Cytological study in the Iraqi species of the genus *Bromus* L. (Poaceae)

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Abstract

This paper is a part of a taxonomical and cytological studies of the genus Bromus L. in Iraq . Chromosome number for 103 specimens representing 12 species were determined . The course of meiosis including an analysis of chromosome configuration for 27 collections belonging to 8 species were investigated . Meiosis was regular in most species . Tetraploid for B.scoparius L. (new cytotype) and hexaploid for B.unioloides Kunth. were reported for the first time . The cytological findings were supported the view of maintaining the closely related species B.danthoniae Trin. and B.lanceolatus Roth. as distinct species .

INTRODUCTION

The genus *Bromus* L. comprising about 130-150 species found throughout the temperate regions of both hemispheres , but mainly in the north (1,2) . About eighteen species have been reported by Bor (3) from Iraq , most of them form a considerable portion of the forage in mountains , dry – steppe , and sub-desert zones . Since Avdulov (4) realized the taxonomic significance of the grass chromosomes , recent cytological data obtained by different workers such as chromosome numbers , karyotypes and chromosome behavior provided valuable additional information to evaluation and taxonomy of Poaceae and confirmed the conclusions reached by Avdulov

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(4) and Stebbins (5) , about the phylogenetic of the family Poaceae .With regard to the genus *Bromus* L. , chromosome numbers have been reported by several authors , (Table .1).The majority of the karylogical studies concerning the genus *Bromus* L. have been reported on the euploid series extending from the diploid (2n=14) to 12-ploid (2n=84) (6,7,8,9,10,2) .

The objective of the present paper was to investigates the cytology and distribution of polyploids of the genus *Bromus* L. , and to determined the relationships between *B.danthoniae* and *B.lancealatus* .

Materials and Methods

Seed obtained from the field collections , and from recently collected herbarium specimens were grown in pots or petri-dishs . Root tips of seedling and plants were pretreated in various ways before fixation . The most satisfactory results were obtained with ice-water for 20-24 hours at 0-1°C . After pretreatment , roots were washed in water and fixed in freshly prepared acetic – alcohol (1:3) for 24 hours in a refrigerator Squash preparations were made in 2% aceto-orcein after hydrolysis in 5N HCl at room temperature for 5-10 minutes according to schedule described by Löve & Löve (11) with some modification . For meiotic examination , young panicles were fixed in fresh solution of one part glacial acetic acid and three parts absolute alcohol for 24 hr , then stored in 70% ethanol at approximately 0 °C . Anthers were smeared in 2% aceto-orcein according to Darligton & La Cour (12) . Good cells were photographed from temparary and permanents slides . Voucher specimens of all counts are deposited in the Babylon University Herbarium .

Table 1. *Bromus* spp. Chromosome counts made by previous workers on material of known and unknown origin

Taxon	Origin	Chromosome Number 2n	n	Author and Date
B.danthoniae	Russia	14		Avdulov ,1928*,1931
	Germany	14		Stählin,1929
	Germany	14		Schulz-Schaeffer,1957* and
	Cheshmashivin;Bakhtiari;	14		Markarian
	Iran	14		Schulz-Schaeffer,1960*
	Pakistan , Afghanistain & Iran	14		Sakamoto,Muramatsu,
		14		1963*
B.japonicus		14		Hill H.D.,1965*
	Mediterranean&South-West			Smith,1972
	Asian	14		Avdulov,1928,1931
	Russia	14		Nielsen E.L,1937*
		14	7	Humphrey
		14		Tateoka,1953*,1954*
	Japan			Barnett,1955
	Portugal	28		Schulz-Schaeffer,1956, 1960
		14		Schulz-Schaeffer.1956
	Botanic Gardens of the	14		
	University of Marburg-Lahn	14		Walters M.S.,1963*
B.scoparius	, Germany .	14	7	Smith,1972
		14		Pillay and Hilu,1995
B.sericeus	Asia	14		Barnett,1955
	Belgium			Smith,1955*
B.tectorum	Portugal	14		Sokopovskia,Stripkova
	Mediterranean & South-,	14		,
	West Asian	14		1939*
	Russia	14		Avdulov,1928
		14		Stähin,1929
	Russia			Tischler,1934*
	Germany	14		Titova,1935*
		14		Cugnac,Simonet,1941
		14		
		14		Knowles P.F.,1944*
		14		a,.Felföldy 1947*
		14		Heiser,Whitakar,1948*
				Polya,1950*
	California	14	7	Hubbard,1954*
	Hungarian	14		Tateoka,1954,1955*,
	Britain	14		1956*
	Soil coservation service	14		Barnett,1955
		14		Love and Love,1956*
	Los Angeles	14		Walters,1958*
B.sterilis		14		Bowden,1960*
		14		Walters M.S.,1963
		14		Pillay and Hilu,1995
	USA			Stählin,1929
	Germany			Tischler,1934
				Cugnac,Simonet,1941

Table 1. Continued

Taxon	Origin	Chromosome Number 2n	n	Author and Date
		14		Delay,1947*
	Britain	14		Hubbard ,1954
	Japan	28		Tateoka,1955,1959*
	Germany	28		Schulz – Schaeffer,1956
		14		Godella , Kliphuis,1967*
	Belgium	14		Pillay and Hilu,1995
B.lanceolatus	Germany	28		Schulz – Schaeffer,1956
	South American	28		Bowden , Seen,1962*
	Mediterranean and South west	28		Smith,1972
B.rubens	-Asian	28		Avdulov,1928,1931
	Russia	28		Stählin,1929
	Germany	28		Beck P. , Horton,1932*
		28		Knowles P.F.,1944
		28		Heiser , Whitaker,1948
	California	28		Reese ,1957*
		28		Walters,1958
	Santa Barbara	28		Walters M.S.,1963
		28		Pillay and Hilu,1995
B.madritensis	USA	28		Avdulov,1928,1931
	Russia	42		Stählin,1929
	Germany	42		Cugnac , Simonet,1941
		28		Knowles P.F.,1944
		28		Delay ,1947
		28		Hubbard,1954
	Britain	28		Love A. , Love D.,1956
	Iceland	14		Sakamoto , Myramatsu,1963
	Pakistan , Afghanistan	28		Walters,1963
	and Iran	28	14	Esnault,1985*
		28		Pillay and Hilu,1995
B.tuniodoides	Algeria and western -	28		Stählin,1929
	France	28		Avdulov,1931
	Belgium	42		Parodi,1946*
	Germany	42	21	Barnett,1955
	Russia	42		Bowdden , Senn,1962
		42		Pillay and Hilu,1990
	Canada	42		Pillay and Hilu,1995
B.diandrus		56		Cugnac, Simonet,1941
	Oregon	56		Hubbard,1954
	USA	56		Gill and Carstairs,1988
	Britain			
	Western Australia			

RESULTS

Chromosome complements : Chromosome number of 103 plants belonging to 12 species of the genus *Bromus* L. are listed in (Table .2) . Ploidy levels of diploid , tetraploid , hexaploid and octoploid have been confirmed by this study . It seems nevertheless evident that the diploid and tetraploid occur more frequently than hexaploid and octoploid . New counts were obtained as follows : tetraploid for *B.scoparius* and hexaploid for *B.unioloides* which recognized as new species for iraq . Chromosomes of the genus *Bromus* L. were found to be metacentric , submetacentric , and rarely subacrocentric or satellited chromosomes according to scheme devised by Levan *et al.* (13) . For example in 28 specimens of *B.danthoniae* were studied , satellite – bearing chromosomes were observed in few specimens . The same situation was observed in the case *B.japonicus* . On the other hand , three species were found to have different chromosome number (2n=14,28) . These are *B.madritensis* , *B.sterilis* and *B.scoparius* . The new tetraploid of *B.scoparius* showed such karyological variation, there are 7 pairs of small metacentric or submetacentric chromosomes , 6 pairs of more or less long metacentric or submetacentric chromosomes , and 1 pair of satellited chromosome (Plate 1,2) . A little correlation was found between the morphlogical characters of diploid and tetraploid races of *B.scoparius* . In the case of *B.madritensis* it was found that the tetraploid is much more widespread and abundant than diploid . Chromosome counts were made for 28 plants of *B.madritensis* growing in different Iraqi districts , of which 26 plants were found to be tetraploid .

The chromosome number was 2n=28 in all specimens of *B.rubens* and *B.laceolatus* . 3 pairs of satellited chromosomes were observed in the chromosome complement of the latter species , and the other chromosomes are metacentric or submetacentric (Plate 2B,C) .

Meiotic Behaviour

Meiotic analysis was studied in 27 specimens belonging to 8 taxa , 8 of which are diploid , 16 tetroploid , 1 hexaploid and 2 octoploid . Data on chromosome associations and chiasma frequency for all taxa are given in (Table .3) .The mean number of chiasma frequencies per cell and per bivalent were estimated from chromosome association at diakinesis and metaphase I . Most plants are found to be cytologically normal and exhibited regular meiosis . Chromosomes paired mostly as bivalents , and the ring bivalents were dominated . In spit of its relatively few and large chromosomes , cytological analysis was found rather difficult , especially when the bivalents were cross-shaped . In diploid species the mean ring bivalents ranged from 2-7 per cell and in tetraploid it varried from 8 to 14 . In hexaploid the mean ring bivalents ranged from 16 to 21 in *B.unioloides* , and in octoploid *B.diandrus* it varied from 12 to 28 . (Plate 3) . Multivalent was observed in one cell in octoploid species (i.e. *B.diandrus*) . (Plate 5A) . Chiasma frequencies per cell in diploid varied from 11.87 in *B.sericeus* (B58) to 13.26 in *B.danthoniae* (B38) , and in tetraploid species it ranged from 22-26.47 in *B.madritensis* (B21) & (B16) respectively . In hexaploid and octoploid species chiasma frequency per cell was 43.08 and 49.5 respectively . From the above there is

Normal separation of the chromosome at anaphase I was observed in most plants studied , but irregularities at anaphase I and subsequent stages were observed . Some cells with unequal chromosome distribution , lagging chromosome or chromosome bridges with or without a fragment occurred in many species of different plody levels (i.e. *B.madritensis* , *B.danthoniae* and *B. diandrus*) (Plate 4,5)

Pollen mitosis was observed in *B.danthoniae* and *B.madritensis* (Plate 4,B 5,D) .

DISCUSSION

Chromosome complements

The chromosomes of the genus Bromus L. are multiple of a basic set of x=7 . with few exception the observed chromosome numbers are in agreements with the published counts (Table.2) . The study has been unable to confirm some counts such as tetraploid of *B.unioloides* , and hexaploid of *B.madritensis* and *B.japonicus*. These counts are either very rare or likely to be due to misidentification or to anomalous cells . The counts 2n=28,42 for *B.japonicus* had been reported by Schulz – Schaeffer (8) and Mehra & Shyam (14) .In this study tetraploid and hexaploid were not observed in *B.japonicus* . Morever Carahan & Hill (10) stated the tetraploid was not reported by previous workers . With regard to *B.madritensis* , the earlier literature counts 2n=14,28 (Table .1) are confirmed by this study , but is not in accordance with an earlier reporte of 2n=42 by Stahlin (15) and Cugnac & Simonet (16) . The count of 2n =28 for *B.scoparias* is new number for this species . One of the most striking differences between the karyotype of this cytotype and other species , is the 7 pairs of unusual small chromosomes . Such karyotype is called bimodel which orginated by so–called Levitsky Principle which caused asymmetrical karyotype by translocation of unequal chromosomal part within the same group . (17) , or derived from symmetrical karytypes rised by polyploidy (18) .

Bor (3) considered the status of *B.lanceolatus* and *B.danthoniae* to be taxonomically unresolved . However , in this study it was found that these two taxa have different chromosome numbers and reprodictivelly isolated in the field . This provides some support for the decision to consider them as distinct species .

Meiosis

Generally speaking kinds of bivalents appear somewhat obscure may be due to the orientation of some of the ring bivalents like rod bivalents (Cross-shaped) . The same situation was encountered with chiasma frequency . This was due to score chiasma frequency at diakinesis and prometaphase rather than at Pachyten and Diplotene . The rage of the mean chiasmata frequency per bivalent , it may be seen that the range of values for different ploidy levels is not very different . The same results obtained by Al-Bermani (19) , in the case *Festuca rubra* aggregate , and the genus *Fallopia* (20) . The variation of chiasma frequency within and between species is likely due to genetic factors or environmental factors or both (21,22,23) . In this study the results obtained from plants grown in greenhouse and in the field , therefore may be the variation explained by environmental factors . The relationship between the breeding system and the chiasma frequency cannot be ignored . Generally outbreeding species have lower chiasma frequency than their inbreeding relations (24,25,17) . Although the breeding system of the

Bromus is beyond the scope of our study , so this variation may be due to breeding factor .

The course of meiosis in most taxa examined was the formation of complete bivalents pairing and rarely multivalent formation in one ploidy level only in one specimen . Such pairing is generally considered to be indicative of allopolyploid or autopolyploid origin in the case of multivalent formation (17) . However bivalents or multivalents cannot be taken as conclusive evidence of allopolyploidy or autopolyploidy respectively , since chromosome pairing in polyploid is affected by chromosome homology , chromosome length and chiasma frequency , and regular meiosis occurs in autopolyploid , or heterozygous reciprocal translocation may cause multivalent association (7) .

Meiotic irregularities in the form of chromatid bridges and fragments , lagging univalents were noticed in first and second anaphase and telphose (Plate 4,5) . Anaphase bridges with or without fragments suggesting heterozygosity for paracentric inversion (17,26) . Sticky chromosomes lead to bridges alone .

Table 2. Chromosome counts for all the *Bromus* examined.

Accession Number	Taxon	Location	Chromosome number
B1	*B.danthoniae*	Near Badra	14
B2	*B.danthoniae*	Babylon University	14
B7	*B.danthoniae*	Jabal Sinjar, 1Km . S. of T.V.Tower	14
B15	*B.danthoniae*	Jabal Sinjar, Near T.V.Tower	14
B24	*B.danthoniae*	Jabal Sinjar , N. of Karsi	14
B27	*B.danthoniae*	25Km, N. of Samarra / Dour Road	14
B31	*B.danthoniae*	Road Side – near Tikrit	14
B34	*B.danthoniae*	Jabal Makhul , 30 Km N. of Zewiya	14
B38	*B.danthoniae*	5Km. N. of Kirkuk , Road-Side	14
B41	*B.danthoniae*	5Km. S. of Dour , Road-Side	14
B45	*B.danthoniae*	8Km. N. of Kirkuk , Road-Side	14
B46	*B.danthoniae*	15Km. N. of Kirkuk , Road-Side	14
B50	*B.danthoniae*	18Km. N. of Kirkuk , Road-Side	14
B51	*B.danthoniae*	20 Km. N. of Kirkuk , Road-Side	14
B60	*B.danthoniae*	Injana-Al-Adhiam	14
B62	*B.danthoniae*	Injana-Near Al-Adhiam river	14
B63	*B.danthoniae*	Injana – Hamrin hills	14
B68	*B.danthoniae*	10 Km. N. of Khalis	14
B70	*B.danthoniae*	2Km. N. of Samarra / Dour Road	14
B72	*B.danthoniae*	5Km. N. of Samarra / Dour Road	14
B76	*B.danthoniae*	10Km. S. of Kirkuk	14
B78	*B.danthoniae*	15Km. S. of Kirkuk	14
B79	*B.danthoniae*	30Km. N. of Kirkuk	14
B84	*B.danthoniae*	20km . S. of Khalis	14
B89	*B.danthoniae*	18 Km. N. of Khalis	14
B91	*B.danthoniae*	20 Km. N. of Kirkuk	14
B94	*B.danthoniae*	10 Km. N. of Kirkuk	14
B95	*B.danthoniae*	Kirkuk City	14
B18	*B.diandrus*	Mosul University –near Science College	56
B19	*B.diandrus*	Mosul University –near Engineering College	56
B33	*B.diandrus*	Jabal Makhul , 30 Km N. of Zewiya	56
B44	*B.diandrus*	8Km. N. of Kirkuk , Road-Side	56
B55	*B.diandrus*	25Km. N. of Kirkuk	56
B81	*B.diandrus*	30Km. N. of Kirkuk	56
B97	*B.diandrus*	10 Km. N. of Kirkuk, on Road Side	56
B99	*B.diandrus*	Mosul University , Science College	56
B100	*B.japonicus*	Jabal Sinjar Near T. V. Tower	14
B22	*B.lanceolatus*	Jabal Sinjar , 3 South of Karsi	28
B26	*B.lanceolatus*	4Km . N. of T.V. Tower	28
B37	*B.lanceolatus*	5Km. N. of Kirkuk , Road-Side	28
B43	*B.lanceolatus*	8Km. N. of Kirkuk , Road-Side	28
B92	*B.lanceolatus*	10 Km. N. of Kirkuk	28
B103	*B.lanceolatus*	Jadriya-Baghdad University / Science College	28
B35	*B.madritensis*	Jabal Makhul , 30 Km N. of Zewiya	14
B36	*B.madritensis*	Agriculture College, Abu Ghirab	14
B3	*B.madritensis*	Babylon University	28
B4	*B.madritensis*	Hilla-Bab Al-Hussin-near Hilla river	28
B16	*B.madritensis*	Mosul University –near Science College	28
B17	*B.madritensis*	Mosul University –near Engineering College	28
B20	*B.madritensis*	Jabal Sinjar , 3Km . S. of Karsi	28

Table 2 : continued

B21	*B.madritensis*	Jabal Sinjar , near Karsi	28
B23	*B.madritensis*	Jabal Sinjar , North of Karsi	28
B25	*B.madritensis*	Near Mosul University	28
B32	*B.madritensis*	Road Side – near Tikrit	28
B40	*B.madritensis*	5Km. N. of Kirkuk , Road-Side	28
B48	*B.madritensis*	15Km. N. of Kirkuk , Road-Side	28
B49	*B.madritensis*	18Km. N. of Kirkuk , Road-Side	28
B54	*B.madritensis*	25Km. N. of Kirkuk	28
B56	*B.madritensis*	25Km. N. of Kirkuk	28
B59	*B.madritensis*	Injana-Al-Adhiam	28
B64	*B.madritensis*	Injana – Hamrin hills	28
B66	*B.madritensis*	10 Km. N. of Khalis	28
B67	*B.madritensis*	15 Km. N. of Khalis	28
B69	*B.madritensis*	2Km. N. of Samarra / Dour Road	28
B71	*B.madritensis*	5Km. N. of Samarra / Dour Road	28
B77	*B.madritensis*	10Km. S. of Kirkuk	28
B80	*B.madritensis*	30Km. N. of Kirkuk	28
B83	*B.madritensis*	20km . S. of Khalis	28
B90	*B.madritensis*	18 Km. N. of Kirkuk	28
B98	*B.madritensis*	Al-Kadisiya University	28
B104	*B.madritensis*	Jadriya-Baghdad University / Science College	28
B29	*B.rubens*	25Km. N. of Samarra / Dour Road	28
B75	*B.rubens*	10Km. S. of Kirkuk	28
B85	*B.rubens*	Kirkuk City	28
B88	*B.rubens*	10 Km. N. of Khalis	28
B105	*B.rubens*	Jadriya-Baghdad University / Science College	28
B5	*B.scoparius*	Al-Kask / Mosul / Sinjar road	14
B39	*B.scoparius*	5Km. N. of Kirkuk , Road-Side	14
B42	*B.scoparius*	5Km. S. of Dour , Road-Side	14
B47	*B.scoparius*	15Km. N. of Kirkuk , Road-Side	14
B53	*B.scoparius*	20 Km. N. of Kirkuk , Road-Side	14
B73	*B.scoparius*	10Km. S. of Altun Kopri	14
B74	*B.scoparius*	10Km. S. of Altun Kopri	14
B87	*B.scoparius*	Near Badra	14
B93	*B.scoparius*	10 Km. N. of Kirkuk	14
B96	*B.scoparius*	Kirkuk City	14
B52	*B.scoparius*	25Km. N. of Kirkuk , Road-Side	28
B61	*B.scoparius*	Injana-Al-Adhiam	28
B82	*B.scoparius*	30Km. N. of Kirkuk	28
B86	*B.scoparius*	Kirkuk City	28
B28	*B.sericeus*	25Km. N. of Samarra / Dour Road	14
B58	*B.sericeus*	Injana-Al-Adhiam	14
B102	*B.sericeus*	Karbala, Education College	14
B8	*B.sterilis*	Jabal Sinjar, 2Km . S. of T.V.Tower	28
B12	*B.sterilis*	Jabal Sinjar, 1Km . S. of T.V.Tower	28
B13	*B.sterilis*	Jabal Sinjar-near T.V. Tower	28
B101	*B.sterilis*	Near Samarra	28
B6	*B.tectorum*	Jabal Sinjar, 1Km . S. of T.V.Tower	14
B10	*B.tectorum*	Jabal Sinjar, 1Km . S. of T.V.Tower	14
B14	*B.tectorum*	Jabal Sinjar, near T.V.Tower	14
B11	*B.tomentellus*	Jabal Sinjar-near T.V. Tower	28
B106	*B.unioloides*	Babylon hospital of Marternity and Pediatries	42
B107	*B.unioloides*	Babylon hospital of Marternity and Pediatries	42

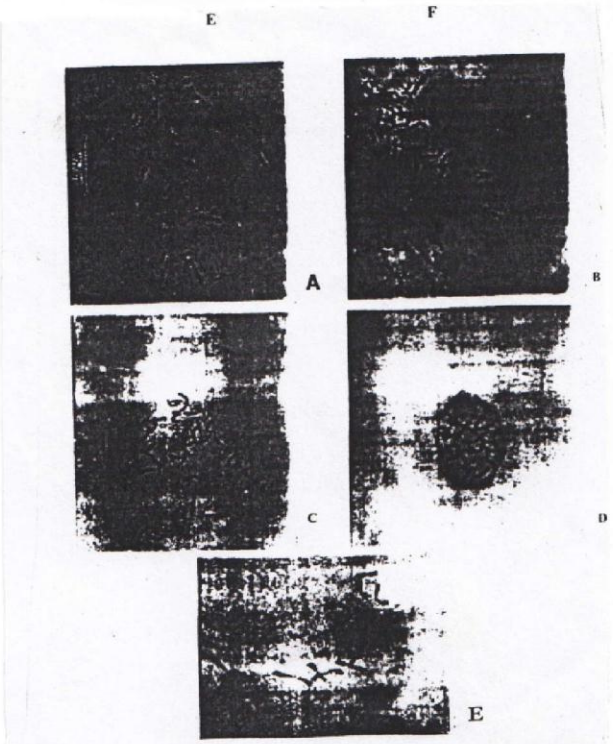


Plate 1. Mitotic Metaphase of *Bromus* spp.
A: *B. diandrus* **B,D: *B. madritensis*** **C: *B. japonicus***
E: *B. unioloides* **F: *B. scoparius***

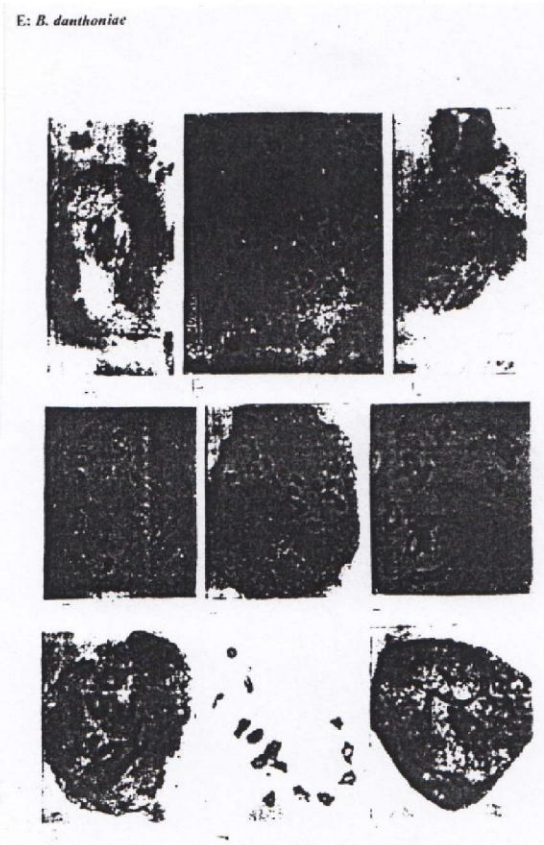
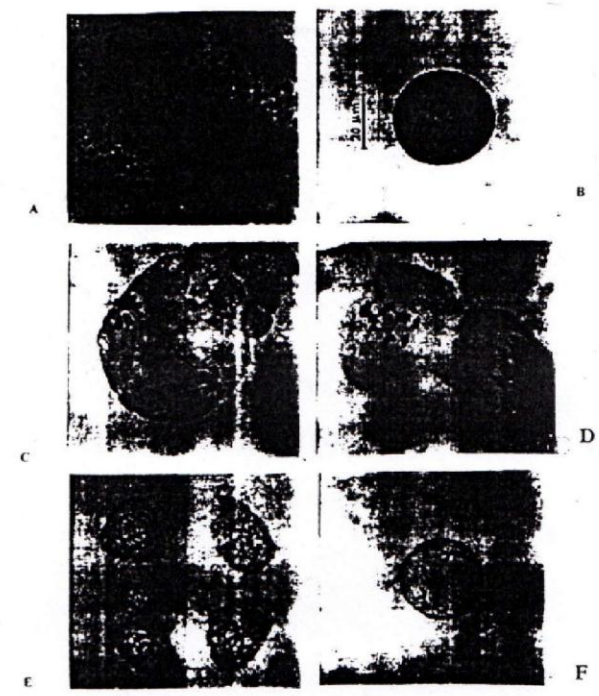
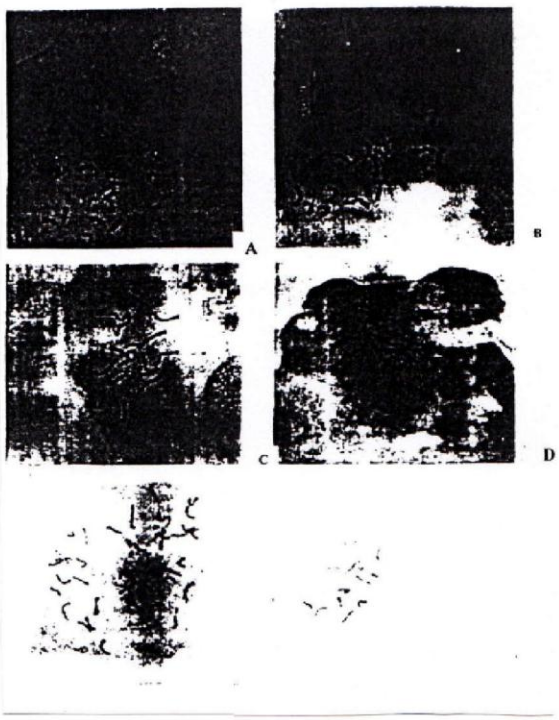


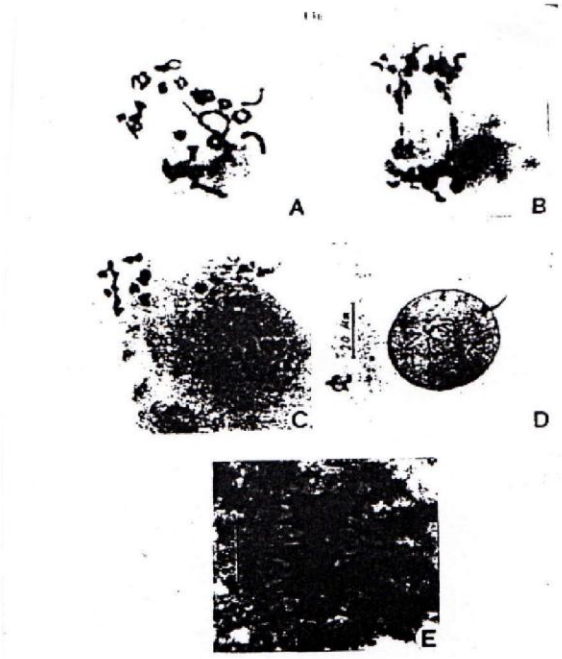
Plate 3. Chromosome pairing in meiosis of *Bromus* spp.



rubens

Plate 4: Meiosis of *Bromus* spp.
A: *B. diandrus* laggared chromosome at Anaphase I
B: *B. madritensis* pollen mitosis
C: *B. danthoniae* laggared fragment
D: *B. scoparius* metaphase I- anaphase I,





E: *B. scoparius* tetrads
F: *B. danthoniae* pollen mitosis

Plate 5. Meiosis of *Bromus* spp.
A-B: *B. diandrus* A: Quadrivalent , B: Two bridges
C-E: *B. madritensis* , C: laggard chromosome at Anaphase I
D: Pollen mitosis E: Metaphase I.

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دراسة خلوية لأنواع الجنس *Bromus L.* العراقية (Poaceae)

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الخلاصة

ان هذا البحث جزء من دراسة تصنيفية و خلوية للجنس Bromus في العراق . و قد تم حساب العدد الكروموسومي لـ ١٠٣ عينة نباتية تمثل ١٢ نوعا ، كما تضمن البحث دراسة الانقسام الاختزالي و تحليل الاقتران الكروموسومي لـ ٢٧ عينة نباتية تعود الى ثمانية انواع ، و كان الانقسام الاختزالي منتظما في معظم الانواع. كما تم تسجيل التعدد المجموعي الرباعي كطراز خلوي جديد للنوع *B.scoparius* L. و التعدد المجموعي السداسي للنوع *B.unioloides Kunth.* لأول مرة . كما دعمت الدراسة الخلوية الابقاء على النوعين *B.danthoniae Trin.* و *B.lanceolatus Roth.* كنوعين مستقلين .