Biolife
AN INTERNATIONAL QUARTERLY JOURNAL OF BIOLOGY & LIFE SCIENCES
4(3):587-594
ISSN (online): 2320-4257
www.biolifejournals.com
BIOLIFE RESEARCH ARTICLE

Male meiosis in structural heterozygotes

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ABSTRACT

The incident of structural heterozygosity is presently noticed for the first time in 9 diploid species, namely, *Artemisia annua* (n=9), *Datura stramonium* (n=12), *Dicliptera bupleuroides* (n=13), *Hemigraphis latebrosa* (n=12), *Leycesteria formosa* (n=9), *Salvia plebeia* (n=8), *Senecio nudicaulis* (n=5), *Sonchus brachyotus* (n=9) and *Verbena officinalis* (n=6, 7). These are heterozygous for chromosomal alternation involving 4-6 chromosomes in reciprocal translocations. All the species show >80% pollen fertility thus indicated the possibility of multivalent formation with alternate disjunction. Almost all the species are involved in some sort of meiotic abnormalities during meiosis I and II.

Key words: Multivalent, reciprocal translocations, structural heterozygosity, pollen fertility.

INTRODUCTION

Structural heretozygosity also known as Chromosomal Rearrangements, which focuses on the two most common forms of rearrangement: translocations and inversions. The presence of multivalent (quadrivalents and hexavalents) in diploids indicates the occurrence of heterozygote translocations between two/ three pairs of chromosomes. The plants with such type of meiotic configurations are termed as structural heterozygotes or structural hybrids. Such type of structural changes in chromosomes may increase the amount of genetic variability in the gametes by forming new genetic linkage groups which may be used for adaptation to adverse environmental conditions (Talukdar, 2009). The more complex situation was found in *Drosophila pseudoobscura* by Dobzhansky (1947) in which the inversion heterozygote contains a gene recombination superior to those of any structural homozygote, has not yet been identified in plants. Terminal association of non-homologous bivalents at meiosis-I has been described in many organisms. In some cases meiotic configurations arise from non-chiasmatic interactions of non-homologues also. It begins by describing the recognizable chromosomal rings, bridges, and fragments at meiosis that are characteristic of such chromosomal variants. Meiotic configurations so formed can lead to irregular segregation and a high rate of non-disjunction with consequent reduction in reproductive potential (Eichenlaub-Ritter and Winking, 1990). Such an existence of natural heterozygosity due to reciprocal translocations has also been reported earlier in many species e.g. *Chrysanthemum boreale, C. carinatum*, *C. coronarium*, *C. sagetum*, *C. indicum*, *C. zawadskii* (Jain and Gupta, 1960; Mehra and Remanandan, 1974; Gill and Gupta, 1981; Kim *et al*., 2009), *Artemisia absinthium* (Malik *et al*., 2010) and *Artemisia parviflora* (Gupta *et al*., 2010b).

During the detailed examination of cytomorphological diversity in 165 gamopetalous species from district Kangra, Himachal Pradesh (Western Himalayas), structural hybridity is reported in 9 species (*Artemisia annua*, *Dicliptera bupleuroides*, *Datura stramonium*, *Hemigraphis latebrosa*, *Leycesteria formosa*, *Salvia plebeia*, *Senecio nudicaulis*, *Sonchus brachyotus* and *Verbena officinalis*). Most of the work has already been briefly published as chromosome data (Bala and Gupta, 2011c, 2012b) and in research articles (Bala and Gupta, 2011a, b, 2012a, 2013; Gupta *et al*., 2010a). The aim and fact of present paper is new and have not been highlighted and conveyed in previous publications.

How to Site This Article:
Santosh Bala, Reyaz A. Malik and R. C. Gupta (2016). Male meiosis in structural heterozygotes. *Biolife*. 4(3), pp 587--594. doi: 10.5281/zenodo.12600135

Received: 2 July 2016; Accepted: 12 August 2016;
Published online: 20 September, 2016

Materials and Methods

All the plant materials are collected from Kangra (Himachal Pradesh) in Western Himalayas (Table-1). For meiotic studies, usual acetocarmine smears of appropriate sized flower buds were made after fixing them in Carnoy's fixative (6:3:1=absolute alcohol: chloroform: glacial acetic acid v/v/v) for 24 hrs and preserved in 70% alcohol at 4˚C. These collected flower buds further subjected to detailed meiotic analysis including meiotic chromosome number, meiotic course, microsporogenesis course, pollen fertility percentage and pollen grain size. Smears of pollen mother cells (PMCs) are prepared in 1% acetocarmine (prepared by fluxing carmine in 45% acetic acid) using standard technique. Pollen fertility was estimated by mounting mature pollen grains in glycerol-acetocarmine (1:1). Voucher specimens are available in Herbarium, Department of Botany, Punjabi University, Patiala (PUN). Photomicrographs of chromosome counts are made from freshly prepared slides using Leica Qwin and Nikon 80*i* Digital Imaging systems.

RESULTS & DISCUSSION

Detailed meiotic behaviour is provided and discussed under each species.

***Artemisia annua* L.**

Meiotic studies showed the occurrence of 2n=18. The presence of 1 chain quadrivalent besides 7 bivalents is noticed in 67.4% PMCs (Table-2) (Figs. 1, 2). The chiasma frequency in PMCs with 1 quadrivalent ranges from 12-16 while PMCs with 9 bivalents show chiasma frequency range of 10-15 (average 14.45). In 7.3% PMCs, the early disjunction of 1-2 bivalents is also noticed. Analysis of 96 PMCs at metaphase-I revealed the chromatin stickiness in 21 PMCs (Fig. 3). Further laggards at telophase-I and telophase-II stages are observed in 6.2% and 9.0% PMCs, respectively (Fig. 4). During microsporogenesis, 1-2 micronuclei are found is 7.5% tetrads (Fig. 5). Pollen fertility is high (94 %) and pollen size ranges from 7.87-11.25 µm. Present chromosome count of diploid cytotype, based on x=9, is in conformity with the former finding of Tyagi and Dubey (1990). Gupta *et al*. (2010b) reported the structural heterozygosity in *Artemisia parviflora*. Similarly, Malik *et al.* (2010) also reported this phenomenon in *Artemisia absinthium.*

***Datura stramonium* L.**

Both the morphotypes with varied flower color: pink and white collected from different localities showed the same chromosome number of 2n=24 at metaphase-I (Fig. 6) and equal segregation to the poles during anaphases. Meiosis is characterised by multiple

Table 1. Data on taxon name, voucher details and chromosome number in presently investigated 9 taxa on population basis.

Name of taxon	Populations: Locality with altitude (m)	Accession number (PUN)*	Chromosome number (2n)
Artemisia annua L.	P-1: Dharamshala, 1500	56426	18
Datura stramonium L.	P-1: Dehra, 490	53200	24
	P-2: Nurpur, 780	57891	24
Dicliptera bupleuroides Nees	P-1: Palampur; Banuri, 1300	53212	26
	P-2: Jawali, 530	53213	26
	P-3: Palampur; Bandla, 1221	53214	26
Hemigraphis latebrosa (Roth) Nees	P-1: Galua, 595	53049	24
	P-2: Jarpali, 501	53074	24+0-2B
Leycesteria formosa Wall.	P-1: Chhota Bhangal; Multhan, 2000	53330	18
Salvia plebeia R.Br.	P-1: Dehra, 490	52994	16
	P-2: Jawali, 530	52995	16
	P-3: Jarpali, 501	52996	16
Senecio nudicaulis Buch.-Ham. ex D.Don	P-1: Palampur, 1221	52607	40
	P-2: Chhota Bhangal; Badagaon, 3300	52809	10
Sonchus brachyotus DC.	P-1: Palampur, 1219	49276	18
	P-2: Dharamshala, 1500	53391	18
	P-3: Shahpur, 780	53393	18
	P-4: Solda, 621	53394	18
Verbena officinalis L.	P-1: Dharamshala, 1500	53179	14
	P-2: Jawali, 530	53180	14
	P-3: Boh, 1670	56036	12

*PUN is the Herbarium Code of Department of Botany, Punjabi University, Patiala as per "Index Herbariorum" by Holmgren and Holmgren (1998).

Table-2. Chiasma frequency in *Artemisia annua*.

Number of PMC observed	Different chromosomal configurations with number of chiasmata			Total chiasmata (T^{Xta})
	IV	II		
	3^{xta}	2^{xta}	1^{xta}	
5	-	7	2	16 x 5
3	1	6	1	16 x 3
3	1	5	2	15 x 3
2	1	3	4	13 x 2
1	1	4	3	14 x 1
1	1	2	5	12 x 1
1	-	6	3	15 x 1
1	-	5	4	14 x 1
1	-	4	5	13 x 1
1	-	3	6	12 x 1
1	-	1	8	10 x1
Total: 20	10	99	61	289
Average frequency/ PMC	0.5	4.95	3.05	14.45
Average frequency/ bivalent	0.05	0.55	0.33	1.60

associations of chromosomes. These associations sometimes are in the form of interbivalent connections (4-8) or secondary associations or multivalents (1-2) (Figs. 7-9). The frequency of PMCs with multivalent/s is found to be 63 %. Cytomixis is also noticed which results into production of hypoploid as well as some hyperploid PMCs (Fig. 10). During anaphase-I, 1-2 laggards are observed (Fig. 11). Besides all these irregularities, microsporogenesis is balanced leading to high (81-84%) pollen fertility. Our report of 2n=24 agrees with the previous finding (Kaur and Singhal, 2010). Only diploid populations are reported from India however from outside India, species is known to have both diploid and tetraploid cytotypes (2n=24 and 48). The chromosomes of *D. stramonium* differ one from another in size and morphological structure (Satina *et al.*, 1941).

***Dicliptera bupleuroides* Nees**

Meiotic analysis on three populations reveals 2n=26 with equal distribution during anaphase-I (Fig. 12). In P-1, laggard is noticed during anaphase-I in 6.42 % PMCs. In Population-2, bivalents show tendency towards association in the form of multivalents (2-4 quadrivalents per PMC) at metaphase-I in 79% PMCs (Figs. 13, 14). P-3 shows normal meiotic course. Further microsporogenesis is normal in all the populations resulting into high pollen fertility (98-99%). Most of the already known reports of species are of diploid cytotype with 2n=26. Its tetraploid cytotype with 2n=52 is also reported (Sareen and Kumari, 1973).

***Hemigraphis latebrosa* (Roth) Nees**

Presently studied two populations show some morphological distinction. P-2 is taller with larger sized leaves than Population-1 (P-1). Meiotically species shows 2n=24 (Fig. 15). In P-2, 2 quadrivalents are seen in 9.33% cells and association of 3 bivalents is also observed in 78% cells (Fig. 16). Microsporogenesis is normal in P-2. Pollen size ranges from 22.50-48.75µm with high pollen fertility (91-92%). The previous chromosome reports in the species from different localities: 2n=26 by Kaur (1965) from South India; 2n=28 by Vasudevan (1976) from Western Himalaya, Sareen and Kumari (1973) from North India and 2n=56 by Bir and Saggoo (1979) from Central India.

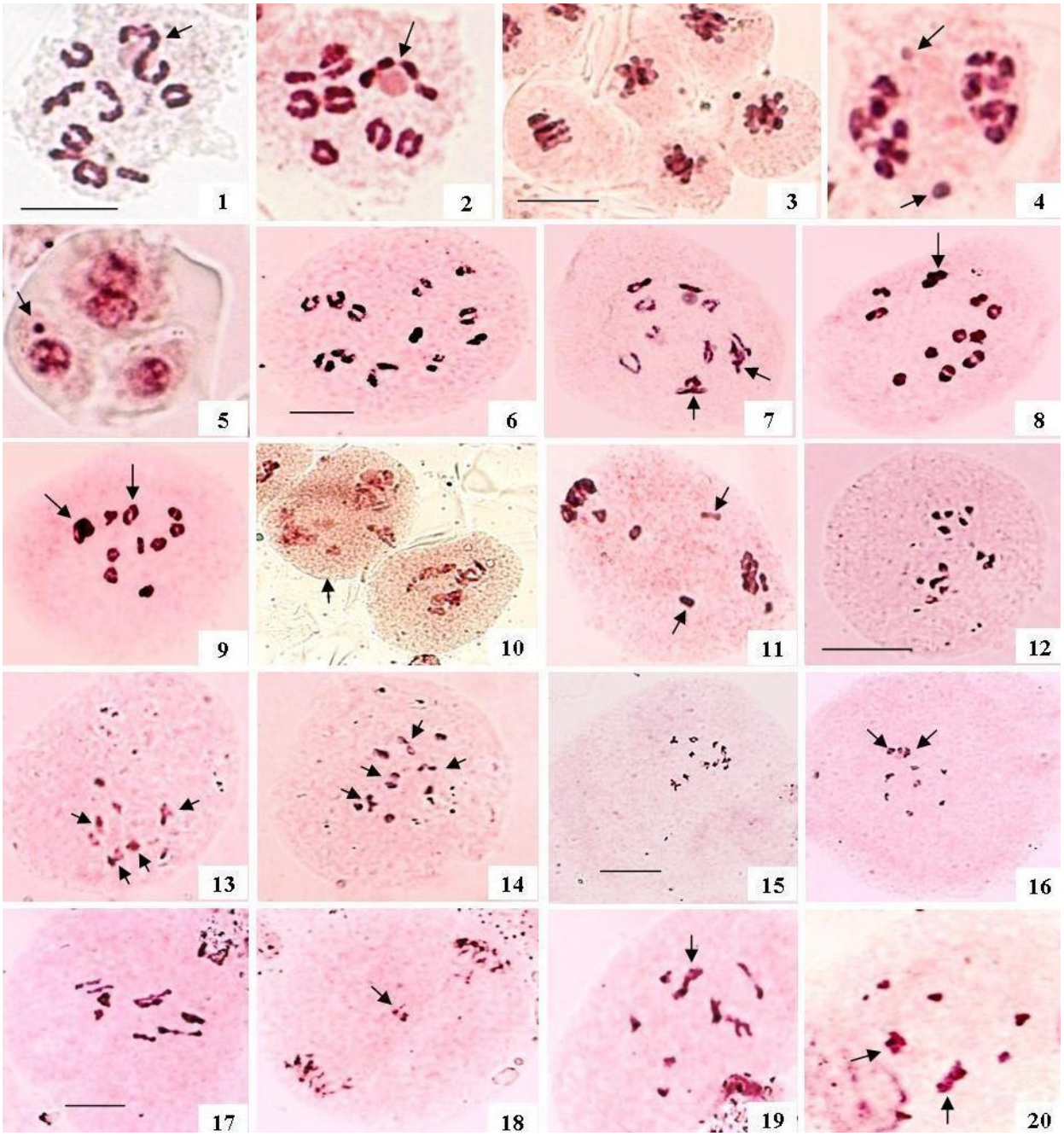
***Leycesteria formosa* Wall.**

Meiotic studies depict the species to be diploid with 2n=18 (Fig. 17) and show balanced distribution of 9:9 chromosomes during anaphase-I. But 4.67% PMCs show laggards at anaphase-I (Fig. 18). Analysis of large number of cells shows the presence of 1-2 quadrivalents in 14.10% PMCs at metaphase-I (Figs. 19, 20). The configuration of $2n=1_{IV}+7_{II}$ is more common and is found in 9.50% PMCs. Further microsporogenesis is found to be normal with 100 % pollen fertility. Pollen size varies from 44.25-46.87µm. The present report confirms the previous chromosome count of Bedi *et al.* (1982).

***Salvia plebeia* R.Br.**

Presently meiotic studies made on three populations from different altitudinal regions and the species show 2n=16 (Fig. 21). Meiosis is abnormal in all the populations. Multivalents in the form of 1-2 quadrivalents are observed in 11.45% cells in P-2 and 14.60% cells in P-3 (Figs. 22, 23). Further meiotic course is normal in all the populations with normal sporad formation leading to high pollen fertility (88-98%). Pollen size varies from 15.00-33.75 µm. Presently reported chromosome number is earlier

Figures 1-20: Photomicrographs of Pollen mother cells (PMCs) of investigated species (Scale bar=10 µm) ***Artemisia annua***: (1, 2) PMCs at diakinesis showing 1_{IV} (chain) + 8_{II}; (3) Some PMCs at metaphase-I showing chromatin stickiness; (4) Anaphase-I with laggards; (5) Tetrad with micronucleus. ***Datura stramonium***: (6) PMC at metaphase-I showing 12_{II}; (7) PMC at diakinesis showing 2_{IV} + 8_{II}; (8) PMC at metaphase-I showing 1_{IV} + 10_{II}; (9) PMC at metaphase-I showing 2_{IV} + 8_{II}; (10) A hyperploid PMC (arrow); (11) PMC at telophase-I showing laggards. ***Dicliptera bupleuroides***: (12) PMC at metaphase-I showing 13_{II}; (13, 14) PMCs at metaphase-I showing 4_{IV} + 5_{II}. ***Hemigraphis latebrosa***: (15) PMC at metaphase-I showing 12_{II}; (16) PMC at metaphase-I showing 2_{IV} + 8_{II}. ***Leycesteria formosa***: (17) PMC at metaphase-I showing 9_{II}; (18) PMC at telophase-I with laggards; (19) PMC at metaphase-I showing 1_{IV} + 7_{II}; (20) PMC at metaphase-I showing 2_{IV} + 5_{II}.



counted by number of workers (Mehra and Gill, 1968; Vij and Kashyap, 1975; Bir and Sidhu, 1980; Gill, 1984). In addition to this, 1B chromosome is reported in diploid cytotype by Gill (1971).

***Senecio nudicaulis* Buch.-Ham. ex D. Don**

Population is found to be diploid (2n=10) with perfect bivalent formation in most of the cells (Fig. 24). But in some PMCs (11.23%), meiotic configuration of $2n=1_{IV} + 3_{II}$ is also noticed (Fig. 25). Early separation of

1-3 bivalents in 54.32% PMCs, late separation of a bivalent in 19% PMCs, laggards (1-3) in 13.42% PMCs and chromatin bridges (1-4) in 29.62% cells are observed (Figs. 26, 27). Further microsporogenesis is normal. Pollen fertility is 87% and pollen size ranges 18.75-22.50µm. This chromosome count is new for the species (Gupta *et al.*, 2010a; Bala and Gupta, 2013).

***Sonchus brachyotus* DC.**

Presently 4 populations of the species have been studied, collected from altitude range from 621-1500m. Meiotic studies revealed them to be diploid with 2n=18. Some PMCs show associations in the form of quadrivalents in all the four populations (Table-3) (Figs. 28, 29). Although the frequencies of PMCs having quadrivalents and quadrivalent per PMC vary in different populations. Though in most of the PMCs there is normal distribution of chromosomes during anaphases, however, some of the PMCs show late disjunction of 1-2 bivalents (Table-4) (Fig. 30). Microsporogenesis is normal in all the populations leading to almost 100% pollen fertility. Earlier workers (Mehra *et al.,* 1965; Gupta and Gill 1989; Gupta *et al*., 1989) reported normal bivalent formation in the species.

***Verbena officinalis* L.**

Meiotic behaviour in the three morphotypes (pink flower in P-1, light purple in P-2 and white in P-3 populations) is given in table-5. Meiotic studies revealed 2n=14 in P-1 and P-2 (Fig. 31) and 2n=12 in P-3 (Fig. 32) with equal segregation of chromosomes during anaphase-I in most of the cells. At metaphase-I in all the three populations, quadrivalents (0-2) are noticed in 28.22%, 31.00%, 39.45% cells, respectively (Figs. 33-35). At anaphase-I, laggards are also noticed in 11.34% PMCs (Fig. 36). Pollen fertility is high (89-99%).

Table 3. Analysis of chromosomal associations at diakinesis/ metapahse-I in *Sonchus brachyotus.*

Populations	Number of PMCs observed	Meiotic configuration	
		IV	II
P-1	11	1	7
	28	-	9
	Total: 39	11	329
	Average frequency /PMC	0.28	8.43
	%age of chromosome involved	6.22	93.66
P-2	8	1	7
	4	3	3
	3	2	5
	21	-	9
	Total: 36	26	272
	Average frequency /PMC	0.72	7.55
	%age of chromosome involved	16.04	83.95
P-3	2	1	7
	6	2	5
	17	-	9
	Total: 25	14	197
	Average frequency /PMC	0.56	7.88
	%age of chromosome involved	12.44	87.55
P-4	3	1	7
	31	-	9
	Total: 34	3	300
	Average frequency /PMC	0.08	8.82
	%age of chromosome involved	1.77	98.03

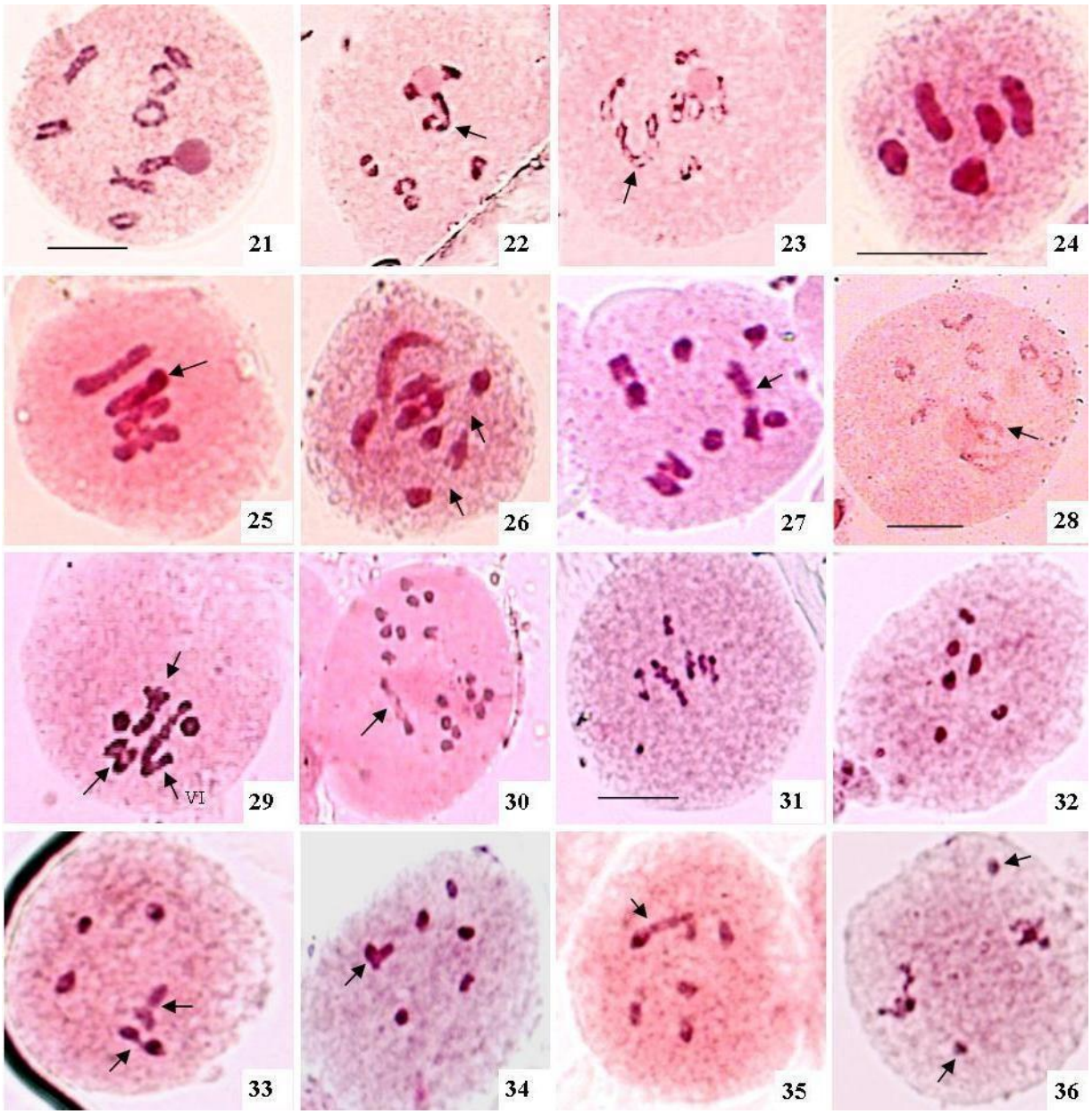
Table 4. Data on late disjunction of bivalent in four populations of *Sonchus brachyotus.*

Populations	Total PMCs observed at A-I	Number of PMCs showing late disjunction of bivalent	%age of PMCs showing late disjunction of bivalent
P-1	68	15	22.05
P-2	61	17	27.86
P-3	72	21	29.16
P-4	54	06	11.11

Table-5. Data on abnormal meiosis in three morphotypes of *Verbena officinalis.*

Population	Flower color	%age of PMCs with multivalent at M-I	%age of PMCs with bridges	%age of PMCs with laggards
P-1 (Morphotype-1)	pink	00.00	06.66	09.33
P-2 (Morphotype-2)	light purple	00.00	00.00	00.00
P-3 (Morphotype-3)	white	39.45	00.00	00.00

Figures 21-36: Photomicrographs of Pollen mother cells (PMCs) of investigated species (Scale bar=10 µm) ***Salvia plebeia***: (21) PMC at diakinesis with 8_{II}; (22, 23) PMCs at diakinesis with $6_{II} + 1_{IV}$. ***Senecio nudicaulis***: (24) PMC at metaphase-I showing 5_{II}; (25) PMC at metaphase-I with $1_{IV} + 3_{II}$; (26) PMC at metaphase-I showing early separation of 3_{II}; (27) PMC at anaphase-I showing late separation of 1_{II}. ***Sonchus brachyotus***; (28) PMC at diakinesis with $1_{IV} + 7_{II}$; (29) PMC at metaphase-I with $1_{VI} + 2_{IV} + 2_{II}$; (30) PMC at anaphase-I showing late disjunction of 1_{II}. ***Verbena officinalis***: (31) PMC at metaphase-I showing 7_{II}; (32) PMC at metaphase-I showing 6_{II}; (33) PMC at metaphase-I with $2_{IV} + 3_{II}$; (34) PMC at metaphase-I with $1_{IV} + 5_{II}$; (35) PMC at metaphase-I with $1_{IV} + 4_{II}$; (36) PMC at anaphase-I showing laggards.



33.75µm). The present chromosome counts of 2n=12 and 14 in *Verbena officinalis* (Bala and Gupta, 2012a) confirm the earlier reports from outside India.

Presence of multivalents in diploids is attributed to the presence of heterozygosity for chromosomal interchanges. Presently, multivalents have been noticed, where structural changes might be one of the reasons for the morphological variation. A well-known example of partial or complete permanent reciprocal translocation heterozygosity was reported in *Oenothera* (Kirk and Tilney-Bassett, 1978). Burnham (1962) stated that the two ends of a chromosome pair are homologous with the ends of different chromosome pairs and such interchanges between non-homologous chromosomes may results into formation of rings. All the presently studied species confirmed with diploid chromosome number. Structural heterozygotes generally show low fertility and seed setting but in present study all the structural hybrids show more than 80% pollen fertility thus indicated multivalent formation with alternate disjunction.

Conflict of Interests

Authors declare that there is no conflict of interests regarding the publication of this paper.

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