported for the first time (KF: karyotype formula, SC: the shortest chromosome length, LC: the longest chromosome length, RL: relative length, CI: centromeric index, THL: total haploid length, MHL: mean haploid length, M_{CA}: mean centromeric asymmetry, CV_{CL}: coefficient of variation of chromosome length).

	G. armeniaca	*G. cappadocica*	*G. siirtica*	*G. tehranica*
KF	14m + 4sm	14m + 4sm	14m + 4sm	12m + 2sm + 2st
SC (µm)	4.80	4.57	3.94	4.01
LC (µm)	7.10	8.51	6.97	8.65
RL (%) SC–LC	9.28–13.73	8.84–16.46	8.88–15.71	7.92–17.08
CI (min–max)	33.82–49.45	25.46–49.30	32.87–49.43	23.02–49.80
THL	51.71	51.71	44.37	50.65
MHL	5.75	5.75	4.93	5.63
M_{CA}	10.02	10.53	9.98	10.63
CV_{CL}	13.82	22.72	21.30	23.75

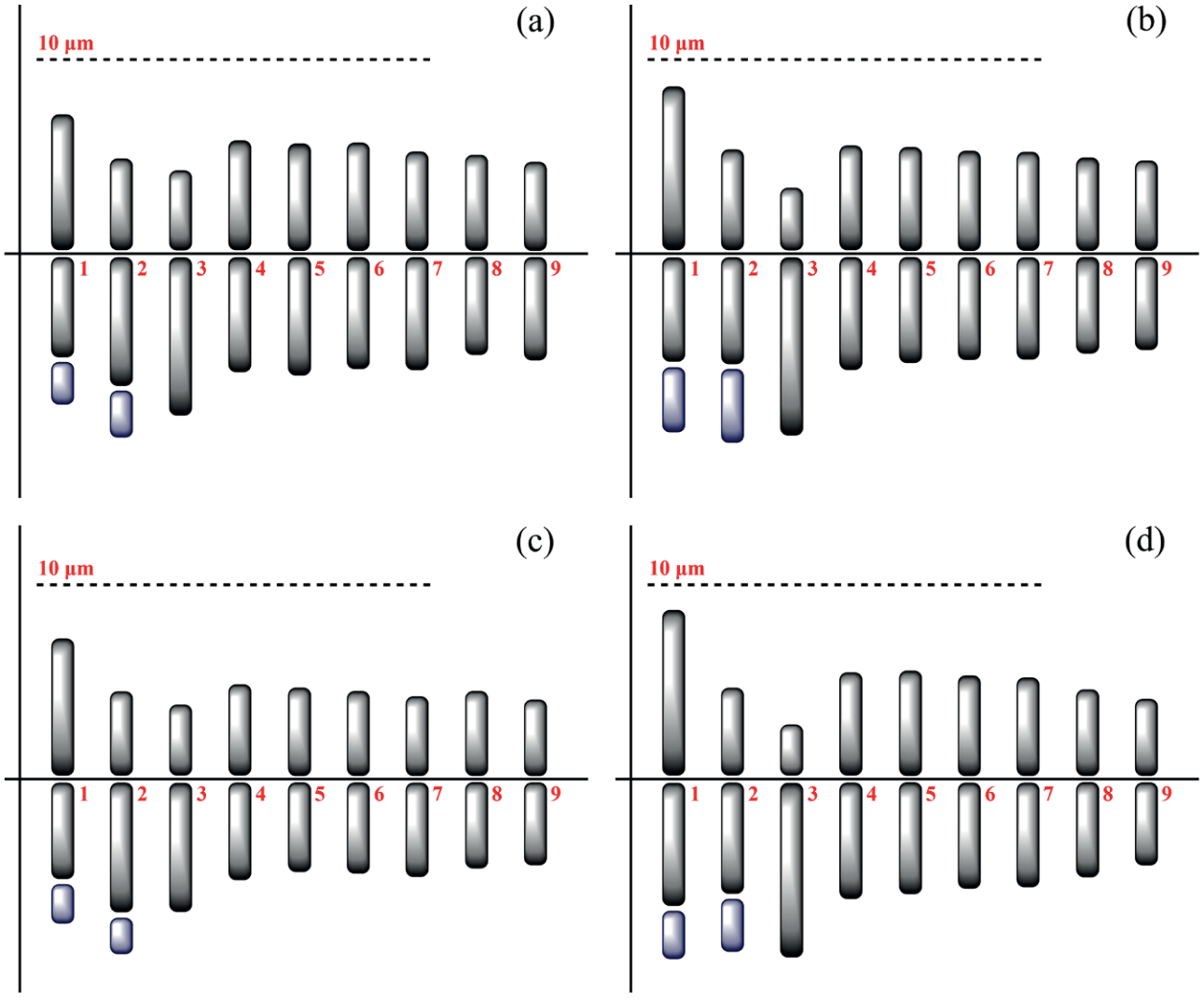


Figure 4. The monoploid ideograms of the species whose chromosome number was reported for the first time. (a) *G. armeniaca*; (b) *G. cappadocica*; (c) *G. siirtica*; (d) *G. tehranica*.

of the long arm, (iii) submetacentric, (iv) submetacentric with secondary constriction in the distal region of the long arm, (v) subtelocentric. In twelve *Gundelia* species, Genç and Fırat (2019) reported that the secondary constrictions at short or long arms of submetacentric chromosomes and in the distal region of long arm of the longest metacentric chromosome.

In intrachromosomal asymmetry, all karyotypes were symmetric. The most symmetric and asymmetrical karyotypes were the karyotypes of *G. siirtica* and *G. tehranica*, respectively. In interchromosomal asymmetry, all karyotypes were symmetric. The most symmetric and asymmetrical karyotypes were the karyotypes of *G. armeniaca* and *G. tehranica*, respectively. Genç and Fırat (2019) reported that *G. rosea* and *G. tournefortii* had the relatively high intrachromosomal asymmetry and low intrachromosomal asymmetry, respectively; also, *G. vitekii* and *G. anatolica* had the high interchromosomal and low interchromosomal asymmetry, respectively.

In the present study, it was recorded only one chromosome number ($2n = 18$) excluding polyploidy ($2n = 36$), the first report for diploid numbers of four species, the first report of polyploidy for the genus, and the same chromosome count with previous report in the twelve species. Thus, the chromosomal data of all Turkish *Gundelia* species were completed. In conclusion, the present study presented new data into the karyological records relating the karyotype evolution and interspecies relations of genus *Gundelia*. In addition, the dysploidy and polyploidy mechanisms probably did not have an important role in the speciation of genus.

ACKNOWLEDGMENTS

This work was supported by the TÜBİTAK under Grant [number 119Z882].

REFERENCES

Al-Taey RA, M. Hossain. 1984. Studies in *Gundelia*: 1 A new species from Iraq. Notes Roy Bot Gard Edinburgh 42:39–44.

Armağan M. 2016. *Gundelia vitekii* (Compositae), a new species from Turkey. Ann Naturhist Mus Wien, B 118:129–134.

Ateş AM, Fırat M, Kaya Z. 2021. Updated-extended molecular time and molecular phylogeny of *Gundelia* species native to Turkey. Plant Syst Evol. 307: 47.

Classen-Bockhoff R, Froebe HA, Langerbeins D. 1989. Die Infloreszenzstruktur von *Gundelia tournefortii* L. (Asteraceae). Flora 182: 463–479.

Eroğlu HE, Altay D, Budak Ü, Martin E. 2020. Karyotypic phylogeny and polyploidy variations of *Paronychia* (Caryophyllaceae) taxa in Turkey. Turk J Bot. 44:245–254.

Eroğlu HE, Martin E, Kahraman A, Gezer Aslan E. 2021. The new chromosomal data and karyotypic variations in genus *Salvia* L. (Lamiaceae): dysploidy, polyploidy and symmetrical karyotypes. Caryologia 74:21–28.

Feinbrun Dothan N. 1978. *Gundelia* L. In: Flora Palaestina: Part 3. Ericaceae to Compositae. Jerusalem: Israel Academy of Sciences and Humanities; p. 360–361

Fırat M. 2016. Four new species of *Gundelia* L. (Asteraceae) from Anatolia: *G. komagenensis*, *G. colemerikensis*, *G. cilicica* and *G. anatolica*. Van: Sitav Yayınevi.

Fırat M. 2017a. *Gundelia rosea* (Asteraceae), a new record for the Flora of Turkey with contributions to its systematics. Acta Biologica Turcica 30:31-35.

Fırat M. 2017b. *Gundelia mesopotamica* (Asteraceae), a new lactiferous species from Mardin (Turkey). Acta Biologica Turcica. 30:64-69.

Fırat M. 2017c. The resurrection and a new status of *Gundelia tournefortii* L. var. *asperrima* Trautv. (*Asteraceae*). OT Sistematik Botanik Dergisi 24:57-67.

Funk VA, Chan R, Keeley SC. 2004. Insights into the evolution of the tribe *Arctoteae* (Compositae: subfamily *Cichorioideae* s.s.) using *trn*L-F, *ndh*F, and ITS. Taxon 53(3): 637-655.

Genç İ, Fırat M. 2019. Karyological study of the genus *Gundelia* (Compositae) in Turkey. Caryologia 72:45-53.

Hind N. 2013. 763. *Gundelia tournefortii*: Compositae. Curtis's Botanical Magazine 30:114–138.

Karis PO, Eldenäs P, Källersjö M. 2001. New evidence for systematic position of Gundelia L. with notes on delimitation of Arctoteae (Asteraceae). Taxon 50:105–114.

Kavcı E, Martin E, Eroğlu HE, Yıldırım FS. 2022. Chromosome number of some *Satureja* species from Turkey. Caryologia 75:13–18.

Komarov VL. 1961. *Gundelia* L. In: Shiskin BK, Bobrov EG, editors. Flora of the USSR [in Russian], Vol. XXVI. Moscow and Leningrad: Izd. Akad. Nauk SSSR.

Kupicha FK. 1975. *Gundelia*. In: Davis PH, Matthews VA, Kupicha FK, Parris BS editors. Flora of Turkey and the East Aegean Islands, Vol. 5. Edinburgh: Edinburgh University Press; p. 325–326.

Levan A, Freda K, Sandberg AA. 1964. Nomenclature for centromeric position on chromosomes. Hereditas. 52:201–220.

Linnaeus C. 1753. Species Plantarum. Tomus II. Salvii, Holmiae.

Martin E, Kahraman A, Dirmenci T, Bozkurt H, Eroğlu HE. 2020. Karyotype evolution and new chromosomal data in *Erodium*: chromosome alteration, polyploidy, dysploidy, and symmetrical karyotypes. Turk J Bot. 44:255–268.

Martin E, Kahraman A, Dirmenci T, Bozkurt H, Eroğlu HE. 2022. New chromosomal data and karyological relationships in *Geranium*: basic number alterations, dysploidy, polyploidy, and karyotype asymmetry. Braz Arch Biol Technol. 65:e22210354.

Meikle RD. 1985. *Gundelia* L. in Flora of Cyprus Vol. 2. Bentham Moxon Trust, Royal Botanic Gardens.

Nersesyan A. 2014. *Gundelia armeniaca* Nersesyan (Compositae) – a new species from Armenia. Ann Naturhist Mus Wien, B 116:191–196.

Panero JL, Crozier BS. 2016. Macroevolutionary dynamics in the early diversification of Asteraceae. Mol Phylogenet Evol. 99: 116–132.

Paszko B. 2006. A critical review and a new proposal of karyotype asymmetry indices. Plant Syst Evol. 258:39–48.

Peruzzi L, Eroğlu HE. 2013. Karyotype asymmetry: again, how to measure and what to measure? Comp Cytogen. 7:1–9.

Rechinger KH. 1989. *Gundelia* L. In: Rechinger KH, editor. Flora Iranica. (Flora des Iranischen Hochlandes und der Umrahmenden Gebirge Persien, Afghanistan, teile von West-Pakistan, Nord-Iraq,Azerbaidjan, Turkmenistan) Compositae VII , Vol. 164. Graz: Akademische Druck- u. Verlangsanstalt; p. 107–109.

Tarıkahya-Hacıoğlu B, Fırat M. 2017. ITS Phylogeny and Molecular Dating of some *Gundelia* (Asteraceae) of Anatolia. AARI 27: 74–77.

Vitek E. 2018. *Gundelia microcephala* (Compositae)–a taxon deserving rank of species. Ann Naturhist Mus Wien, B 120:233–240.

Vitek E, Armağan M. 2023. *Gundelia purpurascens* (Compositae) is a synonym of *G. rosea.* Phyton 62–63:35–39.

Vitek E, Fayvush G, Tamanyan K, Gemeinholzer B. 2010. New taxa of *Gundelia* (Compositae) in Armenia. Ann Naturhist Mus Wien, B 111:85–99.

Vitek E, Yüce E, Çakılcıoğlu U. 2017. *Gundelia glabra* Miller (Compositae) – an ignored taxon. Ann Naturhist Mus Wien, B 119:235–242.

Vitek E, Yüce E, Ergin C. 2014. *Gundelia dersim* and *Gundelia munzuriensis* (Compositae), two new species from Turkey. Phytotaxa 161:130–138.

Vitek E, Noroozi J. 2017. *Gundelia tehranica* (Compositae), a new species from Iran. Ann Naturhist Mus Wien, B 119:243–248.

Caryologia
International Journal of Cytology, Cytosystematics and Cytogenetics

Firenze University Press
www.fupress.com/caryologia

OPEN ACCESS

Citation: Lejla Husić, Adisa Parić, Aner Mesic (2023). Allelopathic and toxicological effects of *Origanum vulgare* L. essential oil. *Caryologia* 76(1): 97-102. doi: 10.36253/caryologia-2132

Received: May 4, 2023

Accepted: July 3, 2023

Published: September, 19, 2023

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

ORCID
AM: 0000-0002-2263-0420

Allelopathic and toxicological effects of *Origanum vulgare* L. essential oil

Lejla Husić, Adisa Parić, Aner Mesic*

University of Sarajevo, Faculty of Science, Department of Biology, Zmaja od Bosne 33-35, 71000 Sarajevo, Bosnia and Herzegovina
*Corresponding author. E-mail: aner.mesic@pmf.unsa.ba

Abstract. *Origanum vulgare* L. has been proven to be the strongest herbal antiseptic in the world, native to the Mediterranean region, but is widely naturalized elsewhere in the temperate Northern Hemisphere. This study aimed to estimate the phytotoxic effect of three different concentrations of oregano essential oil (*O. vulgare*) on three selected plant species namely, wheat, tomato and mint using biotest germination and effects on seedling growth, as well as its toxicological properties using *Allium* test. Our results revealed that oregano essential oil exhibits allelopathic effect on selected species. All three tested concentrations of oregano essential oil caused a significant inhibition of *Allium cepa* L. root growth, as well as a reduction in the mitotic index values in *A. cepa* meristem cells. *O. vulgare* essential oil demonstrated phytotoxic and antiproliferative effects. Further research is needed to confirm our results.

Keywords: allelopathy, phytotoxicity, essential oil, *Origanum vulgare*, toxicological effects, *Allium* test.

INTRODUCTION

The environmental constraints of plant production have sparked many interests in alternative weed control and control strategies. In fact, continued use of synthetic herbicides can endangered sustainable agricultural production, resulting in serious environmental problems such as, increased frequency of weed resistance to important herbicides and increased environmental pollution and health hazards (Owen and Zelaya 2005). The production and accumulation of secondary metabolites (allelochemicals), which inhibit and/or stimulate the germination and development of other plants, is an important mechanism in the interaction between plants. Allelopathy offers the potential for selective biological weeds through the production and release of allelochemicals from leaves, flowers, seeds, stems and roots, living or decomposing plant material (Weston 1996). Together with the investigation of the herbicidal activity of essential oils on weeds, there are some studies on agricultural plants in order do evaluate their effect on adjacent plants (Ibáñez and Blázquez 2020). Under appropriate conditions, allelochemicals can be released in quantities that suppress the development of weed seedlings (Wu

et al. 2001). Allelopathic inhibition is usually a result of the combined action of a group of allelochemicals that interfere with several biochemical interactions between plants, including those mediated by soil microorganisms. Aromatic plants can play an important role in plant-plant interactions and form the primary source of potential allelochemicals. Various allelochemicals have been identified, including essential oils that inhibit seed germination and plant growth (Aliotta et al. 1994).

Essential oils (EOs) are natural, complex compounds characterized by strong odors and formed by aromatic plants as products of secondary metabolism, generally localized in temperate to warm regions where they represent an important part of traditional pharmacopoeia. They are liquid, volatile, clear and rarely colored, soluble in lipids and in organic solvents, have lower density than water, can be synthesized by all plant organs, and are stored in secretory cells, cavities, ducts, epidermal cells or glandular trichomas (Masotti et al. 2003).

O. vulgare belongs to the *Lamiaceae* family, native to Europe, the north of the African continent and most of temperate Asia, but the focus of diversity is found in the Mediterranean region, predominantly in Türkiye. The composition of oregano essential oil varies depending on environmental conditions, geographical area, harvest time and stage of maturity of the plant. Essential oil from plants in different countries has been evaluated in terms of chemical composition and the main components have been identified depending on the area of origin. *O. vulgare* oil contains a large amount of volatile phenylpropanoids, thymol and carvacrol (carvacrol (53.2%) and p-cymen (12.7%), which are known to have a highly herbicidal effect on germination and growth of weed seedlings and cultivated crops (Karalija et al. 2020; Lombrea et al. 2020). According to the available literature, several monoterpenes, such as α and β-pinene, limonene, 1-8-cineol, myrcene, camphor, thymol and carvacrol inhibit seed germination and the growth of primary plant roots, mainly weeds (Singh et al. 2009; De Martino et al. 2010). These studies suggest that monoterpenes, as highly lipophilic compounds, interact with the constituents of biological membranes, altering their densities and fluidity. It has been described that the presence of monoterpenes increases level of malondialdehyde, proline and hydrogen peroxide, which indicates lipid peroxidation and induction of oxidative stress (Witzke et al. 2010).

Therefore, the present study aimed to assess the allelopathic effects of *O. vulgare* essential oil against wheat, tomato and mint, as well as its toxicological properties in *A. cepa* root apical meristem.

MATERIALS AND METHODS

O. vulgare essential oil and chemicals

For testing herbicidal properties against wheat (*Triticum aestivum* L.), tomato (*Lycopersicon esculentum* Mill.) and mint (*Mentha spicata* L.) as well as toxicological effects on *A. cepa*, commercially available essential oil of *O. vulgare* (Biohalilovic, B&H) was used. Stock solution of *O. vulgare* essential oil was made using dimethyl sulfoxide ≥99% (DMSO) (Sigma-Aldrich, St. Louis, MO). The stock solution was diluted with distilled water to the final concentrations of essential oil: 0.2 µg/ml, 0.4 µg/ml and 0.8 µg/ml.

Seed germination and phytotoxicity bioassay

The allelopathic effect of oregano essential oil (EO) on wheat, tomato and mint seeds was carried out by cultivating the seeds of these species in the air chamber. In 36 Petri dishes, with two layers of filter paper, 25 seeds were arranged which were then treated with 5 ml of oregano EO solution in three different test concentrations (0.2 µg/ml, 0.4 µg/ml and 0.8 µg/ml). The test was performed in four replications for each tested oil concentration. Parallel with treatments, four control replications in the form of distilled water, were also performed. Then, the Petri dishes were closed, sealed with parafilm and stored in the air chamber (constant 22°C temperature and 16 h photoperiod) for 4 to 7 days. The following parameters were determined: germination index (Abdul-Baki and Anderson 1973), percentage of seed germination (Scott et al. 1984), percentage of inhibition of germination (Cayuela et al. 2007), and phytotoxicity (Rusan et al. 2015).

Allium test

For the purposes of the *Allium* test, healthy onion (*A. cepa*) seed bulbs were placed onto glass specimens filled with fresh tap water for 72 hours in the dark at room temperature in order to grow. The water was renewed every 24 hours. After 72 hours, prior to the treatment of the *A. cepa* bulbs with the test concentrations of *O. vulgare* essential oil, the length of the roots was measured as suggested by Fiskesjö (1985). The roots were then treated with three different test concentrations of *O. vulgare* eseential oil (0.2 µg/ml, 0.4 µg/ml and 0.8 µg/ml) for 24 hours. Simultaneously, a control (untreated) group was established. For each test concentration and control group, series of three bulbs were used. After

the exposure period, roots length from the experimental sets and control was also measured. The roots were then excised from each bulb and placed in methanol/glacial acetic acid (3:1, v/v) fixative for 24 hours at +4°C. Afterwards, the roots were hydrolyzed in 1 N HCl for 15 minutes at room temperature. After hydrolysis, the root apical 2 mm were cut and placed onto clean glass slide in a drop of 2% acetorcein, and then squashed. For each tested concentration of the *O. vulgare* EO and control group, one slide per bulb was prepared and analyzed.

For cytogenetic analysis, three microscope slides were analyzed for each test concentration, as well as for the control. In this sence, the mitotic index (MI) values were determined, as the measure of cell proliferation. The MI was calculated as the quotient between the number of cells in mitosis (cell division) and the total number of cells analyzed, scoring 1000 cells per slide (3000 cells per each test concentration of essential oil and control group).

Statistical analysis

The obtained results were statistically processed using Microsoft Excel 2019 (Microsoft Corporation, Redmond, USA) and IBM SPSS Statistics for Windows, version 20.0 (Armonk, NY, USA). Mean values and standard deviations for each of the test concentrations and control group for all analyzed parameters, were calculated. In order to determine significant differences between the test substance (*O. vulgare* EO) and control group for all analyzed parameters, one-way ANOVA with post-hoc multiple comparison test (LSD) was carried out. The differences were considered statistically significant at the value of $P \leq 0.05$.

RESULTS

Allelopathic effects of O. vulgare essential oil

The highest value of the germination index (Table 1) was recorded for wheat seeds when treated with the lowest concentration of oregano EO, but with an increase in the tested concentrations, decrease in the germination index was observed. Contrary, in tomato seeds the highest recorded value was in control treatment. Also, the highest value of the germination index in mint seeds was at the lowest concentration of oregano EO.

EO decreased germination in tomatoes, when the highest concentration was applied in comparison with control, while in wheat and mint the percentage of germination increased. Accordingly, the highest percentage of germination inhibition was recorded in tomato seeds when treated with the highest concentration of oregano EO (17.72%). No effects of oregano EO were observed for wheat and mint, when treated with the lowest concentration (0.2 μg/ml) (Table 1). The treatment with the highest concentration of oregano EO (0.8 μg/ml) resulted in the highest phytotoxicity index (0.56%) in tomato seeds.

Table 1. Effect of different concentrations of *O. vulgare* essential oil on the germination index, germination, germination inhibition, phytotoxic index of the tested plant species.

Plant species	Essential oil concentration (μg/ml)	Index of germination	Germination (%)	Germination inhibition **(%)**	Phytotoxic index
T. aestivum	0	94.91±1.52	93.00±3.81	-	-
	0.2	96.08±11.05	93.00±3.82	0.00	-0.07±0.04
	0.4	86.50±2.69*	90.00±2.30*	3.22±0.50*	0.39±0.06
	0.8	86.75±4.64*	97.00±2.00*	-4.30±0.50*	0.29±0.01
L. esculentum	0	47.21±5.00	79.00±7.57	-	-
	0.2	39.08±5.97	75.00±8.24	5.06±1.78	0.03±0.03
	0.4	39.35±6.96	78.00±14.78	1.26±3.20	0.27±0.02
	0.8	25.41±7.13	65.00±6.83	17.72±1.47	0.56±0.01
M. spicata	0	3.20±5.12	45.00±3.82	-	-
	0.2	12.20±5.38	45.00±8.86	0.00	0.15±0.04
	0.4	12.06±6.00	49.00±16.12	-8.89±4.03	0.001±0.006
	0.8	11.53±6.01	50.00±5.16	-11.11±1.29	0.18±0.05

The results represent the mean values of four independent replications ± standard deviation.
* Statistically significant differences compared to the control group ($P \leq 0.05$).

Table 2. Results of the roots length increment and mitotic index values of *A. cepa* after treatment with different concentrations of *O. vulgare* essential oil.

Essential oil concentration (μg/ml)	Roots length increment (cm)	Mitotic index (%)
Control group	0.55±0.05	2.96±0.94
0.2	0.20±0.10*	0.12±0.05*
0.4	0.16±0.15*	0.20±0.10*
0.8	0.13±0.15*	0.23±0.05*

The results represent the mean values ± standard deviation.
* Statistically significant differences compared to the control group ($P \leq 0.05$).

Toxicological effects of O. vulgare essential oil

After the *Allium* test, a significant effect of test concentrations of *O. vulgare* EO on the growth of *A. cepa* roots was observed, as well as on the values of the mitotic index (Table 2). All three tested concentrations of oregano EO caused a significant inhibition of root growth, which was followed by a statistically significant decrease in the mitotic index (MI) value, as compared to the control group. It is important to hightlight that with the increase in the tested concentration of oregano EO, an increase in the inhibitory effect on the growth of roots was detected. As for the MI, the lowest value of the MI was recorded at the concentration of 0.2 μg/ml, while at the concentrations of 0.4 μg/ml and 0.8 μg/ml there was a slight increase in MI values. However, for all three tested concentrations of oregano EO, a statistically significant reduction in the value of the MI when compared to the control group, was demonstrated.

DISCUSSION

It has been observed that essential oils (EOs) from different plant species belonging to the *Lamiaceae* family have allelopathic properties (Verdeguer et al. 2009). The allelopathic effects of EOs are associated with the essential oil itself, its composition, the concentration applied, and the species to which they are applied. It has been recorded that oregano oil contains a large amount of volatile phenylpropanoids, thymol, linalool as well as p-cymene, which are known to have a high herbicidal effect on the germination and growth of weed seedlings and cultivated crops, as well as carvacrol, which is considered the main component of oregano oil that has a strong inhibitory effect on the growth of the stem, its thickness as well as the rate of photosynthesis (Dudai et al. 2004; Argyropoulos et al. 2008; Kadoglidou et al. 2020; Karalija et al. 2020; Lombrea et al. 2020).

Previous studies have shown that monoterpenes act on seeds in very low concentrations (Dudai et al. 2004), and that they possess very powerful inhibitors of wheat seed germination (Dragoeva et al. 2014). The results of our study demonstrated inhibitory effect of *O. vulgare*. EO at the lowest concentration, but stimulated at the highest concentration on the germination of wheat seeds. A similar trend can be noticed for mint, opposite to tomato. Argyropoulos et al. (2008) observed that oregano EO inhibits germination of tomato seeds (88.3%), even when used in the lowest concentration (1 μl/ml), which is attributed to the higher concentration of carvacrol (Ibáñez and Blázquez 2020). Our results indicate that the highest percentage of tomato seeds inhibition was recorded in treatments with the highest concentration of oregano EO (17.72%). Previously was reported that monoterpene compounds of EO are responsible for germination inhibition (Dudai et al. 2004), and that oregano essential oil had the strongest phytotoxic effect on seed germination and growth of tomato seedlings, in comparison with other applied oils (Ibáñez and Blázquez 2020).

The phytotoxic effect of *O. vulgare* EO was significant in monocotyledons (*T. aestivum* and *Hordeum vulgare*), while in dicotyledons the stimulating effect was observed (Grulova et al. 2020). Our results revealed that *O. vulgare* essential oil showed different effects on monocotyledons and dicotyledons, respecting their biological activity. The lowest value of the phytotoxicity index in *T. aestivum* (-0.07%) was recorded at the lowest concentration of oregano oil, while the value of the phytotoxicity index in *L. esculentum* increased with an increase in the oregano EO concentration.

The beneficial effect of oregano (*O. vulgare* L. subsp. *hirtum*) and green mint (*M. spicata* L.) on agronomic characteristics (taller plants with thicker stems), improvement of physiological characteristics of tomato (higher index of chlorophyll content and speed of photosynthesis), was also observed as an increased yield and improved quality characteristics of tomato fruits (Kadoglidou et al. 2020). This study revealed that direct incorporation of oregano or spearmint plant material into the soil can improve tomato resistance to soil-borne fungi, soil fertility, and consequently increase yield and product quality.

It has been proven that root growth is regulated by a cell division in the zone of active meristem and subsequent cell elongation, which represents independent events (Shishkova et al. 2007). In fact, the rate of root growth is directly affected by the disruption of either of

these two processes (Obroucheva 2008). Therefore, it is reasonable to assume that the decrease in *A. cepa* root growth caused by oregano essential oil (*O. vulgare*) is a consequence of a reduced mitotic index (cell division) in *A. cepa* root apical meristem.

The results of the present study are supportive to the results of a recent study which demonstrated that oregano *O. vulgare* EO led to a decrease in the value of the mitotic index (MI) in onion meristem cells (*A. cepa*) (Grondona et al. 2014). Similarly, one study evidenced that a significant decrease in *Vicia faba* L. mitotic activity caused by oregano oil was more pronounced than that caused by rosemary oil. According to cytotoxicity limit values, both oils showed subletal cytotoxic effects (Hamedo and Abdelmigid 2009). Furthermore, our results are consistent with the results of an investigation in which the antiproliferative potential of ethanol extract of oregano on Caco-2 cells of adenocarcinoma of the colon, through an increased proapoptotic activity, was reported (Savini et al. 2009). Begnini et al. (2014) observed that EOs from *O. vulgare* inhibited cell proliferation in breast adenocarcinoma cells (MCF-7) and colon adenocarcinoma (HT-29). Similarly, Marrelli et al. (2016) showed antiproliferative effect of deer grass essential oil (*O. dictamnus*) on the cell line of colon cancer (LoVo), as well as on the hepatocarcinoma (HepG2) cell line.

In conclusion, the essential oil of oregano showed a phytotoxic effect on selected species. In addition, it exhibited antiproliferative effects in *A. cepa*. Studies like this can contribute to a better understanding of the allelopathic and toxicological potential of essential oils, but further research is needed to evaluate their potential use for natural weed control.

REFERENCES

Abdul-Baki AA, Anderson JD. 1973. Vigor determination in soybean seed by multiple criteria. Crop Science. 13(6): 630-633.

Aliotta G, Cafiero G, De Feo V, Sacchi R. 1994. Potential allelochemicals from *Ruta graveolens* L. and their action on radish seeds. J Chem Ecol. 20(11): 2761–2775.

Argyropoulos EI, Eleftherohorinos Lias, Vokou D. 2008. In vitro evaluation of essential oils from Mediterranean aromatic plants of the *Lamiaceae* for weed control in tomato and cotton crops. Allelopathy J. 22(1): 69-78.

Begnini KR, Nedel F, Lund RG, Carvalho PH, Rodrigues MR, Beira FT, Del-Pino FA 2014. Composition and antiproliferative effect of essential oil of *Origanum vulgare* against tumor cell lines. J Med Food. 17(10): 1129–1133.

Cayuela ML, Millner P, Slovin J, Roig A. 2007. Duckweed (*Lemna gibba)* growth inhibition bioassay for evaluation the toxicity of olive mill wastes before and during compositing. Chemosphere. 68(1): 1985-1991.

De Martino L, Mancini E, Almeida LFR, De Feo V. 2010. The antigerminative activity of twenty-seven monoterpenes. Molecules. 15: 6630–6637.

Dudai N, Ben-Ami M, Chaimovich R, Chaimovitsh D. 2004. Essential oils as allelopathic agents: Bioconversion of monoterpenes by germinating wheat seeds. Acta Hortic. 629: 505–508.

Dragoeva A., Koleva VP, Nanova ZD, Kaschieva MZ, Yotova IR. 2014. Allelopathic and cytotoxic activity of *Origanum vulgare ssp. vulgare* growing wild in Bulgaria. Chem Bulg J Sci Educ. 23(1): 914-923.

Fiskesjö G. 1985. The Allium test as a standard in environmental monitoring. Hereditas. 102: 99-112.

Grondona E, Gatti G, López AG, Sánchez LR, Rivero V, Pessah O, Zunino MP, Ponce AA. 2014. Bio-efficacy of the essential oil of oregano (*Origanum vulgare Lamiaceae*. SSP. *Hirtum*). Plant Foods Hum Nutr. 69(4): 351–357.

Hamedo HA, Abdelmigid HM. 2009. Use of antimicrobial and genotoxicity potentiality for evaluation ofessential oils as food preservatives. Open Biotechnol J. 3(1): 50–56.

Ibáñez MD, Blázquez MA. 2020. Phytotoxic effects of commercial essential oils on selected vegetable crops: Cucumber and tomato. Sustain Chem Pharm. 15: 100209.

Karalija E, Dahija S, Parić A, Ćavar Zeljković S. 2020. Phytotoxic potential of selected essential oils against *Ailanthus Altissima* (mill.) Swingle, an invasive tree. Sustain Chem Pharm. 15: 100219.

Kadoglidou K, Chatzopoulou P, Maloupa E, Kalaitzidis A, Ghoghoberidze S, Katsantonis D. 2020. Mentha and oregano soil amendment induces enhancement of tomato tolerance against soilborne diseases, yield and quality. Agronomy. 10(3): 406.

Lombrea A, Antal D, Ardelean F, Avram S, Pavel IZ, Vlaia L, Mut AM, Diaconeasa Z, Dehelean CA, Soica C, et al. 2020. A recent insight regarding the phytochemistry and bioactivity of *Origanum vulgare* L. Essential Oil. Int J Mol Sci. 21(24): 9653.

Marrelli M, Conforti F, Formisano C, Rigano D, Arnold NA, Menichini F, Senatore F. 2016. Composition, antibacterial, antioxidant and antiproliferative activities of essential oils from three origanum species growing wild in Lebanon and Greece. Nat Prod Res. 30(6): 735–739.

Masotti V, Juteau F, Bessière JM, Viano J. 2003. Seasonal and phenological variations of the essential oil from the narrow endemic species *Artemisia molinieri* and its biological activities. J Agric Food Chem. 51(24): 7115–7121.

Obroucheva N. 2008. Cell elongation as an inseparable component of growth in terrestrial plants. Russ J Dev Biol. 39(1): 13–24.

Owen MD, Zelaya IA. 2005. Herbicide-resistant crops and weed resistance to herbicides. Pest Manag Sci. 61(3): 301–311.

Rusan MJ, Albalasmeh AA, Zuraiqi S, Bashabsheh M. 2015. Evaluation of phytotoxicity effect of olive mill wastewater treated by different technologies on seed germination of barley (*Hordeum vulgare* L.). Environ Sci Pollut Res. 22(12): 9127–9135.

Savini I, Arnone R, Catani MV, Avigliano L. 2009. *Origanum vulgare* L. induces apoptosis in human colon cancer Caco-2 cells. Nutr Cancer. 61(3): 381–389.

Scott SJ, Jones RA, William WA. 1984. Review of data analysis methods for seed germination. Crop Science. 24(4): 1192-1199.

Shishkova S, Rost TL, Dubrovsky JG. 2007. Determinate root growth and meristem maintenance in angiosperms. Ann Bot. 10(3): 319–340.

Singh HP, Kaur S, Mittal S, Batish DR, Kohli RK. 2009. Essential oil of Artemisia scoparia inhibits plant growth by generating reactive oxygen species and causing oxidative damage. J Chem Ecol. 3: 154–162.

Verdeguer M, Blázquez MA, Boira H. 2009. Phytotoxic effects of *Lantana camara*, eucalyptus camaldulensis and eriocephalus africanus essential oils in weeds of Mediterranean summer crops. Biochem Syst Ecol. 37(4): 362–369.

Weston LA. 1996. Utilization of allelopathy for weed management in Agroecosystems. J Agron. 88(6): 860–866.

Witzke S, Duelund L, Kongsted J, Petersen M, Mouritsen OG, Khandelia H. 2010. Inclusion of terpenoid plant extracts in lipid bilayers investigated by molecular dynamics simulations. J Phys Chem. 114: 15825–15831.

Wu H, Pratley J, Lemerle D, Haig T, An M. 2001. Screening Methods for the Evaluation of Crop Allelophatic Potential. Bot Rev. 67: 403-415.

Caryologia
International Journal of Cytology, Cytosystematics and Cytogenetics

Firenze University Press
www.fupress.com/caryologia

OPEN ACCESS

Citation: Mauricio Barros Fernandes, Jamille de Araújo Bitencourt, Joandson Calixto dos Santos, José Henrique Galdino, Paulo Roberto Antunes de Mello Affonso (2023). Cytogenetic analysis in *Tetragonopterus franciscoensis* (Characiformes): another piece to the karyoevolutionary puzzle of tetra fishes. *Caryologia* 76(1): 103-118. doi: 10.36253/caryologia-2145

Received: May 14, 2023

Accepted: July 9, 2023

Published: September, 19, 2023

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Cytogenetic analysis in *Tetragonopterus franciscoensis* (Characiformes): another piece to the karyoevolutionary puzzle of tetra fishes

Mauricio Barros Fernandes, Jamille de Araújo Bitencourt, Joandson Calixto dos Santos, José Henrique Galdino*, Paulo Roberto Antunes de Mello Affonso

Department of Biological Sciences, Universidade Estadual do Sudoeste da Bahia (UESB), Jequié - BA, Brazil
*Corresponding author. E-mail: galdino.jhsgb@gmail.com

Abstract. *Tetragonopterus* is a taxonomically complex genus in Characidae, being currently represented by nine species according to integrative approaches. One of them, *T. franciscoensis* was recently validated in rivers from northeastern Brazil. Even though molecular and morphological data have been collected in *Tetragonopterus*, the cytogenetic analyses in this group are scarce despite of the role of chromosomal variation in speciation. Herein, we present the first detailed karyotypic study in *T. franciscoensis* along with a comparative analysis with published cytogenetic data in characin fish. All specimens shared 2n=52 distributed in 12 metacentric (m), 12 submetacentric (sm), and 28 subtelocentric/acrocentric (st/a) chromosomes for both sexes as well as single nucleolus organizer regions on short arms of pair 8 and several GC-rich sites. The mapping of telomeric sequences (TTAGGG)n revealed no telomeric interstitial signals. While subtle cytogenetic differences were observed between samples from northeastern basins in Brazil, corroborating a recent genetic divergence, distinct karyotypes were detected in relation to congeneric taxa from other Brazilian regions. Therefore, the origin of large biarmed pairs in species with low 2n values should be related to occurrence of centric fusions.

Keywords: Characidae, Characins, cytotaxonomy, neotropical fish, Tetragonopterinae.

INTRODUCTION

The genus *Tetragonopterus* (Characidae) was proposed by Cuvier (1816) to describe the species *T. argenteus* based on a unique specimen from South America. In the second half of the 19th century, Günther (1864) added 32 new species to this taxon and proposed the subfamily Tetragonopterinae which would include most of small characins or tetras (e.g., *Astyanax, Hemigrammus, Moenkhausia, Psalidodon*).

Over the following decades, the group was extensively revised and it turned to be one of the most intriguing taxa among Characidae. In a series of studies carried out by Carl H. Eigenmann, several species previously allo-

cated in *Tetragonopterus* were reassigned to different genera, like *Bryconamericus, Ctenobrycon,* and *Deuterodon* (Eigenmann, 1917; Eigenmann, 1918; Eigenmann, 1921; Eigenmann and Myers, 1929). Later, the number of species in *Tetragonopterus* was reduced to four evolutionary units, comprising *T. argenteus, T. chalceus. T. gibossus*, and *T. huberi*. On that occasion, the reassignment of *T. georgiae* and *T. rarus* to *Moenkhausia*, for example, was justified by the lack of a complete lateral line greatly bent downwards at the anterior portion, a common feature of *Tetragonopterus*. Follow-up taxonomic reviews reallocated *T. argenteus* and *T. chalceus* as the only representatives of this genus (Reis *et al.,* 2003). However, this scenario has changed considerably, as DNA-based studies provided important insights about the taxonomic relationships of *Tetragonopterus* and other tetras (Araújo and Lucinda, 2014; Mirande, 2019).

Accordingly, molecular analysis recognized eight previously described species in *Tetragonopterus* (*T. anostomus, T. araguaiensis, T. argenteus, T. carvalhoi, T. chalceus, T. denticulatos. T. georgiae*, and *T. rarus)* and cases of cryptic diversity (Silva *et al.,* 2016). These authors revealed that the populations of *T. chalceus* from São Francisco, Paraguaçu, and Itapicuru river basins actually encompassed a distinct species, referred to as *T. fransciscoensis* (Silva *et al*., 2016). In addition, three new species were also described (*T. jurema, T. kulene*, and *T. ommotus*) and new evidence reallocated *Moenkhausia georgiae* back to *Tetragonopterus* (*T. georgiae*), as also supported by other authors (Silva *et al.,* 2016; Melo *et al.,* 2016; Terán *et al*., 2020).

Even though the abovementioned studies were particularly informative to resolve the taxonomic uncertainties in *Tetragonopterus*, cytotaxonomic analyses that could add new pieces of evidence to this subject remain limited to a few reports based on conventional analyses in *T. argenteus* Cuvier, 1816 and *T. chalceus* Spix & Agassiz, 1829. Both species shared a modal diploid number of 2n = 52, a single NOR system and few heterochromatin regions, but they diverge in their karyotype formulae (Portela *et al.,* 1988; Alberdi and Fenocchio, 1997). Interestingly, populations of *T. argenteus* from Cuiabá River were differentiated by the presence of two cytotypes (1 and 2). While the cytotype 1 is represented by specimens with 2n=50 and a karyotype of 14m+4sm+4st+28a, the cytotype 2 presents 2n=52 distributed into 14m+4sm+4st+30a chromosomes (Miyazawa, 2015).

A striking cytogenetic feature commonly reported in small characins is the presence of a large first metacentric pair when compared to other chromosomes in the karyotype (Scheel, 1973). In fact, this metacentric pair and a modal number of 2n=50 have been regarded as plesiomorphies for this fish group (Morelli *et al*., 1983; Portela-Castro *et al*.,1998; Tenório *et al*., 2013), being also observed in Bryconidae (Almeida-Toledo *et al.,* 1996; Mariguela *et al.,* 2010; Yano *et al.,* 2021).

In turn, the highly conserved morphology of small characins, including *Tetragonopterus* (Eigenmann, 1917), indicates that species complexes or cryptic species might be present, thus hindering reliable estimates of richness and endemicity rates in these Neotropical fishes. In this context, cytogenetic methods can help reveal such overlooked diversity, as exemplified by studies in the genus *Psalidodon* (e.g. Bertaco *et al*., 2006; Ferreira-Neto *et al.,* 2012). Therefore, the goal of the present study was to report the first detailed cytogenetic characterization of *T. franscicoensis* from an isolated drainage from Northeastern Brazil to shed some light on the taxonomy and species delimitation in *Tetragonopterus*. In addition, we carried out a comprehensive comparative cytogenetic analysis in characin species to provide insights about the karyoevolutionary trends in the subfamily Tetragonopterinae.

MATERIAL AND METHODS

Thirteen individuals of *T. franciscoensis* Silva, Melo, de Oliveira & Benine, 2016 (8 males and 5 females) were collected at the Itapicuru-Mirim River (Itapicuru River Basin) in the municipality of Tucano, state of Bahia, northeastern Brazil (11°12'15.3"S/40°29'15.1"W) (Fig. 1). The collection license was granted by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio/SISBIO n. 26752-2). The procedures and experiments were approved by the Committee of Ethics in Experimentation with Animals from the State University of Southwestern Bahia (CEUA/UESB 32/2013).

To stimulate cell division, the fish specimens were inoculated with fungal antigens and kept in tanks for 48 to 72 hours (Lee and Elder, 1980). Afterwards, the specimens were euthanized in cold water (Blessing *et al*., 2010), and the anterior kidney was removed to obtain metaphase cells, according to Netto *et al*., (2007). The cell suspension containing the mitotic chromosomes were dropped on glass slides, air dried and stained with 10% Giemsa in phosphate buffer (pH 6.8).

The heterochromatin was visualized by C-banding technique (Sumner, 1972), and active nucleolar organizer regions (Ag-NOR) were detected by silver staining (Howell and Black, 1980). Sequential staining with the base-specific fluorochromes Chromomycin A_3 (CMA_3) and 4'-6-diamino-2-fenilindole (DAPI) to detect GC-

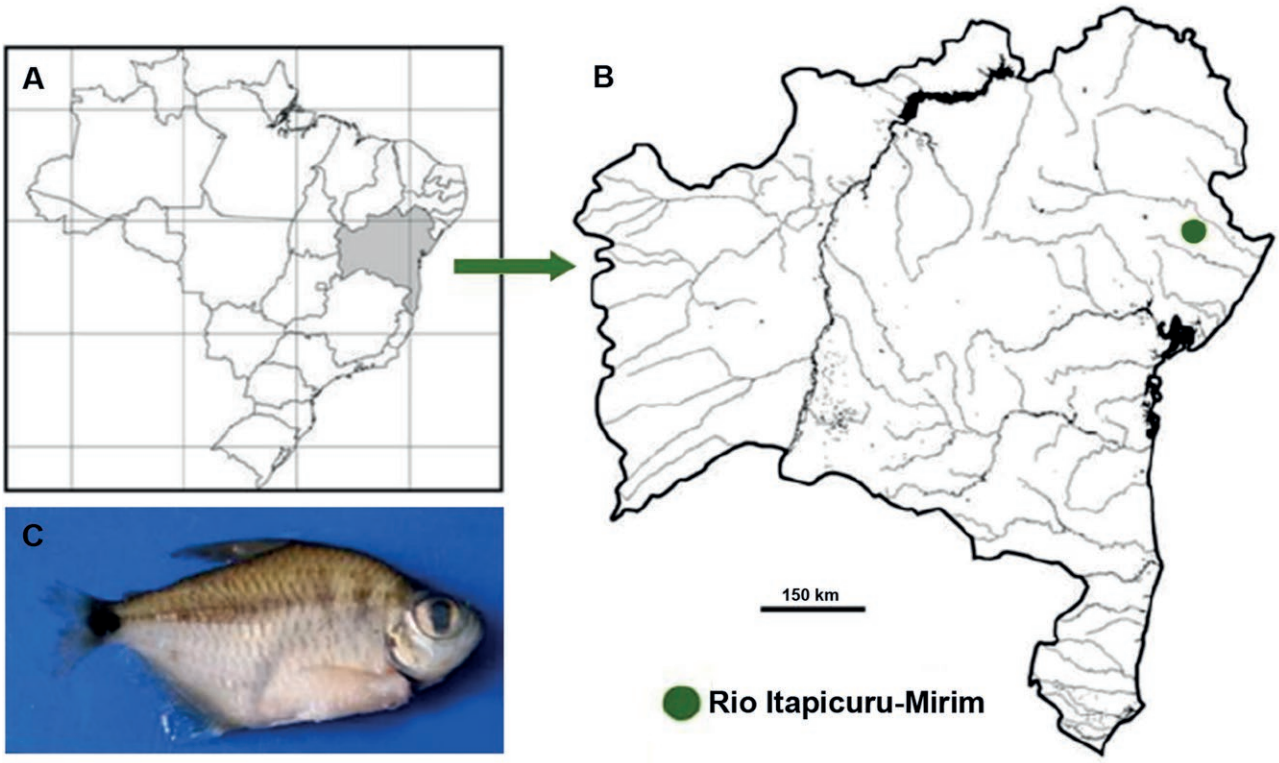


Figure 1. Map of Brazil highlighting the state of Bahia (a) and the collection site in Itapicuru-Mirim River (b) of *T. franciscoensis* (c).

and AT-rich regions, respectively, were carried out according to Schmid (1980).

The physical mapping of telomers was performed based on fluorescence *in situ* hybridization (FISH) according to Pinkel *et al.* (1986) under high stringency (77%) conditions to evaluate the putative presence of internal telomere sequences (ITS) that could reveal structural rearrangements. The telomere (TTAGGG) n probes were obtained via PCR without template DNA (Ijdo et al., 1991). The probes were labeled with digoxigenin-11-dUTP and detected with anti-digoxigenin-Rhodamine conjugate, according to the manufacturer's instructions (Roche). The chromosomes were counterstained with DAPI and the slides were mounted in a Vectashield medium.

A mean number of 10 metaphase spreads per specimen were analyzed using an epifluorescence microscope (Olympus BX-51) attached to a digital camera and equipped with the software Image-Pro Plus® v. 6.2 for photo documentation. The chromosomes were measured using the software Easy Idio 1.0 (Diniz and Melo, 2006). Then, they were classified according to their arm ratio (Levan *et al.* 1964), and the chromosomal pairs were systematically organized into karyotypes in decreasing size order within each morphological category.

RESULTS

A modal diploid number of 2n = 52 was observed in all specimens of *T. franciscoensis*, while the karyotype was invariably organized into 12 metacentric (m), 12 submetacentric (sm), and 28 subtelocentric/acrocentric (st/a) chromosomes (Figure 2a). No heteromorphic sex chromosomes were detected.

The silver staining revealed a single NOR-bearing pair (8) with heteromorphic ribosomal cistrons at interstitial regions on short arms. On the other hand, the C-banding revealed few heterochromatin blocks restricted to centromeres (Figure 2b). The GC-rich sites (CMA_3^+/$DAPI^-$) were coincident with Ag-NORs on pair 8 (Figure 3). Furthermore, additional CMA_3 signals were observed in, at least, three other chromosomal pairs (Figure 3). The mapping of (TTAGGG)n sequences by FISH revealed conspicuous signals on telomeres of all chromosomes and no internal telomere sequences (ITS) (Figure 4).

DISCUSSION

The karyotype macrostructure of *T. franciscoensis* (2n=52 and a karyotype formula of 12m+12sm+28st/a)

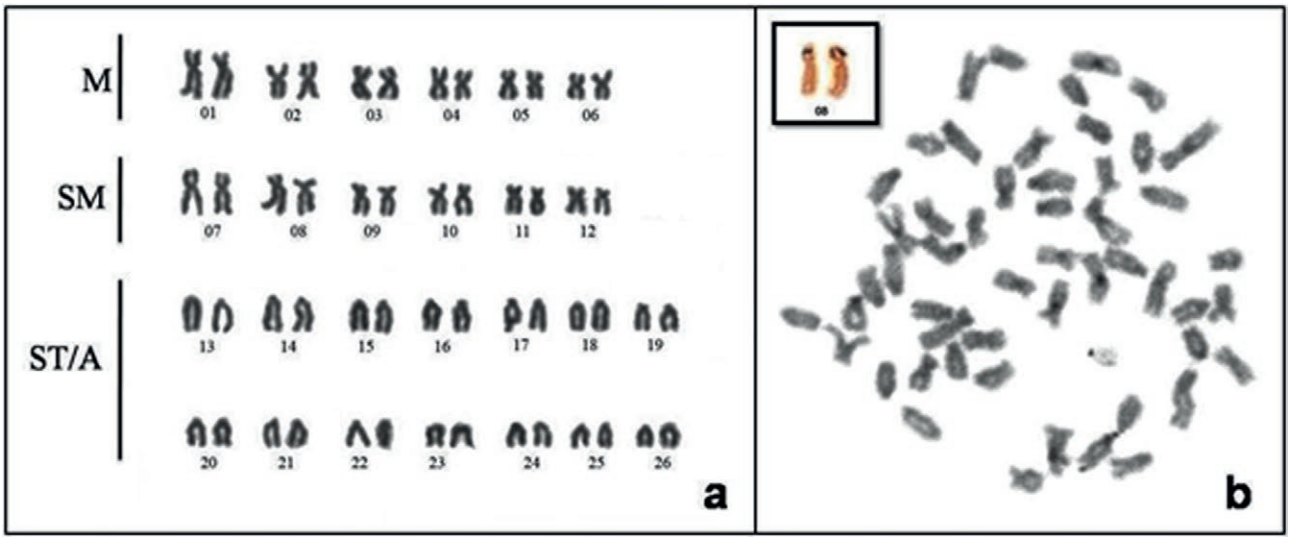


Figure 2. Giemsa-stained (a) and C-banded (b) karyotypes of *T. franciscoensis*. The NOR-bearing pair after silver nitrate in shown inbox.

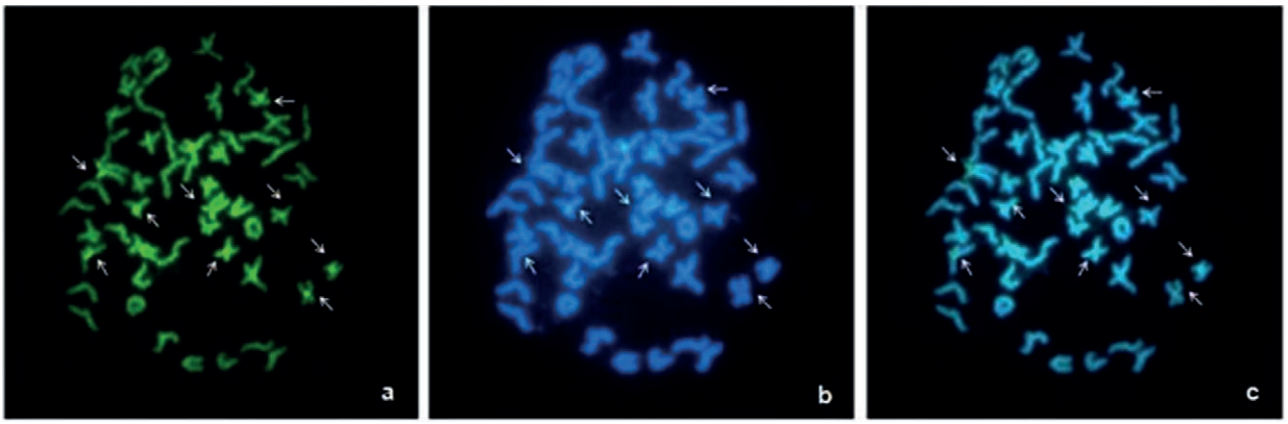


Figure 3. Somatic metaphases after CMA_3 (a); DAPI (b); and overlapped CMA_3/DAPI (c) staining, showing the GC-rich (CMA_3^+/$DAPI^-$ signals) indicated by arrows.

is similar to that reported in populations of *T. chalceus* (=*T. franscicoensis* sensu Silva *et al.*, 2016) from São Francisco River (26m/sm+26st/a) (Portela *et al*., 1988). The only difference refers to the presence of an additional subtelocentric/acrocentric pair in specimens from Itapicuru-Mirim (present study). This result suggests a genetic divergence among these lineages from each hydrographic system driven by pericentric inversions in a chromosome pair. Nevertheless, artifactual effects could also account for these such as distinct levels of chromosome condensation or the criteria for determining the chromosomal morphology between authors.

On the other hand, remarkable macrostructural differences are observed when the cytogenetic data in *T. franciscoensis* from the present study are compared to those reported in closely related species, such as *T. argenteus* from Paraná (16m/sm+2st+34a), Paraguay (14m+4sm+4st+28a), and (De La Plata) river basins (Alberdi and Fenocchio, 1997; Miyazawa, 2015). In the latter, the specimens presented interindividual variation in both number and morphology of chromosomes (2n-50, 14m+4sm+4st+28a and 2n=52, 16m+4sm+4st+28a) (Miyazawa, 2015; Supplementary Table 1). These findings indicate that inversions and fusions/fissions are involved in the karyoevolution of *Tetragonopterus* and that cryptic species are likely to be present in this group, as commonly observed in small characins (Medrado *et al*., 2018).

Furthermore, *T. franciscoensis* lacks the typical large metacentric pair described in other tetras, a condition putatively associated with the presence of 2n=52

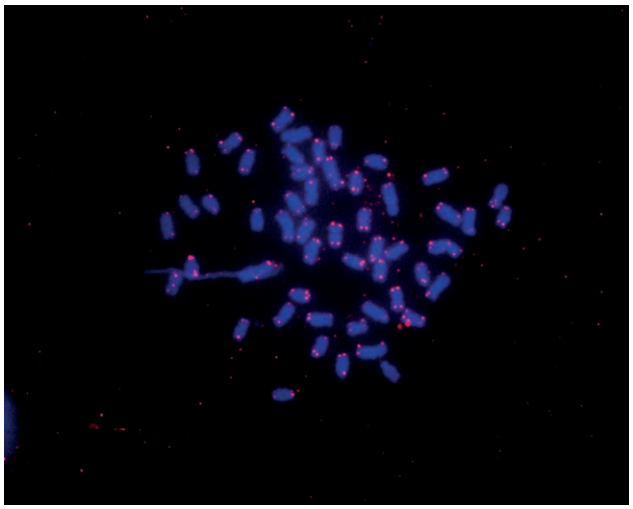


Figure 4. Metaphase of *T. franciscoensis* after FISH with (TTAGGG) n probes, revealing the positive signals (in magenta) on telomeres.

(Figure 2a), thus diverging from the pattern observed in several genera of Characidae like *Astyanax, Psalidodon*, *Moenkhausia*, and *Cheirodon* (Tenório *et al*., 2013; Soto *et al.,* 2018; Nascimento *et al.,* 2020). Such difference reinforces the divergence of *T. franciscoensis* and congeners in relation to other small characin lineages, corroborating their allocation in a distinct subfamily (Tetragonopterinae) (Mirande, 2018; Terán *et al*., 2020).

In fact, a distinctive first large metacentric pair is also found in representatives from other closely related and basal families of Characiformes (Supplementary Table 1), such as Bryconidae (Almeida-Toledo *et al.,* 1996, Mariguela *et al.,* 2010; Silva *et al.,* 2012), indicating that this is a plesiomorphic condition. Moroever, this condition (presence or absence of large metacentric pairs) varies remarkably among distinct taxonomic units in Characidae. Such variation has been reported even within some genera such as *Astyanax, Psalidodon*, and *Hyphessobrycon*, and within species, like *Bryconamericus* aff. *exodon* and *Bryconamericus* aff. *iheringii*, indicating putative species complexes or cryptic diversity (Supplementary Table 1).

On the other hand, the absence of a long metacentric pair appears to be ubiquitous in *Odontostilbe, Piabina, Serrapinnus,* and *Knodus* (Supplementary Table 1). Moreover, according to the present revision, the lack of this large metacentric pair is correlated with species characterized by 2n=52 (Supplementary Table 1). Therefore, it is reasonable to hypothesize that independent chromosomal fusion events could account for the very large size of the first pair of biarmed chromosomes and the reduction of diploid numbers (2n < 50) in characins. However, these findings are insufficient to fully understand the karyoevolutionary trends in Characidae because several genera and species in this family remain poorly studied in relation to their cytogenetic traits. Therefore, further basic chromosomal studies should be carried out to test the role of centric fusions in the karyoevolution of small characins and the utility of the largest metacentric pair as a cytotaxonomic marker in tetras.

Similarly, the number and distribution of NORs in *T. franciscoensis* (Figure 2b) resembles that of *T. chalceus* (Portela *et al*., 1988) and *T. argenteus* (Miyazawa, 2015), following a common trend among characins (Medrado *et al*., 2008). In addition, the presence of GC-rich (CMA_3^+) sites co-located with NORs are considered a basal trait for fish and amphibianli (Schmid, 1980; Tenório *et al*., 2013; Monteiro *et al*., 2022). On the other hand, the presence of additional GC-rich sites at centromeric regions (Figure 3) represent a unique and putatively apomorphic condition since AT-rich sites near centromeres are more frequently reported in small characins (Sánchez *et al*., 2021), thus indicating a heterogenous composition of satellite DNAs. These results show the importance of detailed chromosomal analyses to infer the dynamics of genome organization and the role of microarrangements in speciation of tetra fishes.

The mapping of telomeric sequences on chromosomes of *T. franciscoensis* (Figure 4) followed the expected pattern in vertebrates, revealing positive signals at terminal portions of chromosomal pairs (Meyne *et al*., 1989; Ferro *et al.,* 2003; Schmid *et al*., 2006) and no evidence of ITS. Nonetheless, this pattern should not reject the occurrence of chromosomal rearrangements in the analyzed species. Actually, ITS are often lost or degenerated in rearranged chromosomes, particularly when the chromosomal changes have occurred in early stages of differentiation among clades (Meyne *et al.,* 1990; Bolzan, 2017).

In general, the present study revealed subtle cytogenetic differences in *Tetragonopterus* from São Francisco and Itapicuru River basins in northeastern Brazil, contrasting with the distinct karyotypes of congeneric species from other Brazilian regions (e.g., *T. argenteus*). These findings provide additional support to the validation of these populations as *T. franciscoensis* as proposed by morphological data (Silva *et al.,* 2016). At last, the lack of the typical large metacentric pair and the predominance of 2n=52 in *Tetragonopterus* when compared to other small characins reinforced the status of Tetragonopterinae as a monophyletic subfamily. In addition, cytotaxonomic markers were reported for *T. franciscoensis* that can be properly used to resolve taxonomic uncertainties in Neotropical tetras.

FUNDING

This work was supported by Fundação de Amparo à Pesquisa do Estado da Bahia (FAPESB) under Grant [RED0009/2013]; and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) under Grant [001].

ACKNOWLEDGMENTS

The authors are thankful to Débora Diniz for adding efforts to conduct fluorescence *in situ* hybridization (FISH).

REFERENCES

Alberdi AJ, Fenocchio, AS. 1997. Karyotypes of Five Tetragonopterinae Species (Pisces, Characidae) from Argentina. Cytologia. 62:171-176.

Almeida-Toledo LFD, Bigoni AP, Bernardino G, Foresti F, Toledo-Filho SDA. 1996. Karyotype and NOR conservatism with heterochromatin reorganization in Neotropical Bryconids. Caryologia. 49(1):35-43.

Alves AL, Martins-Santos IC. 2002. Cytogenetics studies in two populations of *Astyanax scabripinnis* with 2n= 48 chromosomes (Teleostei, Characidae). Cytologia. 67(2):117-122.

Araujo L, Lucinda PHF. 2014. A new species of the genus *Tetragonopterus* Cuvier, 1816 (Ostariophysi: Characiformes: Characidae) from the rio Tocantins drainage, Brazil. Neotropical Ichthyology. 12:309-315.

Arefjev VA. 1990. Problems of karyotypic variability in the family Characidae (Pisces, Characiformes) with the description of somatic karyotypes for six species of tetras. Caryologia. 43(3-4):305-319.

Barreto SB, Cioffi MB, Medrado AS, Silva AT, Affonso, PR, Diniz D. 2016. Allopatric chromosomal variation in *Nematocharax venustus* Weitzman, Menezes & Britski, 1986 (Actinopterygii: Characiformes) based on mapping of repetitive sequences. Neotropical Ichthyology. 14(2):e150141.

Blessing JJ, Marshall JC, Balcombe SR. 2010. Humane killing of fishes for scientific research: a comparison of two methods. Journal of Fish Biology. 76(10):2571-2577.

Bolzan DA. 2017. Interstitial telomeric sequences in vertebrate chromosomes: Origin, function, instability and evolution. Mutation Research/Reviews in Mutation Research. 773:51-65.

Capistano TG, Portela-Castro ALDB, Júlio-Junior HF. 2008. Chromosome divergence and NOR polymorphism in *Bryconamericus* aff. *iheringii* (Teleostei, Characidae) in the hydrographic systems of the Paranapanema and Ivaí Rivers, Paraná, Brazil. Genetics and Molecular Biology. 31:203-207.

Carvalho ML, Oliveira C, Foresti F. 2002. Cytogenetic analysis of five species of the subfamily Tetragonopterinae (Teleostei, Characiformes, Characidae). Caryologia. 55(3):181-188.

Centofante L, Bertollo LAC, Miyazawa CS, Moreira-Filho, O. 2003. Chromosomal differentiation among allopatric populations of *Hyphessobrycon anisitsi* (Pisces, Tetragonopterinae). Cytologia. 68(3):283-288.

Coutinho-Sanches N, Dergam JA. 2015. Cytogenetic and molecular data suggest *Deuterodon pedri* Eigenmann, 1907 (Teleostei: Characidae) is a member of an ancient coastal group. Zebrafish. 12(5):357-365.

Cunha MS. 2021. Repetitive DNA mapping on *Oligosarcus acutirostris* (Teleostei, Characidae) from the Paraíba do Sul River Basin in southeastern Brazil. Caryologia. 74(4):121-128.

Cuvier G. 1816. Le règne animal distribué d'après son organisation pour servir de base à l'histoire naturelle des animaux et d'introduction à l'anatomie comparée. Les reptiles, les poissons, les mollusques et les annélides. Edition 1 v.2., Déterville, Paris, xviii+532.

Da Silva LLL, Dos Santos AR, Giuliano-Caetano L, Dias, AL. 2016. Chromosomal characterization in two species of an *Astyanax bimaculatus* complex (Characidae, Characiformes) using different techniques of chromosome banding. Cytotechnology. 68:1277-1286.

Da Silva LLL, Giuliano-Caetano L, Dias AL. 2014. Karyotypic diversity in a population of *Bryconamericus* aff. *iheringii* (Characidae). Genetics and Molecular Research. 13(1):2069-2081.

Da Silva LLL, Giuliano-Caetano L, Dias AL. 2012. Chromosome studies of *Astyanax jacuhiensis* Cope, 1894 (Characidae) from the Tramandai River Basin, Brazil, using in situ hybridization with the 18S rDNA probe, DAPI and CMA3 staining. Folia Biologica (Kraków). 60(3-4):135-140.

Da Silva LL, Dos Santos AR, Giuliano-Caetano L, Dias AL. 2016. Chromosomal characterization in two species of an *Astyanax bimaculatus* complex (Characidae, Characiformes) using different techniques of chromosome banding. Cytotechnology. 68:1277-1286.

Diniz D, Xavier PM: 2006. Easy Idio. Available: http://geocities.yahoo.com.br/easyidio/.

Domingues MDS, Vicari MR, Abilhoa V, Wamser JP, Cestari MM, Bertollo LA, Artoni RF. 2007. Cytogenetic and comparative morphology of two allopatric populations of *Astyanax altiparanae* Garutti & Britski, 2000 (Teleostei: Characidae) from upper rio Paraná basin. Neotropical Ichthyology. 5:37-44.

Dos Santos R, Medrado AAS, Diniz D, Oliveira C, Affonso PRAM. 2016. ZZ/ZW sex chromosome system in the endangered fish *Lignobrycon myersi* Miranda-Ribeiro, 1956 (Teleostei, Characiformes, Triportheidae). Comparative Cytogenetics. 10(2):245.

Dos Santos AR, Rubert M, Giuliano-Caetano L, Dias, AL. 2012. Sympatric occurrence of four cytotypes and one extra chromosome in *Bryconamericus ecai* (Characidae): 18S rDNA polymorphism and heterochromatin composition. *Hereditas*. 149(1):24-33.

Duarte MA, Giugliano LG, de Aquino PDPU, Grisolia CK, Milhomem-Paixão SSR. 2018. Cytogenetic studies in *Hasemania crenuchoides* (Characiformes: Characidae) and molecular investigations into kinship relationships of the genus. Caryologia. 71(4):446-452.

Eigenmann CH. Catalogue of the fresh-water fishes of tropical and south temperate America. 1910.

Eigenmann CH. 1903. New genera of South American fresh-water fishes, and new names for some old genera. Smithsonian Miscellaneous Collections.

Eigenmann CH. 1908. Preliminary descriptions of new genera and species of tetragonopterid characins (Zoölogical Results of the Thayer Brazilian expedition.). Bulletin of the Museum of Comparative Zoology. 52(6):91-106.

Eigenmann CH. 1917. The American Characidae, Part 1. Memoirs of the Museum of Comparative Zoology. 43:1–102.

Eigenmann CH. 1918. The American Characidae, Part 2. Memoirs of the Museum of Comparative Zoology. 43:103–208.

Eigenmann CH. 1921. The American Characidae, Part 3. Memoirs of the Museum of Comparative Zoology. 43:209–310.

Eigenmann CH, Myers G. 1929. The American Characidae, Part 5. Memoirs of the Museum of Comparative Zoology. 43:429–558.

Fernandes CA, Piscor D, Bailly D, Da Silva VFB, Martins-Santos IC. 2010. Cytogenetic studies comparing three Characidae fish species from the Iguatemi River Basin, Brazil. Cytologia. 75(4):329-333.

Ferreira Neto M, Vicari, MR, Camargo EFD, Artoni RF, Moreira-Filho O. 2009. Comparative cytogenetics among populations of Astyanax altiparanae (Characiformes, Characidae, Incertae sedis). Genetics and Molecular Biology. 32:792-796.

Ferro MAD, Moreira-Filho O, Bertollo CAL. 2003. B Chromosome Polymorphism in the Fish, *Astyanax scabripinnis*. Genetica. 119:147-53.

Foresti F, Almeida-Toledo LF, Toledo SA. 1989. Supernumerary chromosome system, C-banding pattern characterization and multiple nucleolus organizer regions in *Moenkhausia sanctaefilomenae* (Pisces, Characidae). Genetica. 79(2):07-114.

Freitas PD, Galetti-Jr PM. 1998. Chromosome characterization of a neotropical fish *Poptella paraguayensis* from Paraguay river basin (Stethaprioninae, Characidae). Cytologia. 63(1):73-77.

Gavazzoni M, Paiz LM, Oliveira CA, Pavanelli CS, Graca WJ, Margarido, VP. 2018. Morphologically cryptic species of the *Astyanax bimaculatus* "caudal peduncle spot" subgroup diagnosed through cytogenetic characters. Zebrafish. 15(4):382-388.

Goes CAG, Daniel SN, Piva LH, Yasui GS, Artoni RF, Hashimoto DT, Porto-Foresti F. 2020. Cytogenetic markers as a tool for characterization of hybrids of *Astyanax* Baird & Girard, 1854 and *Hyphessobrycon* Eigenmann, 1907. Comparative Cytogenetics. 14(2):231.

Günther ACLG. 1864. Catalogue of the fishes in the British Museum. Catalogue of the Physostomi, containing the families Siluridae, Characinidae, Haplochitonidae, Sternoptychidae, Scopelidae, Stomiatidae in the collection of the British Museum. Catalogue of the fishes in the British Museum. 5:1-455.

Guimarães IN, Foresti de Almeida-Toledo L, Oliveira C, Foresti F, De Almeida Toledo Filho S. 1995. Cytogenetic studies of three species of Glandulocaudinae (Pisces, Characiformes, Characidae). Revista Brasileira de Genética. 18(2):185-189.

Hattori RS, Zambelli FDSM. 2007. Foresti, A.T.L. Karyotype characterization and gene mapping of 5S and 18S rDNA in three species of *Oligosarcus* (Teleostei: Characidae). Caryologia. 60(4):372-378.

Howell WMT, Black DA. 1980. Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method. Experientia 36(8):1014-1015.

Ijdo JW, Wells RA, Baldini A, Reeders ST. 1991. Improved telomere detection using a telomere repeat probe (TTAGGG) n generated by PCR. Nucleic acids research. 19(17):4780.

Kantek DLZ, Noleto RB, Maurutto FAM, Bertollo LAC, Moreira-Filho O, Cestari MM. 2008 Cytotaxonomy of *Astyanax* (Characiformes, Characidae) from the Upper Iguaçu River Basin: confirmation of the occurrence of distinct evolutionary units. Journal of Fish Biology. 73(8):2012-2020.

Kavalco KF, Pazza R, Almeida-Toledo LF. 2008. *Astyanax bockmanni* Vari and Castro, 2007: an ambiguous karyotype in the *Astyanax* genus. Genetica. 136:135-139.

Kavalco KF, Pazza R, Bertollo LA, Moreira-Filho O. 2005. Molecular cytogenetics of *Oligosarcus hepsetus* (Teleostei, Characiformes) from two Brazilian locations. Genetica. 124:85-91.

Kavalco KF, Moreira-Filho O. 2003. Cytogenetical analyses in four species of the genus *Astyanax* (Pisces, Characidae) from Paraíba do Sul River basin. Caryologia. 56(4):453-461.

Klassmann JP, Dos Santos ICM. 2017. Karyotype diversity in *Astyanax scabripinnis* (Pisces, Characiformes) from the São Francisco River basin, Brazil. Cytologia. 82(2):199-203.

Krinski D, Centofante L, Miyazawa CS. 2008. Multiple NORs in *Knodus* cf. *chapadae* (Pisces: Characidae: *incertae sedis*) from Upper Paraguay Basin, Mato Grosso State, Brazil. The Nucleus. 51(3):229-238.

Krinski D, Miyazawa CS. 2012. First description of the karyotype and Ag-NORs localization of *Brachychalcinus retrospina* (Pisces: Characidae: Stethaprioninae) from Upper Paraguay Basin, Mato Grosso State, Brazil. Caryologia. 65(1):82-85.

Lee MR, Elder FFB. 1980. Yeast stimulation of bone marrow mitosis for cytogenetic investigations. Cytogenetic and Genome Research. 26(1):36-40.

Levan, A. 1964. Nomenclature for centromeric position on chromosomes. Hereditas. 52:201-220.

Maistro EL, Foresti F, Oliveira C, de Almeida Toledo LF. 1992. Occurrence of macro B chromosomes in *Astyanax scabripinnis paranae* (Pisces, Characiformes, Characidae). Genetica. 87:101-106.

Mari-Ribeiro IP, Scorsim B, Oliveira AVD, Portela-Castro ALDB. 2022. Cytogenetic and Molecular Characterization of *Oligosarcus pintoi* (Characidae): A New Record of Supernumerary Chromosome in this Species. Zebrafish. 19(2):71-80.

Mariguela T, Nirchio M, Ron E, Gaviria J, Foresti F, Oliveira C. 2010. Cytogenetic characterization of *Brycon amazonicus* (Spix et Agassiz, 1829) (Teleostei: Characidae) from Caicara del Orinoco, Venezuela. Comparative Cytogenetics. 4(2):185-193.

Martinez ER, Alves AL, Silveira SM, Foresti F, Oliveira C. 2012. Cytogenetic analysis in the *incertae sedis* species *Astyanax altiparanae* Garutti and Britzki, 2000 and *Hyphessobrycon eques* Steindachner, 1882 (Characiformes, Characidae) from the upper Paraná River basin. Comparative Cytogenetics. 6(1):41.

Medrado AS, Affonso PRMA, Carneiro PL, Vicari MR, Artoni RF, Costa MA. 2015. Allopatric divergence in *Astyanax* aff. *fasciatu*s Cuvier, 1819 (Characidae, Incertae sedis) inferred from DNA mapping and chromosomes. Zoologischer Anzeiger-A Journal of Comparative Zoology. 257:119-129.

Medrado AS, Figueiredo AVA, Waldschmidt AM, Affonso PRAM, Carneiro PLS. 2008. Cytogenetic and morphological diversity in populations of *Astyanax fasciatus* (Teleostei, Characidae) from Brazilian northeastern river basins. Genetics and Molecular Biology. 31:208-214.

Melo BF, Benine RC, Silva GS, Avelino GS, Oliveira C. 2016. Molecular phylogeny of the Neotropical fish genus *Tetragonopterus* (Teleostei: Characiformes: Characidae). Molecular Phylogenetics and Evolution. 94:709-717.

Mestriner CA, Galetti PM, Valentini SR, Ruiz IR, Abel LD, Moreira-Filho O, Camacho JP. 2000. Structural and functional evidence that a B chromosome in the characid fish *Astyanax scabripinnis* is an isochromosome. Heredity. 85(1):1-9.

Mendes MM, Rosa R, Giuliano-Caetano L, Dias AL. 2011. Karyotype diversity of four species of the *incertae sedis* group (Characidae) from different hydrographic basins: analysis of AgNORs, CMA3 and 18S rDNA. Genetics and Molecular Research. 10(4):3596-3608.

Meyne J, Baker RJ, Hobart, HH, Hsu TC, Ryder OA, Ward, OG, Moyzis RK. 1990. Distribution of non-telomeric sites of the (TTAGGG) n telomeric sequence in vertebrate chromosomes. Chromosoma. 99:3-10.

Meyne J, Ratliff RL, MoYzIs RK. 1989. Conservation of the human telomere sequence (TTAGGG) n among vertebrates. Proceedings of the National Academy of Sciences. 86(18):7049-7053.

Mirande JM. 2019. Morphology, molecules and the phylogeny of Characidae (Teleostei, Characiformes). Cladistics. 35(3):282-300.

Miyazawa C. 2015. Diferentes citótipos de *Tetragonopterus argenteus* Cuvier, 1817 (Characiformes, Tetragonopterinae) e descrição cariotípica de *Markiana nigripinnis* Perugia, 1891 (Characiformes, Characidae, *incertae sedis*) de rios da bacia do rio Paraguai. Scientia Vitae. 2:17– 27.

Monteiro ABGF, Takagui FH, Baldissera JNDC, Jerep FC, Giuliano-Caetano L. 2022. Classical and molecular cytogenetics of *Markiana nigripinnis* (Pisces-Characiformes) from brazilian Pantanal: a comparative analysis with cytotaxonomic contributions. Biologia. 77(8):2371-2382.

Moreira PWA, Bertollo LAC, Moreira-filho O. 2007. Comparative cytogenetics between three Characidae fish species from the São Francisco River basin. Caryologia. 60(1-2):64-68.

Morelli S, Bertollo LAC, Foresti F, Moreira-Filho O, De Almeida Toledo FS. 1983. Cytogenetic considerations on the genus *Astyanax* (Pisces, Characidae). I. Karyotypic variability. Caryologia. 36(3):235-244.

Nanda I, Schneider-Rasp S, Winking H, Schmid M. 1995. Loss of telomeric sites in the chromosomes of *Mus musculus domesticus* (Rodentia: Muridae) dur-

ing Robertsonian rearrangements. Chromosome Research. 3:399-409.

Nascimento CND, Troy WP, Alves JCP, Carvalho ML, Oliveira C, Foresti F. 2020. Molecular cytogenetic analyses reveal extensive chromosomal rearrangements and novel B chromosomes in *Moenkhausia* (Teleostei, Characidae). Genetics and Molecular Biology. 43(4): e20200027.

Netto MRCB, Paula E, Affonso PRAMA. 2007. standard protocol for obtaining fish chromosomes under post-mortem conditions. Micron. 38(3):214-217.

Nishiyama PB, Rossi MM, Porto FE, Borin LA, Castro ALDBP, dos Santos ICM. 2015. Estudos citogenéticos em espécies de peixes de riachos: *Hyphessobrycon vinaceus*, *Bryconamericus* aff. *iheringii* e *Odontostilbe pequira* (PISCES: CHARACIDAE). Evolução e Conservação da Biodiversidade. 6(1):13-22.

Pacheco RB, Giuliano-Caetano L, Julio Junior HF, Dias AL. 2010. Cytogenetic data on *Astyanax jacuhiensis* (Characidae) in the lago Guaíba and tributaries, Brazil. Neotropical Ichthyology. 8:667-671.

Paintner-Marques TR, Giuliano-Caetano L, Dias AL. 2002. Karyotypic diversity in a *Bryconamericus* aff. *exodon* population (Characidae, Tetragonopterinae). Cytologia. 67(4):397-402.

Pazza R, Kavalco SAF, Penteado PR, Kavalco KF, DeAlmeida-Toledo LF. 2008. The species complex *Astyanax fasciatus* Cuvier (Teleostei, Characiformes) – a multidisciplinary approach. Journal of Fish Biology. 72(8):2002-2010.

Pazian MF, Pereira LHG, Shimabukuru-Dias CK, Oliveira C, Foresti F. 2012. Cytogenetic and molecular markers reveal the complexity of the genus *Piabina* Reinhardt, 1867 (Characiformes: Characidae). Neotropical Ichthyology. 10:329-340.

Peres WAM, Bertollo LAC, Buckup PA, Blanco DR, Kantek DLZ, Moreira-Filho O. 2012. Invasion, dispersion and hybridization of fish associated to river transposition: Karyotypic evidence in *Astyanax* "*bimaculatus* group" (Characiformes: Characidae). Reviews in Fish Biology and Fisheries. 22:519-526.

Peres WAM, Buckup PA, Kantek DLZ, Bertollo LAC, Moreira-Filho O. 2009. Chromosomal evidence of downstream dispersal of *Astyanax fasciatus* (Characiformes, Characidae) associated with river shed interconnection. Genetica. 137:305-311.

Pinkel D, Straume T, Gray JW. 1986. Cytogenetic analysis using quantitative, high-sensitivity, fluorescence hybridization. Proc Natl Acad Sci. 83:2934-2938.

Piscor D, Paiz LM, Baumgärtner L, Cerqueira FJ, Fernandes CA, Lui RL, Margarido VP. 2020. Chromosomal mapping of repetitive sequences in *Hyphessobrycon eques* (Characiformes, Characidae): a special case of the spreading of 5S rDNA clusters in a genome. Genetica. 148(1):25-32.

Piscor D, Centofante L, Parise-Maltempi PP. 2017. Distinct classical and molecular cytogenetics of *Astyanax marionae* and *A. fasciatus* (Characiformes: Characidae): a comparative study of the organization of heterochromatin and repetitive genes. Journal of genetics. 96:665-671.

Piscor D, Parise-Maltempi. 2015. First description of B chromosomes in the *Hyphessobrycon* (Characiformes, Characidae) genus: a hypothesis for the extra element of *Hyphessobrycon eques* Steindachner, 1882. Comparative Cytogenetics. 9(3):325.

Piscor D, Ribacinko-Piscor DB, Fernandes CA, Parise-Maltempi PP. 2013. Cytogenetic analysis in three *Bryconamericus* species (Characiformes, Characidae): first description of the 5S rDNA-bearing chromosome pairs in the genus. Molecular Cytogenetics. 6:1-8.

Portela BA, Galetti-Jr. PM, Bertollo LAC. 1988. Considerations on the chromosome evolution of Tetragonopterinae (Pisces, Characidae). Braz. J. Genet. 11(2):307-316.

Portela-Castro ALB, Júlio–Jr, HF. 2002. Karyotype relationships among species of the subfamily Tetragonopterinae (Pisces, Characidae): Cytotaxonomic and evolution aspects. Cytologia. 67:329-336.

Prestes AB, Nardelli A, Paiz LM, Gavazzoni M, Margarido VP. 2019. Cytogenetic markers as tools in delimiting species of the highly diverse neotropical fish *Bryconamericus* (Characiformes: Characidae). Neotropical Ichthyology. 17(3):e190057.

Reis RE, Kullander SO, Ferraris-Jr CJ. 2003. Check list of the freshwater fishes of South and Central America. Porto Alegre: Edipucrs.

Rubert M, Margarido VP. 2007. Cytogenetic studies in three species of the genus *Oligosarcus*. Brazilian Archives of Biology and Technology. 50:127-135.

Salvador LB, Moreira-Filho O. 1992. B chromosomes in *Astyanax scabripinnis* (Pisces, Characidae). Heredity. 69(1):50-56.

Santos ARD, Usso MC, Gouveia JG, Araya-Jaime C, Frantine-Silva W, Giuliano-Caetano L, Dias AL. 2017. Chromosomal mapping of repetitive DNA sequences in the genus *Bryconamericus* (Characidae) and DNA barcoding to differentiate populations. Zebrafish. 14(3):261-271.

Sánchez KI, Takagui FH, Fenocchio AS. 2021. Cytogenetic analyses in three species of *Moenkhausia* Eigenmann, 1903 (Characiformes, Characidae) from

Upper Paraná River (Misiones, Argentina). Caryologia. 74(4):3-10.

Sánchez-Romero O, Abad CQ, Cordero PQ, de Sene VF, Nirchio M, Oliveira C. 2015. First description of the karyotype and localization of major and minor ribosomal genes in *Rhoadsia altipinna* Fowler, 1911 (Characiformes, Characidae) from Ecuador. Comparative cytogenetics. 9(2):271.

Scheel JJ. 1973. Fish chromosomes and their evolution. Internal Report of Danmarks Akvarvum, 22.

Schmid M. 1980. Chromosome banding in amphibia. IV. Differentiation of GC-and AT-rich chromosome regions in Anura. Chromosoma. 77(1):83-103.

Schmid M, Ziegler CG, Steinlein C, Nanda I, Schartl M. 2006. Cytogenetics of the bleak (*Alburnus alburnus*), with special emphasis on the B chromosomes. Chromosome Research, 14, 231-242.

Silva GS, Melo BF, Oliveira C, Benine RC. 2016. Revision of the South American genus *Tetragonopterus* Cuvier, 1816 (Teleostei: Characidae) with description of four new species. Zootaxa. 4200(1):1-46.

Silva DM, Pansonato-Alves JC, Utsunomia R, Daniel SN, Hashimoto DT, Oliveira C, Foresti F. 2013. Chromosomal organization of repetitive DNA sequences in *Astyanax bockmanni* (Teleostei, Characiformes): dispersive location, association and co-localization in the genome. Genetica. 141:329-336.

Silva PC, Santos U, Travenzoli NM, Zanuncio JC, Cioffi MDB, Dergam JA. 2012. The unique karyotype of *Henochilus wheatlandii*, a critically endangered fish living in a fast-developing region in Minas Gerais State, Brazil. Plos One. 7(7): e42278.

Soares LB, Paim FG, Ramos LP, Foresti F, Oliveira C. 2021. Molecular cytogenetic analysis and the establishment of a cell culture in the fish species *Hollandichthys multifasciatus* (Eigenmann & Norris, 1900) (Characiformes, Characidae). Genetics and Molecular Biology. 44(2):e20200260.

Soto MÁ, Castro JP, Walker LI, Malabarba LR, Santos MH, de Almeida MC, Artoni RF. 2018. Evolution of trans-Andean endemic fishes of the genus *Cheirodon* (Teleostei: Characidae) are associated with chromosomal rearrangements. Revista chilena de historia natural. 91(1):1-8.

Sousa CRP, Dos Santos JLA, Silva-Oliveira GC, Furo IDO, de Oliveira EHC, Vallinoto M. 2023. Characterization of a new cytotype and ocurrence of a B microchromosome in two spot *Astyanax*, *Astyanax bimaculatus* Linnaeus, 1758 (Characiformes: Characidae). Journal of Fish Biology. 102(2):520-524.

Sumner ATA. 1972. simple technique for demonstrating centromeric heterochromatin. Exp. Cell Res. 75:304-306.

Tenório RCCDO, Vitorino CDA, Souza IL, Oliveira C, Venere PC. 2013. Comparative cytogenetics in *Astyanax* (Characiformes: Characidae) with focus on the cytotaxonomy of the group. Neotropical Ichthyology. 11:553-564.

Terán GE, Benitez MF, Mirande JM. 2020. Opening the Trojan horse: phylogeny of *Astyanax*, two new genera and resurrection of *Psalidodon* (Teleostei: Characidae). Zoological Journal of the Linnean Society. 190(4):1217-1234.

Tonello S, Blanco DR, Cerqueira FJ, Lira NL, Traldi JB, Pavanelli CS, Lui RL. 2022. High rDNA polymorphisms in *Astyanax lacustris* (Characiformes: Characidae): new insights about the cryptic diversity in *A. bimaculatus* species complex with emphasis on the Paraná River basin. Neotropical Ichthyology. 20(2):e210147.

Torres RA, Motta TS, Nardino D, Adam ML, Ribeiro J. 2008. Chromosomes, RAPDs and evolutionary trends of the Neotropical fish *Mimagoniates microlepis* (Teleostei: Characidae: Glandulocaudinae) from coastal and continental regions of the Atlantic Forest, Southern Brazil. Acta Zoologica. 89(3):253-259.

Torres-Mariano AR, Morelli S. 2008. B chromosomes in a population of *Astyanax eigenmanniorum* (Characiformes, Characidae) from the Araguari River Basin (Uberlândia, MG, Brazil). Genetics and Molecular Biology. 31:246-249.

Troy WP, Pacheco ÉB, Oliveira C, Miyazawa CS. 2010. Chromosomic number characterization of *Odontostilbe* and *Serrapinnus* species (Characidae: Cheirodontinae) from Paraguay River basin, Brazil. Publicatio UEPG-Ciências Biológicas e da Saúde. 16(1):57-61.

Usso MC, Mortati AF, Morales-Blanco AG, Giuliano-Caetano L, Dias AL. 2018. Molecular cytogenetics in different populations of *Oligosarcus paranensis* (Characidae): comparative analysis of the genus with 5S and 18S rDNA probes. Caryologia. 71(2):103-108.

Voltolin DTHTA, de Arruda ADNV, Foresti F, Bortolozzi J, Porto-Foresti F. 2012. Cytogenetic analysis of B chromosomes in one population of the fish *Moenkhausia sanctaefilomenae* (Steindachner, 1907) (Teleostei, Characiformes). Comparative Cytogenetics. 6(2):141-151.

Wasko AP, Galetti Jr PM. 1998. Karyotype diversity in the neotropical fish *Bryconamericus* (Characidae, Tetragonopterinae). Cytobios. 94:185-193

Wasko AP, Vênere PC, Galetti Jr PM. 1996. Chromosome divergence between two simpratic characid fishes of the genus *Bryconamericus.* Braz J. Genet. 19:225-230.

Yano CF, Sember A, Kretschmer R, Bertollo LAC, Ezaz T, Hatanaka T, de Bello Cioffi M. 2021. Against the

mainstream: exceptional evolutionary stability of ZW sex chromosomes across the fish families Triportheidae and Gasteropelecidae (Teleostei: Characiformes). Chromosome Research. 29(3-4):391-416.

Yano CF, Bertollo LAC, Liehr T, Troy WP, Cioffi MDB. 2016. W chromosome dynamics in *Triportheus* species (Characiformes, Triportheidae): an ongoing process narrated by repetitive sequences. Journal of Heredity. 107(4):342-348.

Table 1. Cytogenetic data of small characins and closely related groups, according to Mirande (2018) and Terán *et al.* (2020) (the asterisks indicate the taxa whose scientific names were updated).

Species	2n	Karyotype	Locality	Distinctive large m pair	Reference
Family Characidae					
Subfamily Aphyocharacinae					
Prionobrama filigera	52	12m/sm+ 40st/a	Not Informed	Present	Arefjev (1990)
Subfamily Characinae					
Phenacogaster cf. pectinatus	46	12m+2st+32a	São Francisco stream - AC	Present	Carvalho *et al.* (2002)
Subfamily Cheirondotinae					
Cheirodon australe	52	8m+6sm+4st+33a	La Poza Lake - Chile	Present	Soto *et al.* (2018)
Cheirodon galusdae	52	6m+6sm+4st+34a	Andalién River - Chile	Present	Soto *et al.* (2018)
Cheirodon interruptus	52	6m+6sm+4st+34a	Marga-Marga River - Chile	Present	Soto *et al.* (2018)
Cheirodon kiliani	52	8m+6sm+4st+33a	Calle-Calle River - Chile	Present	Soto *et al.* (2018)
Cheirodon pisciculus	52	6m+6sm+4st+34a	Angostura River - Chile	Present	Soto *et al.* (2018)
Odontostilbe pequira	52	14m+20sm+14st+4a	Onça stream - MS	**Absent**	Nishiyama *et al.* (2015)
Odontostilbe pequira	52	24m+12sm+12st+4a	Cuiabá River - MT	**Absent**	Troy *et al.* (2010)
Serrapinnus calliurus	52	36m+12sm+6st	Bento Gomes River - MT	**Absent**	Troy *et al.* (2010)
Serrapinnus heterodon	52	16m+20sm+14st+2a	São Francisco River - MG	**Absent**	Peres *et al.* (2007)
Serrapinnus kriegi	52	24m+18sm+10st	Cuiabá River - MT	**Absent**	Troy *et al.* (2010)
Serrapinnus microdon	52	30m+12sm+8st+4a	Bento Gomes River - MT	**Absent**	Troy *et al.* (2010)
Serrapinnus piaba	52	16m+20sm +14st+2a	São Francisco River- MG	**Absent**	Peres *et al.* (2007)
Subfamily Stethaprioninae					
Astyanax abramis	50	4m+30sm+8st+8a	Iguaçu River - PR	Present	Gavazzoni *et al.* (2018)
Astyanax altiparanae	50	6m+28sm+4st+12a	Pântano Stream and Jordão River - PR, Feijão Stream - MG	Present	Ferreira-Neto *et al.* (2009)
Astyanax altiparanae	50	6m+28sm+8st+8a	Tibagi River - PR	Present	Domingues *et al.* (2007)
Astyanax altiparanae	50	6m+30sm+8st+6s	Iguaçu River - PR	Present	Domingues *et al.* (2007
Astyanax altiparanae	50	16m+24sm+4st+6a	Queixada River - PR	Present	Da Silva *et al.* (2016)
Astyanax altiparanae	50	16m+20sm+4st+10a	Esperança stream - PR	Present	Da Silva *et al.* (2016)
Astyanax altiparanae	50	16m+20sm+4st+10a	Jacutinga River - PR	Present	Da Silva *et al.* (2016)
Astyanax altiparanae	50	6m+28sm+4st+12a	Paraná River - PR	Present	Gavazzoni *et al.* (2018)
Astyanax asuncionensi	50	18m+22sm+6st+4a	Miranda River - MS	Present	Da Silva *et al.* (2016)
Astyanax asuncionensis	50	8m+24sm+6st	Iguaçu River - PR	Present	Gavazzoni *et al.* (2018)
Astyanax aff. *bimaculatus*	50	4m+14sm+24st+8a	Dois de Agosto stream - PA	Present	Sousa *et al.* (2023)
Astyanax bimaculatus	50	8m+34sm+2st+6a	São Francisco River - PR	Present	Peres *et al.* (2012)
Astyanax bimaculatus	50	8m+32sm+2st+8a	Grande River - PR	Present	Peres *et al.* (2012)
Astyanax bimaculatus	50	8m+33sm+2st+7a	Piumhi River - PR	Present	Peres *et al.* (2012)
Astyanax bimaculatus	50	8m+31sm+2st+9a	Piumhi River - PR	Present	Peres *et al.* (2012)
Astyanax bimaculatus	50	8m+30sm+2st+10a	Piumhi River - PR	Present	Peres *et al.* (2012)
Astyanax bimaculatus	50	10m+18sm+12 st+10a	Aguapeí River - SP	Present	Alberdi and Fenocchio (1997)
Astyanax bimaculatus	50	6m+28sm+8st+8a	Caeté River - PA	Present	Sousa *et al.* (2023)
Astyanax lacustris	50	10m+24sm+6st+10a	Itaipu Lake, Paraná River basin - PR	Present	Tonello *et al.* (2022)

Species	2n	Karyotype	Locality	Distinctive large m pair	Reference
Astyanax lacustris	50	6m+12sm+14st+18a	Pirassununga River - SP	Present	Goes *et al.* (2020)
Astyanax jacuhiensis	50	10m+26sm+6st+8a	Tramandaí River basin - RS	Present	Da Silva *et al.* (2012)
Astyanax jacuhiensis	50	8m+30sm+4st+8a	Guaíba Lake - RS	Present	Pacheco *et al.* (2010)
Astyanax jacuhiensis	50	8m+28sm+6st+8a	Ijuá River - PR	Present	Gavazzoni *et al.* (2018)
Astyanax scabripinnis	50	8m+20s+8st+14a	Macacos River - PR	Present	Kavalco and Moreira-Filho (2003)
Astyanax scabripinnis	50	6m+22sm+10st+12a	Córrego das Pedras stream - SP	Present	Mestriner *et al.* (2000)
Astyanax scabripinnis	50	8m+22sm+12st+6a	Mogi-Guaçu River basin - SP	Present	Pazza *et al.* (2008)
Astyanax scabripinnis	50	12m+20sm+10st+4a	Paranapanema River basin - SP	Present	Pazza *et al.* (2008)
Astyanax scabripinnis	50	6m+22sm+10st+12a	Córrego das Pedras stream - SP	Present	Salvador and Moreira-Filho (1992)
Astyanax scabripinnis	50	10m+20sm+8st+12a	São Francisco River - PR	Present	Klassmann and Martins-Santos (2017)
Astyanax scabripinnis	48	11m+18sm+9st+10a	São Francisco River - PR	Present	Klassmann and Martins-Santos (2017)
Astyanax scabripinnis	48	10m+20sm+8st+10a	Ivaí River - PR	Present	Alves and Martins-Santos (2002)
Astyanax sp.	50	4m+22sm+8st+16a	Piraquara, Upper Paraná River basin - PR	Present	Kantek *et al.* (2008)
Astyanax sp.	50	4m+22sm+8st+16a	Bicudo River, Upper Paraná River basin - PR	Present	Kantek *et al.* (2008)
Astyanax sp.	50	4m+24sm+6st+16a	Bicudo River, Upper Paraná River basin - PR	Present	Kantek *et al.* (2008)
Astyanax sp.	52	22m+26sm+4a	Upper Paraná River basin - PR	**Absent**	Tenório *et al.* (2013)
Brachychalcinus retrospina	50	6m+24sm+6st+4a	Angelim River - MT	Present	Krinski and Miyazawa (2012)
Ctenobrycon hauxwellianus	50	10m+6sm+34st	São Francisco stream - AC	Present	Carvalho *et al.* (2002)
Deuterodon (*Astyanax*) *giton**	50	6m+8sm+8st+28a	Paraitinga River - SP	Present	Kavalco and Moreira-Filho (2003)
Deuterodon (*Astyanax*) *intermedius**	50	6m+18sm+12st+10a	Paraitinga River - SP	Present	Kavalco and Moreira-Filho (2003)
Deuterodon (*Astyanax*) *janeiroensis**	50	6m+14sm+14st+16a	Betari River - SP	Present	Carvalho *et al.* (2002)
Deuterodon pedri	50	12m+12sm+20st+6a	Santo Antônio - River	Present	Coutinho-Sanches and Dergam (2015)
Deuterodon pedri	50	14m/sm+36st/a	Pedri River - SP	Present	Portela *et al.* (1988)
Deuterodon stigmaturus	50	8m+6sm+2st+34a	Maquiné River - RS	Present	Mendes *et al.* (2011)
Gymnocorhimbus ternetzi	50	14m+12sm+6st	Paraná River - PR	Present	Alberdi and Fenocchio (1997)
Hasemania crenuchoides	50	6m+26sm+16st+2a	Alto-Tocantins River	Present	Duarte *et al.* (2018)
Hasemania marginata	50	12m+18sm +10st+10a.	Not Informed	Present	Arefjev (1990)
Hasemania nana	50	8m+42sm	São Francisco River basin - MG	Present	Moreira *et al.* (2007)
Hemigrammus hyanuary	52	22m/sm+30st/a.	Not Informed	Present	Arefjev (1990)
Hemigrammus marginatus	50	10m+34sm+6a	Upper Paraná River basin -PR	Present	Portela-Castro and Júlio-Jr (2002)
Hollandichthys multifasciatus	50	8m+10sm+32st	Iguapé River - SP	Present	Soares *et al.* (2021)
Hollandichthys multifasciatus	50	10m+12sm+28st	Grande River - SP	Present	Carvalho *et al.* (2002)
Hyphessobrycon anisitsi	50	6m+16sm+12st+16a	Upper Paraná River - PR	Present	Centofante *et al.* (2003)
Hyphessobrycon anisitsi	50	10m+2sm+20st+18a	Pirassununga River -SP	Present	Goes *et al.* (2020)
Hyphessobrycon anisitsi	50	18m+10sm+6st+16a	Tibagí River - PR	Present	Mendes *et al.* (2011)
Hyphessobrycon eques	52	10m+20sm+8st+14a	Piracicaba River - SP	**Absent**	Piscor *et al.* (2020)
Hyphessobrycon eques	52	14m+16sm+4st+18a	Capivara River - SP	**Absent**	Martinez *et al.* (2012)
Hyphessobrycon eques	52	12m+26sm+8st+14a	Ribeirão Claro River - SP	**Absent**	Piscor and Parise-Maltempi (2015)
Hyphessobrycon flammeus	52	18m/sm+32st+2a.	Not informed	Present	Arefjev (1990)
Hyphessobrycon herbertaxelrodi	52	10m/sm+42st/a.	não informado	**Absent**	Arefjev (1990)
Hyphessobrycon luetkenii	50	6m + 8sm + 36a	Maquiné River - RS	Present	Mendes *et al.* (2011)
Hyphessobrycon reticulatus	50	14m+20sm+16st	Jequiá River - SP	Present	Carvalho *et al.* (2002)

Species	2n	Karyotype	Locality	Distinctive large m pair	Reference
Hyphessobrycon scholzei	50	8m+20sm+14a	Not Informed	Present	Arefjev (1990)
Hyphessobrycon vinaceus	50	8m+12sm+30a	Pardo River - BA	Present	Nishiyama *et al.* (2015)
Moenkhausia cosmops	50	14m+30sm+6st	Verde River - MT	Present	Nascimento *et al.* (2020)
Moenkhausia costae	50	50m/sm	São Francisco River – MG	Present	Portela *et al.* (1988)
Moenkhausia dichroura	50	22m+22sm+6st	Upper Paraná River - Argentina	Present	Sánchez *et al.* (2021)
Moenkhausia forestii	50	10m+32sm+8st	Sangue River - MT	Present	Nascimento *et al.* (2020)
Moenkhausia intermedia	50	16m+28sm+6st	Upper Paraná River - Argentina	Present	Sánchez *et al.* (2021)
Moenkhausia intermedia	50	50m/sm	Lagoa do Mato - SP	Present	Portela *et al.* (1988)
Moenkhausia intermedia	50	16m+34sm	Paraná River - PR	Present	Portela-Castro and Júlio-Jr (2002)
Moenkhausia nigromarginata	50	14m+32sm+4a	Verde River - MT	Present	Nascimento *et al.* (2020)
Moenkhausia oligolepis	50	12m+32sm+6st	Xapuri River- AC	Present	Nascimento *et al.* (2020)
Moenkhausia sanctaefilomenae	50	6m+23sm+12st	Batalha River, Tietê River basin - SP	Present	Voltolin *et al.* (2012)
Moenkhausia sanctaefilomenae	50	48m/sm+2a	Capivara and Tietê River - SP	Present	Forestii *et al.* (1989)
Moenkhausia sanctaefilomenae	50	12m+36sm+2a	Paraná River - PR	Present	Portela-Castro and Júlio-Jr (2002)
Moenkhausia sanctaefilomenae	50	12m+32sm+6st	Upper Paraná River - Argentina	Present	Sánchez *et al.* (2021)
Moenkhausia sanctaefilomenae	50	48m/sm+2st/a	Aguapeí River - Argentina	Present	Alberdi and Fenocchio (1997)
Nematobrycon palmeri	50	8m/sm+10st+32a	Not Informed	Present	Arefjev (1990)
Nematocharax venustus	50	8m+26sm+14st+2a	Contas River - BA/Jequitinhonha - MG	Present	Barreto *et al.* (2016)
Oligosarcus acutirostris	50	4m+14sm+18st	Paraibuna River - ES	Present	Cunha *et al.* (2021)
Oligosarcus hepsetus	50	6m+10sm+16+18a	Paraitinga and Paraíba do Sul River basin -SP	Present	Kavalco *et al.* (2005)
Oligosarcus hepsetus	50	2m+16sm+16st+16a	Paraíba do Sul River - SP	Present	Hattori *et al.* (2007)
Oligosarcus jenynsii	50	2m+24sm+10st+14a	Uruguay River - SC	Present	Hattori *et al.* (2007)
Oligosarcus longirostris	50	4m+10sm+16st+20a	Iguaçu River - PR	Present	Rupert and Margarido (2007)
Oligosarcus paranensis	50	8m+18sm+10st+14a	Tibagi River basin - PR	Present	Usso *et al.* (2018)
Oligosarcus paranensis	50	4m+10sm+16st+20a	Piquiri River basin - PR	Present	Rupert and Margarido (2007)
Oligosarcus pintoi	50	4m+10sm+16st+20a	Piquiri River basin - PR	Present	Rupert and Margarido (2007)
Oligigosarcus pintoi	50	4m+12sm +14st +20a	Ivaí River - PR	Present	Mari-Ribeiro *et al.* (2022)
Oligosarcus pintoi	50	2m+20sm+12st+16a	Mogi-Guaçu River - SP	Present	Hattori *et al.* (2007)
Oligosarcus sp.	50	6m+14sm+18st+12a	Velhas River basin - Ouro Preto - MG	Present	De-Barros *et al.* (2015)
Oligosarcus sp.	50	4m+14sm+20st+12a	Doce River basin - MG	Present	De-Barros *et al.* (2015)
Orthospinus franciscensis	50	22m+20sm+2st+6a	São Francisco River - MG	Present	Moreira *et al.* (2007)
Poptella paraguayensis	50	10m+26sm+8st+6a	Miranda River - MT	Present	Freitas and Galetti (1998)
Psalidodon (*Astyanax*) *bockmanni**	50	10m+12sm+12st+16a	Paranapanema River basin, São Miguel Arcanjo and Pilar do Sul - SP	Present	Kavalco *et al.* (2008)
Psalidodon (*Astyanax*) *bockmanni**	50	8m+14sm+12st+16a	Capivara River, Tietê River basin - SP	Present	Silva *et al.* (2013)
Psalidodon (*Astyanax*) *bockmanni**	50	8m+14sm+14st+14a	Água Madalena stream, Paranapanema River basin - SP	Present	Silva *et al.* (2013)
Psalidodon (*Astyanax*) *bockmanni**	50	6m+20sm+8st+16a	Iguatemi River basin - MS	Present	Fernandes *et al.* (2010)
Psalidodon (*Astyanax*) aff. *fasciatus**	50	16m+12sm+6st+16a	Tributary of Cabeça River - SP	Present	Piscor *et al.* (2017)
Psalidodon (*Astyanax*) aff. *fasciatus**	48	10m+20sm+8st+10a	Tributary of Ribeirão Claro River - SP	Present	Piscor *et al.* (2017)
Psalidodon (*Astyanax*) *eigenmanniorum**	48	14m+24sm+4st+10a	Araguari River Basin - MG	Present	Torres-Mariano and Morelli (2008)
Psalidodon (*Astyanax*) *eigenmanniorum**	48	10m+16sm+10st+12a	Laguna dos patos - RS	Present	Mendes *et al.* (2011)

Species	2n	Karyotype	Locality	Distinctive large m pair	Reference
Psalidodon (*Astyanax*) *fasciatus**	48	8m+20sm+16st+4a	São Francisco River - MG	Present	Peres *et al.* (2009)
Psalidodon (*Astyanax*) aff. *fasciatus**	50	8m+26sm+6st+10a	Afluente do rio Corumbataí - SP	Present	Piscor *et al.* (2017)
Psalidodon (*Astyanax*) aff. *fasciatus**	48	10m+12sm+12st+14a	Pirassununga - SP	Present	Goes *et al.* (2020)
Psalidodon (*Astyanax*) aff. *fasciatus**	48	8m+20sm+12st+8a	Preto do Costa River - BA	Present	Medrado *et al.* (2015)
Psalidodon (*Astyanax*) aff. *fasciatus**	48	8m+18sm+14st+8a	Mutum River- BA	Present	Medrado *et al.* (2015)
Psalidodon (*Astyanax*) aff. *fasciatus**	48	8m+24sm+10st+6a	Oricó River - BA	Present	Medrado *et al.* (2015)
Psalidodon (*Astyanax*) aff. *fasciatus**	48	8m+28sm+8st+4a	Criciúma River - BA	Present	Medrado *et al.* (2015)
Psalidodon (*Astyanax*) aff. *fasciatus**	48	8m+18sm+16st+6a	Gongogi River - BA	Present	Medrado *et al.* (2015)
Psalidodon (*Astyanax*) aff. *fasciatus**	48	8m+16sm+16st+8a	Mineiro River - BA	Present	Medrado *et al.* (2015)
Psalidodon (*Astyanax*) aff. *fasciatus**	48	8m+16sm+18st+6a	Itapicuru River - BA	Present	Medrado *et al.* (2015)
Psalidodon (*Astyanax*) aff. *fasciatus**	48	8m+24sm+10st+6a	Braço River - BA	Present	Medrado *et al.* (2015)
Psalidodon (*Astyanax*) aff. *fasciatus**	48	8m+22sm+10st+8a	Cachoeira River - BA	Present	Medrado *et al.* (2015)
Psalidodon (*Astyanax*) aff. *fasciatus**	48	8m+20sm+16st+4a	Contas River - BA	Present	Medrado *et al.* (2015)
Psalidodon (*Astyanax*) *marionae**	48	4m+24sm+10st+6a	Rio claro stream, Paraguai River basin - MS	Present	Piscor *et al.* (2017)
Psalidodon (*Astyanax*) *parabybae**	48	8m+18sm+12st+10a	Paraitinga River - SP	Present	Kavalco and Moreira-Filho (2003)
Psalidodon (*Astyanax scabripinnis paranae*) *paranae**	50	4m+34sm+4st+6a	Araquá River - SP	Present	Maistro *et al.* (1992)
Psalidodon (*Astyanax*) *schubarti**	36	10m+10sm+10st+6a	Pirassununga -SP	Present	Goes *et al.* (2020)
Psalidodon (*Astyanax*) *schubarti**	36	14m+14sm/6st+2a	Paraná River - PR	Present	Alberdi and Fenocchio (1997)
Rhoadsia altipinna	50	10m+26+14a	Das Bocas River - Equador	Present	Sanchez-Romero *et al.* (2015)
Subfamily Stevardiinae					
Bryconamericus aff. *exodon*	52	16m+12sm+6st+18a	Tibagí River - PR	**Absent**	Paintner-Marques *et al.* (2002)
Bryconamericus aff. *exodon*	52	10m+24sm+6st+12a	Tibagí River - PR	**Absent**	Paintner-Marques *et al.* (2002)
Bryconamericus aff. *iheringii*	52	12m+10sm+16st+14a	Três Bocas Stream - PR	**Absent**	Da Silva *et al.* (2014)
Bryconamericus aff. *iheringii*	52	18m+14sm+10st+10a	Três Bocas Stream - PR	**Absent**	Da Silva *et al.* (2014)
Bryconamericus aff. *iheringii*	52	20m+18sm+4st+10a	Três Bocas Stream - PR	**Absent**	Da Silva *et al.* (2014)
Bryconamericus aff. *iheringii*	52	20m+14sm+12st+6a	Três Bocas Stream - PR	**Absent**	Da Silva *et al.* (2014)
Bryconamericus aff. *iheringii*	52	22m+18sm+8st+4a	Três Bocas Stream - PR	**Absent**	Da Silva *et al.* (2014)
Bryconamericus aff. *iheringii*	52	18m+24sm+6st+4a	Três Bocas Stream - PR	**Absent**	Da Silva *et al.* (2014)
Bryconamericus aff. *iheringii*	52	12m+18sm+8st+ 14a	Maringá stream, Paraná River basin - PR	**Absent**	Capistano *et al.* (2008)
Bryconamericus aff. *iheringii*	52	8m+28sm+6st+ 10a	Keller River, Paraná River basin - PR	**Absent**	Capistano *et al.* (2008)
Bryconamericus aff. *iheringii*	52	8m+20sm+8st+16a	Tatupeba stream, Paraná River basin - PR	**Absent**	Capistano *et al.* (2008)
Bryconamericus aff. *iheringii*	52	10m+16sm+14st+12a	Upper Uruguai River basin	Present	Prestes *et al.* (2009)

Species	2n	Karyotype	Locality	Distinctive large m pair	Reference
Bryconamericus aff. *iheringii*	52	12m+18sm+8st+14a	Ocoí River - PR	**Absent**	Nishiyama *et al.* (2015)
Bryconamericus aff. *iheringii*	52	10m+14sm+18st+10a	Corumbataí River - SP	**Absent**	Piscor *et al.* (2013)
Bryconamericus coeruleus	52	14m+20sm+8st+10a	Upper Paraná River basin - PR	Present	Prestes *et al.* (2009)
Bryconamericus ecai	52	10m+16sm+8st +18a	Forquetinha River - RS	**Absent**	Santos *et al.* (2017)
Bryconamericus ecai	52	8m+16sm+14st+14a	Forquetinha River - RS	**Absent**	Santos *et al.* (2017)
Bryconamericus ecai	52	10m+16sm+8st+18a	Forquetinha River - RS	**Absent**	Santos *et al.* (2017)
Bryconamericus ecai	52	10m+10sm+8st+24a	Forquetinha River - RS	**Absent**	Dos Santos *et al.* (2012)
Bryconamericus ecai	52	10m+18sm+8st+16a	Forquetinha River - RS	**Absent**	Dos Santos *et al.* (2012)
Bryconamericus ecai	52	14m+14sm+6st+18a	Forquetinha River - RS	**Absent**	Dos Santos *et al.* (2012)
Bryconamericus ecai	52	10m+24sm+14st+4a	Forquetinha River - RS	**Absent**	Dos Santos *et al.* (2012)
Bryconamericus ecai	52	10m+16sm+14st+12a	Upper Uruguai River basin - PR	**Absent**	Prestes *et al.* (2009)
Bryconamericus eigenmanni	52	6m+16sm+16st+14a	Upper Uruguai River basin - PR	Present	Prestes *et al.* (2009)
Bryconamericus sp.	52	16m+14sm+10st+12a	Vermelho stream, Ivaí River basin - PR	**Absent**	Santos *et al.* (2017)
Bryconamericus sp.	52	2m+12sm+20st+20a	Cambuta River, Ivaí River basin - PR	**Absent**	Santos *et al.* (2017)
Bryconamericus sp. A	52	6m+30sm+6st+10a	Piracicaba river - SP	**Absent**	Wasko and Galetti-Jr (1998)
Bryconamericus sp. B	52	6m+10sm+20st+16a	Piracicaba river - SP	**Absent**	Wasko and Galetti-Jr (1998)
Bryconamericus sp. C	52	6m+18sm+14st+14a	Tibagi River - PR	**Absent**	Wasko and Galetti-Jr (1998)
Bryconamericus sp. D	52	8m+14sm+16st+14a	Garças River - MT	**Absent**	Wasko and Galetti-Jr (1998)
Bryconamericus sp. E	54	10m+16sm+22st+6a	Garças River - MT	**Absent**	Wasko and Galetti-Jr (1998)
Bryconamericus sp. A	52	6m+30sm+ 6st+10a	Piracicaba river - SP	**Absent**	Wasko *et al.* (1996)
Bryconamericus sp. B	52	10m+6sm+18st+18a	Piracicaba river - SP	**Absent**	Wasko *et al.* (1996)
Bryconamericus stramineus	52	26m/sm+26st/a	Mogi Guaçu River - SP	**Absent**	Portela *et al.* (1988)
Bryconamericus stramineus	52	6m+10sm+16st+20a	Iguatemi River basin - MS	**Absent**	Fernandes *et al.* (2010)
Bryconamericus stramineus	52	6m+10sm+16st+20a	Guaçu stream, Iguatemi River basin - MS	**Absent**	Piscor *et al.* (2013)
Bryconamericus turiuba	52	8m+10sm+14st+20a	Passo-Cinco River - SP	**Absent**	Piscor *et al.* (2013)
Glandulocauda melanogenys	52	4m+12sm+22st+14a	Paranapiacaba - SP	**Absent**	Guimarães *et al.* (1995)
Knodus cf. *chapadae*	52	14m+14sm+ 14st+10a	Tangará da Serra - MT	**Absent**	Krinski *et al.* (2008)
Markiana nigripinnis	52	8m+22sm+22st/a	Miranda River - MT	**Absent**	Monteiro *et al.* (2022)
Mimagoniates laterallis	52	6m+20sm+16st+10a	Itanhém - SP	**Absent**	Guimarães *et al.* (1995)
Mimagoniates microlepis	52	12m+18sm+14+8a	Iguaçu River basin and Piraquara River - PR	**Absent**	Torres *et al.* (2008)
Piabina anhembi	52	8m+10sm+16st+18a	Salesópolis - SP	**Absent**	Pazian *et al.* (2012)
Piabina argentea	52	26m/sm+26st/a	Mogi Guaçu - SP	**Absent**	Pazian *et al.* (2012)
Piabina argentea	52	8m+14sm+16st+14a	São Francisco - MG	**Absent**	Pazian *et al.* (2012)
Piabina argentea	52	4m+22sm+10s+16a	Itatinga - SP	**Absent**	Pazian *et al.* (2012)
Piabina argentea	52	8m+18sm+18st+10a	Botucatu - SP	**Absent**	Pazian *et al.* (2012)
Piabina argentea	52	4m+24sm+10st+14a	Bauru - SP	**Absent**	Pazian *et al.* (2012)
Piabina argentea	52	26m/sm+26st/a	Mogi Guaçu River - SP	**Absent**	Portela *et al.* (1988)
Piabina argentea	52	8m+14sm+16st+14a	São Francisco River - MG	**Absent**	Moreira *et al.* (2007)
Piabina argentea	52	6m+24sm+12st+10a	Iguatemi River - MS	**Absent**	Fernandes *et al.* (2010)
Subfamily Tetragonopterinae					
Tetragonopterus argenteus	52	16m+4sm+4st+28a	Cuiabá River - MT	**Absent**	Miyazawa (2015)
Tetragonopterus argenteus	52	24m+8sm+4st+16a	Bento Gomes River - MT	**Absent**	Miyazawa (2015)
Tetragonopterus argenteus	50	14m+4sm+4st+28a	Cuiabá River - MT	**Absent**	Miyazawa (2015)
Tetragonopterus argenteus	52	16m/sm+2st+34a	Paraná River - PR	**Absent**	Alberdi and Fenocchio (1997)
Tetragonopterus franciscoensis	52	12m+26sm+14a	Itapicuru River - BA	**Absent**	Present study
Tetragonopterus franciscoensis (*chalceus*)*	52	13m/sm+13st/a	São Francisco River - MG	**Absent**	Portela *et al.* (1988)

Species	2n	Karyotype	Locality	Distinctive large m pair	Reference
Family Bryconidae					
Brycon amazonicus	50	22m+14sm+14st	Orinoco basin -Venezuela	Present	Mariguela *et al.* (2010)
Brycon cf. *cephalus*	50	26m+24sm/st	Amazon basin - AM	Present	Almeida-Toledo *et al.* (1996)
Brycon cf. *reinhardti*	50	22m+28sm/st	Paraíba do Sul River - SP	Present	Almeida-Toledo *et al.* (1996)
Brycon insignis	50	24m+21sm/st	Paraíba do Sul River - SP	Present	Almeida-Toledo *et al.* (1996)
Henochilus wheatlandii	50	26m+12sm+12st	Santo Antônio River - MG	Present	Silva *et al.* (2012)
Family Gasteropelecidae					
Carnegiella strigata	50	Not Informed	Manaus - MA	**Absent**	Yano *et al.* (2021)
Gasteropelecus levis	54	Not Informed	Manaus - MA	**Absent**	Yano *et al.* (2021)
Thoracocharax stellatus	54	Not Informed	Barra do Bugres - MT	**Absent**	Yano *et al.* (2021)
Family Triportheidae					
Agoniates halecinus	52	Not Informed	Manaus - AM	**Absent**	Yano *et al.* (2021)
Lignobrycon myersi	52	28m+18sm+6a	Almada River - BA	**Absent**	Dos-Santos et al. (2016)
Triportheus auritus	52	Not Informed	Ponta do Araguaia - MT	**Absent**	Yano *et al.* (2021)
Triportheus nematurus	52	13m+23sm+16st	Piracicaba River - SP	**Absent**	Diniz *et al.* (2008)
Triportheus pantanensis	52	Not Informed	Paraguay basin	**Absent**	Yano *et al.* (2016)
Triportheus aff. *rotundatus*	52	Not Informed	Paraguay basin	**Absent**	Yano *et al.* (2016)

Finito di stampare da
Logo s.r.l. – Borgoricco (PD) – Italia

OPEN ACCESS POLICY

Caryologia provides immediate open access to its content. Our publisher, Firenze University Press at the University of Florence, complies with the Budapest Open Access Initiative definition of Open Access: By "open access" , we mean the free availability on the public internet, the permission for all users to read, download, copy, distribute, print, search, or link to the full text of the articles, crawl them for indexing, pass them as data to software, or use them for any other lawful purpose, without financial, legal, or technical barriers other than those inseparable from gaining access to the internet itself. The only constraint on reproduction and distribution, and the only role for copyright in this domain is to guarantee the original authors with control over the integrity of their work and the right to be properly acknowledged and cited. We support a greater global exchange of knowledge by making the research published in our journal open to the public and reusable under the terms of a Creative Commons Attribution 4.0 International Public License (CC-BY-4.0). Furthermore, we encourage authors to post their pre-publication manuscript in institutional repositories or on their websites prior to and during the submission process and to post the Publisher's final formatted PDF version after publication without embargo. These practices benefit authors with productive exchanges as well as earlier and greater citation of published work.

PUBLICATION FREQUENCY

Papers will be published online as soon as they are accepted, and tagged with a DOI code. The final full bibliographic record for each article (initial-final page) will be released with the hard copies of *Caryologia*. Manuscripts are accepted at any time through the online submission system.

COPYRIGHT NOTICE

Authors who publish with *Caryologia* agree to the following terms:

- Authors retain the copyright and grant the journal right of first publication with the work simultaneously licensed under a Creative Commons Attribution 4.0 International Public License (CC-BY-4.0) that allows others to share the work with an acknowledgment of the work's authorship and initial publication in Caryologia.
- Authors are able to enter into separate, additional contractual arrangements for the non-exclusive distribution of the journal's published version of the work (e.g., post it to an institutional repository or publish it in a book), with an acknowledgment of its initial publication in this journal.
- Authors are permitted and encouraged to post their work online (e.g., in institutional repositories or on their website) prior to and during the submission process, as it can lead to productive exchanges, as well as earlier and greater citation of published work (See The Effect of Open Access).

PUBLICATION FEES

Open access publishing is not without costs. *Caryologia* therefore levies an article-processing charge of € 150.00 for each article accepted for publication, plus VAT or local taxes where applicable.

We routinely waive charges for authors from low-income countries. For other countries, article-processing charge waivers or discounts are granted on a case-by-case basis to authors with insufficient funds. Authors can request a waiver or discount during the submission process.

PUBLICATION ETHICS

Responsibilities of *Caryologia*'s editors, reviewers, and authors concerning publication ethics and publication malpractice are described in *Caryologia*'s Guidelines on Publication Ethics.

CORRECTIONS AND RETRACTIONS

In accordance with the generally accepted standards of scholarly publishing, *Caryologia* does not alter articles after publication: "Articles that have been published should remain extant, exact and unaltered to the maximum extent possible".

In cases of serious errors or (suspected) misconduct *Caryologia* publishes corrections and retractions (expressions of concern).

Corrections

In cases of serious errors that affect or significantly impair the reader's understanding or evaluation of the article, *Caryologia* publishes a correction note that is linked to the published article. The published article will be left unchanged.

Retractions

In accordance with the "Retraction Guidelines" by the Committee on Publication Ethics (COPE) *Caryologia* will retract a published article if:

- there is clear evidence that the findings are unreliable, either as a result of misconduct (e.g. data fabrication) or honest error (e.g. miscalculation)
- the findings have previously been published elsewhere without proper crossreferencing, permission or justification (i.e. cases of redundant publication)
- it turns out to be an act of plagiarism
- it reports unethical research.

An article is retracted by publishing a retraction notice that is linked to or replaces the retracted article. *Caryologia* will make any effort to clearly identify a retracted article as such.

If an investigation is underway that might result in the retraction of an article *Caryologia* may choose to alert readers by publishing an expression of concern.

COMPLYNG WITH ETHICS OF EXPERIMENTATION

Please ensure that all research reported in submitted papers has been conducted in an ethical and responsible manner, and is in full compliance with all relevant codes of experimentation and legislation. All papers which report in vivo experiments or clinical trials on humans or animals must include a written statement in the Methods section. This should explain that all work was conducted with the formal approval of the local human subject or animal care committees (institutional and national), and that clinical trials have been registered as legislation requires. Authors who do not have formal ethics review committees should include a statement that their study follows the principles of the Declaration of Helsinki

ARCHIVING

Caryologia and Firenze University Press are experimenting a National legal deposition and long-term digital preservation service.

ARTICLE PROCESSING CHARGES

All articles published in *Caryologia* are open access and freely available online, immediately upon publication. This is made possible by an article-processing charge (APC) that covers the range of publishing services we provide. This includes provision of online tools for editors and authors, article production and hosting, liaison with abstracting and indexing services, and customer services. The APC, payable when your manuscript is editorially accepted and before publication, is charged to either you, or your funder, institution or employer.

Open access publishing is not without costs. *Caryologia* therefore levies an article-processing charge of € 150.00 for each article accepted for publication, plus VAT or local taxes where applicable.

FREQUENTLY-ASKED QUESTIONS (FAQ)

Who is responsible for making or arranging the payment?

As the corresponding author of the manuscript you are responsible for making or arranging the payment (for instance, via your institution) upon editorial acceptance of the manuscript.

At which stage is the amount I will need to pay fixed?

The APC payable for an article is agreed as part of the manuscript submission process. The agreed charge will not change, regardless of any change to the journal's APC.

When and how do I pay?

Upon editorial acceptance of an article, the corresponding author (you) will be notified that payment is due.

We advise prompt payment as we are unable to publish accepted articles until payment has been received. Payment can be made by Invoice. Payment is due within 30 days of the manuscript receiving editorial acceptance. Receipts are available on request.

No taxes are included in this charge. If you are resident in any European Union country you have to add Value-Added Tax (VAT) at the rate applicable in the respective country. Institutions that are not based in the EU and are paying your fee on your behalf can have the VAT charge recorded under the EU reverse charge method, this means VAT does not need to be added to the invoice. Such institutions are required to supply us with their VAT registration number. If you are resident in Japan you have to add Japanese Consumption Tax (JCT) at the rate set by the Japanese government.

Can charges be waived if I lack funds?

We consider individual waiver requests for articles in Caryologia on a case-by-case basis and they may be granted in cases of lack of funds. To apply for a waiver please request one during the submission process. A decision on the waiver will normally be made within two working days. Requests made during the review process or after acceptance will not be considered.

I am from a low-income country, do I have to pay an APC?

We will provide a waiver or discount if you are based in a country which is classified by the World Bank as a low-income or a lower-middle-income economy with a gross domestic product (GDP) of less than $200bn. Please request this waiver of discount during submission.

What funding sources are available?

Many funding agencies allow the use of grants to cover APCs. An increasing number of funders and agencies strongly encourage open access publication. For more detailed information and to learn about our support service for authors.

APC waivers for substantial critiques of articles published in OA journals

Where authors are submitting a manuscript that represents a substantial critique of an article previously published in the same fully open access journal, they may apply for a waiver of the article processing charge (APC).

In order to apply for an APC waiver on these grounds, please contact the journal editorial team at the point of submission. Requests will not be considered until a manuscript has been submitted, and will be awarded at the discretion of the editor. Contact details for the journal editorial offices may be found on the journal website.

What is your APC refund policy?

Firenze University Press will refund an article processing charge (APC) if an error on our part has resulted in a failure to publish an article under the open access terms selected by the authors. This may include the failure to make an article openly available on the journal platform, or publication of an article under a different Creative Commons licence from that selected by the author(s). A refund will only be offered if these errors have not been corrected within 30 days of publication.

2023
Vol. 76 – n. 1

Caryologia

International Journal of Cytology, Cytosystematics and Cytogenetics

Table of contents