Cytological Investigations on Some North Indian Bicarpellatae

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Summary The present investigations have been made on 37 wild species including 76 accessions in 29 genera covering 3 families—Acanthaceae, Lamiaceae and Scrophulariaceae of the Bicarpellatae series of subclass gamopetalae. Chromosome numbers and detailed meiotic course, along with pollen fertility, have been studied for each species on its accession basis. New reports are recorded as B-chromosomes: *Ajuga bracteosa* ($n=32+0-2B$), *Calamintha clinopodium* ($n=18+0-1B$) and *Hemigraphis latebrosa* ($n=12+0-2B$) and *Veronica anagallis-aquatica* ($n=18+2B$); varied chromosome counts: *Calamintha clinopodium* ($n=18$), *Hemigraphis latebrosa* ($n=12$), *Justicia diffusa* ($n=16$) and *Lindenbergia indica* ($n=25$); euploid cytotypes: *Lepidagathis hyalina* ($n=30$) and *Veronica persica* ($n=7$). Two species, *Veronica persica* ($n=7, 14$) and *Veronica anagallis-aquatica* ($n=18+2B$), have been counted for the first time in India. A comparison of morphological characters in the above listed interesting taxa has been made.

Key words Acanthaceae, B-Chromosomes, Varied chromosome numbers, Euploid, Lamiaceae, Scrophulariaceae.

Acanthaceae, Lamiaceae and Scrophulariaceae are important families of the Bicarpellatae series of subclass gamopetalae of dicot angiosperms. With its 2,500 species in 250 genera (Airyshaw 1973), Acanthaceae (or Acanthus family) is one of the larger families in the plant kingdom. Members of this family are chiefly distributed in the tropics and sub-tropics and show much variation in habit. Medicinally important plants, such as *Adhatoda vasica*, *Asteracantha longifolia*, *Barleria cristata*, grow throughout India. The *Strobilathes alatus* is grown as an ornamental plant.

Lamiaceae (the mint family) comprises over 240 genera and 6,500 species worldwide and its members produce a wide array of aromatic compounds (terpenoids), and they have been used for a number of medicinal purposes. Common wild species used for medicinal purposes are: *Ajuga bracteosa*, *Anisomeles indica*, *Calamintha clinopodium*, *Leonurus cardiaca*, *Leucas cephalotes*, *Nepeta hindostana*, *Origanum vulgare* and *Prunella vulgaris*; common as ornamentals are: *Lamiun album* and *L. amplexicaule*.

The Scrophulariaceae (or Figwort family) embraces 220 genera and around 3,000 species, comprising mostly of herbaceous plants distributed throughout the world. Scrophulariaceae is best known for its ornamental species e.g. *Antirrhinum* (snapdragon), *Penstemon*, *Mimulus*, *Majus japonicas*, *Cymbalaria*, and *Digitalis* (foxglove). *Digitalis* is also famous as the original source of digitalin and digitoxin, 2 drugs used in the treatment of heart problems. *Lindenbergia indica* and *Verbascum thapsus* are used for medicinal purposes.

Most of the chromosome studies on these families were carried out from South and Central India with scattered reports from North India. There are reports on chromosome numbers but no detailed cytogenetical investigations seem to have been carried out on these families from North India. The present attempt has been made in view of the economic importance of members of these families. The present paper deals with the detailed cytological analysis of 76 natural populations of

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37 species of 3 families: Acanthaceae, Lamiaceae and Scrophulariaceae.

Materials and methods

For meiotic studies, usual acetocarmine smears of appropriate sized young flower buds were made after fixing them in Carnoy's fixative. Pollen fertility was estimated by mounting mature pollen grains in glycerol-acetocarmine. Materials for the present studies were collected from the Kangra district of Himachal Pradesh, covering altitude range between 490–3,300 m. Specific localities along with the altitude of various taxa are presented in Table 1. Voucher specimens are available in the Herbarium, Department of Botany, Punjabi University, Patiala (PUN).

Results and discussion

The haploid (n) chromosome numbers in the investigated species along with specific locality, accession numbers (PUN) and previous chromosome reports are presented in the Table 1. The families, genera and species are arranged alphabetically under their respective families. Only the interesting taxa are described in detail.

Hemigraphis latebrosa (Roth) Nees

It is a common herb mostly distributed between 500–1,600 m altitudes. Two accessions from Galua and Jarpali exhibited 12 bivalents. Additionally, in some cells 2 B-chromosomes were observed (Fig. 1). This varied chromosome count has been made for the first time on a worldwide basis, as the present report is not accordance with the previous reports of $n=7$ (Vasudevan 1976) and $n=14$ (Sareen and Sanjota 1976, Bir and Saggoo 1979). Earlier 3 species of the genus have been worked out which showed $n=7$, 14, 15 and 16. The chromosome number presently found ($n=12$) is new to the species as well as the genus.

The meiotic course in the Galua accession is abnormal due to the occurrence of laggards and bridges during anaphases/telophases, with high pollen fertility (96%). On the other hand, the accession from Jarpali has no meiotic abnormality and has cent per cent pollen fertility. Morphologically, the Jarpali population shows robust habit in terms of plant height, leaf size and petiole length.

Justicia diffusa Willd.

Meiotic studies revealed 16 bivalents at metaphase-I (Fig. 2). This chromosome number has been worked out for the first time on a worldwide basis, as the previous reports are of $n=9$ from India (Bir and Sidhu 1980, Vasudevan 1976, Valsala and Mathew 1982) and from outside India (Khatoon and Ali 1993). The common gametic chromosome numbers known in the genus are $n=9$, 11, 13 and 14. The chromosome number presently found ($n=16$) is new to the species as well as the genus.

Meiosis in both accessions is normal with high pollen fertility.

Lepidagathis hyalina Nees

This is common in Sal forests between 400–1500 m. The present studies showed the presence of $n=30$ at metaphase-I (Fig. 3). This is the first record of the hexaploid cytotype on a worldwide basis, as the previous record for the species is of $n=10$ (Bir and Saggoo 1981).

Ajuga bracteosa Wall.

The species is common in dry, sandy places and on rocks between altitudes of 500–800 m. The present investigations have been made on 3 accessions of the species which represents 3 morpho-

Table 1. Information on taxa, voucher data, accession number, chromosome number and previous chromosome reports of presently investigated species of the Bicarpellatae series.

Name of taxa	Voucher data (Locality with altitude)	Accession number (PUN)	Meiotic chromosome number (<i>n</i>)	Previous chromosome reports (2 <i>n</i>)	
				India	World
FAMILY: ACANTHACEAE					
<i>Adhatoda vasica</i> Nees	Bankhandi, 557 m Shahpur, 780 m Trilokpur, 615 m Jarpali, 501 m	53050 53051 53052 53053	17 17 17 17	34	56
<i>Asteracantha longifolia</i> Nees	Shahpur, 780 m	53034	16	32	
<i>Barleria cristata</i> Linn.	Shahpur, 780 m	53211	19	34, 36, 38, 40	40
<i>Dicliptera bupleroides</i> Nees	Banuri, 1300 m Nagrota Surian, 530 m Shahpur, 780 m	53212 53213 53214	13 13 13	26, 52	
<i>Eranthemum nervosum</i> (Vahl) R.Br. ex. Roem. & Schult.	Jarpali, 501 m	53042	11	42, 44	
* <i>Hemigraphis latebrosa</i> (Roth) Nees	Galua, 595 m Jarpali, 501 m	53049 53074	12 12+0–2B	14, 28	
* <i>Justicia diffusa</i> Willd.	Dharamkot, 2200 m Shahpur, 780 m	53046 53047	16 16	18	
<i>Lepidagathis cuspidata</i> Nees	Mecleodganj, 1780 m Shahpur, 780 m Salli, 1500 m	53039 53040 53041	11 11 11	22	
* <i>Lepidagathis hyalina</i> Nees	Galua, 595 m 32 Meel, 620 m Dehra Gopipur, 490 m	53043 53044 53045	30 30 30	20	
<i>Peristrophe bicalyculata</i> Retz.	Nagrota Surian, 530 m	53048	15	20, 30	30
<i>Strobilanthes alatus</i> Nees	Mecleodganj, 1780 m Dharamkot, 2200 m Multhan, 2000 m Swar, 2500 m	53035 53036 53037 53038	16 16 16 16	32	
FAMILY: LAMIACEAE					
* <i>Ajuga bracteosa</i> Wall.	Dehra Gopipur, 490 m Masroor, 617 m Nagrota Surian, 530 m	53014 53015 53016	32 32 32+0–2B	32, 64	32
<i>Ajuga macrosperma</i> Wall. ex Benth.	Masroor, 617 m	53011	16	32	16
<i>Ajuga parviflora</i> Benth.	Palampur, 1219 m Bir, 1300 m	53012 53013	16 16		
<i>Anisomeles indica</i> (Linn.) O. Kuntze	Shahpur, 780 m Dehra Gopipur, 490 m Nagrota Surian, 530 m	52988 52989 52990	17 17 17	32, 32+0-2B	
* <i>Calamintha clinopodium</i> Benth.	Bir, 1300 m Palampur, 1219 m Multhan, 2000 m	53017 53018 53019	18+0–1B 18 18	20, 34 20	20
<i>Lamium amplexicaule</i> Linn.	Nagrota Surian, 530 m	53000	9	18	18
<i>Leonurus cardiaca</i> Linn.	Lohardi, 2200 m	53038	9	18	18

Table 1. (Continued)

Name of taxa	Voucher data (Locality with altitude)	Accession number (PUN)	Meiotic chromosome number (<i>n</i>)	Previous chromosome reports (2 <i>n</i>)	
				India	World
<i>Leucas cephalotes</i> (Roth) Spreng.	Nagrota Surian, 530 m	53020	11	18, 22, 28	
<i>Leucas lanata</i> Benth.	Banuri, 1300 m	52998	14	28	
	Shahpur, 780 m	52999	14		
<i>Micromeria biflora</i> (Buch.-Ham.) Benth.	Banuri, 1300 m	53027	15	30	30
	Nagrota Surian, 530 m	53028	15		
<i>Nepeta hindostana</i> (Roth) Haines	Tiara, 810 m	53001	18	18, 36	
	Sakri-Bilaspur, 527 m	53003	18		
	Dyot, 2037 m	53004	18		
<i>Nepeta leucophylla</i> Benth.	Multhan, 2000 m	53361	18	36	
<i>Plectranthus rugosus</i> (Wall.)	Salli, 1500 m	53022	12	24	
<i>Plectranthus striatus</i> Benth.	Salli, 1500 m	53021	12	24	
<i>Pogostemon benghalensis</i> (Bum. f.) O. Kuntze	Bankhandi, 557 m	53030	32	42, 64	
<i>Prunella vulgaris</i> Linn.	Multhan, 2000 m	53023	14	28	28, 32
<i>Salvia coccinia</i> Jull. ex Murr.	Gaggal, 770 m	21827	11	22	20, 22
	Masroor, 617 m	21828	11		
<i>Salvia plebeia</i> R.Br.	Dehra Gopipur, 490 m	52994	8	16, 32	16
	Nagrota Surian, 527 m	52995	8		
	Jarpali, 501 m	52996	8		
<i>Scutellaria repens</i> Buch.-Ham. ex D. Don	Tiara, 810 m	53072	10	20, 20+1B	
<i>Stachys sericea</i> Wall.	Lohardi, 2200 m	53007	15	30	
	Badagaon, 3300 m	53009	15		
FAMILY: SCROPHULARIACEAE					
* <i>Lindenbergia indica</i> (Linn.) Vatke	Tiara, 810 m	53067	25	30	30
	Dharamshala, 1500 m	53068	25		
	Dyot, 2037 m	53070	25		
	Nagrota Surian, 530 m	53071	25		
<i>Mazus japonicus</i> (Thumb.) O. Kuntze	Nagrota Surian, 530 m	53056	20	40, 52	38, 40
	Bassa, 490 m	53057	20		
	Galua, 595 m	53058	20		
<i>Scrophularia himalensis</i> Royle ex Benth.	Lohardi, 2200 m	53060	24	48+2-4B	
<i>Verbascum thapsus</i> Linn.	Harchhakian, 600 m	53061	18	34, 36	34, 36
	Bir, 1300 m	53062	18		
	Palampur, 1219 m	53064	18		
	Dyot, 2037 m	53065	18		
	Badagaon, 3300 m	53066	18		
* <i>Veronica anagallis-aquatica</i> Linn.	Nagrota Surian, 530 m	53059	18+0-2B		18, 36
* <i>Veronica persica</i> Poir	Nagrota Surian, 530 m	53054	7		28
	Palampur, 1219 m	53055	14		

* New records

Figs. 1–9. Meiotic analysis. (Scale bar=10 μ m). 1. *Hemigraphis latebrosa*. PMC at Metaphase-I with $n=12+0-2B$ (arrows). 2. *Justicia diffusa*. PMC at Metaphase-I with 16 bivalents. 3. *Lepidagathis hyalina*. PMC at Metaphase-I with 30 bivalents. 4. *Ajuga bracteosa*. PMC at Metaphase-I with $n=32+0-2B$ (arrows). 5. *Calamintha clinopodium*. PMC at diakinesis with $n=18+0-1B$ (arrow). 6. *Lindenbergia indica*. PMC at Metaphase-I with 25 bivalents. 7. *Veronica anagallis-aquatica*. PMC at diakinesis with $n=18+0-2B$ (arrows). 8, 9. *Veronica persica* 8. PMC at Metaphase-I with 7 bivalents. 9. PMC at Metaphase-I with 14 bivalents.

types on flower color basis (white blue, pure white and white purple).

All the accessions are tetraploid with $n=32$. The Nagrota Surian population shows up to 2 B chromosomes in some cells (Fig. 4). The present report of $n=32$ is in accordance with previous records (Bir and Saggoo 1981, Chuang *et al.* 1963, Gill 1984), but the B-chromosomes are reported for the first time. Already the B-chromosomes are known in *A. parviflora* ($2n=32+0-2B$) by Bir and Saggoo (1982).

Meiosis in all the accessions is abnormal with the presence of univalents, unoriented bivalents, secondary chromosomal associations and cytomixis in the Nagrota Surian accession, and only a few laggards in the Masroor and Dehra Gopipur accessions.

Calamintha clinopodium Benth.

The present study on the accessions of Bir and Palampur revealed 18 bivalents. In addition to 18 bivalents, 1B-chromosome is also reported (Fig. 5). This chromosome number has been worked out for the first time on a worldwide basis, as the present report is not accordance with the previous reports of $n=10$ from India (Gill 1984, Saggoo 1983) and from outside India (Löve 1954, Majovsky

et al. 1970, Reese 1953, Scheerer 1939). The various chromosome numbers already known in the genus *Calamintha* are: $n=5, 9, 10, 11, 12, 18, 19, 21, 23$ and 24 . Thus, the chromosome number $n=18$ is already known in the genus but is newly reported for the present species. Meiosis in both accessions is normal with high pollen fertility.

Lindenbergia indica (Linn.) Vatke

It is commonly distributed in open slopes between altitudes of 500–2010 m. All 4 accessions from various localities of the Kangra District showed $n=25$ (Fig. 6). The previous reports are of $n=15$ from India (Thakur 1978, Trivedi and Trivedi 1992) and from outside India (Khattoon and Ali 1993).

Tiara and Dyot accessions show normal meiosis with a high percentage of pollen fertility (96–98%). However, the Dharamshala and Nagrota Surian accessions are abnormal with the presence of laggards; monads, dyads, triads and tetrads with or without micronuclei. In these, the pollen fertility is slightly low (86–89%).

Veronica anagallis-aquatica Linn.

It is a cosmopolitan species, very commonly found on marshy grounds and along banks of streams. The Indian population of the species has been worked out for the first time with $n=18$. In addition this 2 B-chromosomes were also observed (Fig. 7) and the present record is in conformity with previous chromosome reports (Bjorkqvist *et al.* 1969, Ozturk and Fischer 1982, Dalgaard 1985). Presence of B-chromosomes is reported for the first time. Meiotic course is mostly normal except for the presence of some laggards and with high pollen fertility (98%).

Veronica persica Poir

It is a common species, distributed on moist and shady places between 500–2000 m. Here, the Indian population of the species has been worked out for the first time. Two cytotypes have been observed, diploid ($n=7$, Fig. 8) from Nagrota Surian and tetraploid ($n=14$, Fig. 9) from Palampur.

The present chromosome count of $n=7$ in the species is the first ever record of diploid cytotype on a worldwide basis. The chromosome count of $n=14$ for the species has been counted for the first time in India and is in conformity with the previous records from outside India (Hill 1989, Chuang and Heckard 1992, Mulligan 1959, Yamashita 1937). Meiosis in both accessions is normal. The pollen fertility is cent per cent in diploid and low (84%) in the tetraploid cytotype.

Morphologically, the tetraploid cytotype shows taller plants with fewer branches per plant and dark green colored leaves as compared to the diploid cytotype.

Conclusion

New chromosome data for these North Indian species showed B-chromosomes, varied chromosome counts, euploid cytotypes and 2 species counted for the first time from India. A total of 11 species of Acanthaceae family, presently investigated from the study area, show 7 diploids and 4 polyploids. Previous chromosome data reveals that the family is polybasic ($x=7, 9, 10, 11, 13, 15, 16$ and 17). The present study adds up 1 euploid cytotype ($2n=6x=60$) for *Lepidagathis hyalina* and 2 varied chromosome numbers *i.e.* $2n=24, 24+0-2B$ for *Hemigraphis latebrosa* and $2n=32$ for *Justicia diffusa*. This is the first time that $n=12$ has been reported in the *H. latebrosa* and $n=16$ in *J. diffusa*.

In the family Lamiaceae, a total of 20 species are presently investigated, out of which 16 are diploids and 4 polyploids. Like Acanthaceae, Lamiaceae is also polybasic ($x=8, 9, 10, 11, 12, 14, 15, 16$ and 17). The present study adds up 1 new chromosome number: $2n=36; 36+0-1B$ for *Calamintha clinopodium* and B-chromosomes ($2n=64+0-2B$) for *Ajuga bracteosa*.

In the Scrophulariaceae family, a total of 6 species are presently investigated, out of which 3 are diploids, 2 polyploids and 1 species with diploid as well as polyploid cytotypes. A new chromosome number ($2n=50$) for *Lindenbergia indica*; a new cytotype ($2n=2x=14$) for *Veronica persica*; and B-chromosomes ($2n=36+2B$) for *Veronica anagallis-aquatica* have been recorded.

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