Cytological Studies in Some North Indian Scrophulariaceae

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Worldwide in distribution, the Scrophulariaceae (220 genera and 3,000 species, cf. Willis 1973), is well known for its ornamental and medicinal values. Cytological data is available for only 16% of the described species (Fedorov 1969, Moore 1973). Of a sizeable number of Indian members (275 species in 54 genera; cf. Hooker 1885), 59 species belonging to 31 genera are North Indian in distribution. The present communication deals with the cytological aspects of some of these.

Material and methods

The material was collected in nature in the North Indian plains and hills within an altitude of 300 m–2,200 m. The source of the taxa investigated is given in Table 1.

Cytological studies were carried out in the pollen mother cells, for which purpose, the young flower buds were fixed for 24 hours in Carnoy's fluid and stored in 70% alcohol until use. The anthers were squashed in 1% acetocarmine containing traces of iron-acetate. Pollen grain fertility was judged by their stainability with glycerocarmine.

The photomicrographs and the camera lucida drawings are at a uniform magnification of $\times 1510$ except where otherwise mentioned. The voucher specimens have been deposited in the Herbarium of Panjab University, Botany Department, Chandigarh.

Results and discussion

Cytological studies were made in 14 taxa belonging to 10 genera (Table 1). Normal bivalent formation and their subsequent segregation leading to 100% pollen fertility was characteristics of all the taxa except where otherwise mentioned. Some of the other cytological features in these are discussed below.

Verbascum L.

Two investigated species *V. celsioides* Benth. and the morphologically variable *V. thapsus* L. uniformly revealed $n=18$ (Figs. 1, 2). Both these species were characterised by unequal sized bivalents. Some of the similar sized bivalents were secondarily associated, probably indicating chromosomal homologies. Further meiotic course was normal.

V. celsioides was cytologically uninvestigated earlier, whereas, *V. thapsus* is in conformity with the earlier cytological records (Mulligan 1961, Packer 1964, Gadella

Table 1.

Species worked out	Voucher no.	Locality	Chromosome number	Level of ploidy
Tribe: Verbasceae				
<i>Verbascum thapsus</i> L.	5001	Kasauli, 2,000 m	n=18	4x
<i>V. celsioides</i> Benth.	5014	Mandi, 1,000–1,500 m	n=18	4x
Tribe: Antirrhineae				
<i>Kickxia ramosissima</i> Janchen	5002	Pinjore, Mugal Garden, 300 m	n=9	2x
<i>Antirrhinum orontium</i> L.	5004	Chandigarh, 280 m	n=8	2x
<i>A. majus</i> Linn.	5003	P.U. Bot. Garden, 280 m	n=8	2x
Tribe: Gratioleae				
<i>Mazus rugosus</i> Lour.	5005	Chandigarh, 280 m	n=20	4x
<i>Lindenbergia urticaefolia</i> Link and Otto.	5006	Mussoorie, 1974 m	n=25	2x ₂ ?
<i>Torenia cordifolia</i> Roxb.	5007	—do—	n=16	4x
<i>Vandellia crustacea</i> Benth.	5013	Mandi, 1,500 m	n=7	2x
<i>V. crustacea</i> Benth.	5012	Chandigarh, 280 m	n=14	4x
Tribe: Digitaleae				
<i>Veronica anagallis</i> L.	5008	Surajpur, 300 m	n=27	6x
<i>V. agrestis</i> L.	5009	Kasauli, 2,000 m	n=14	4x
Tribe: Gerardiaceae				
<i>Striga euphrasioides</i> Benth.	5010	Chandigarh 280 m	n=20	4x
<i>Centranthera hispida</i> R.Br.	5011	Mandi, 1,000 m	n=15	2x

and Kliphuis 1966, Verma and Dhillon 1967, Gill 1971). Håkansson (1926) reported $2n=34$ and 36 for this species. Darlington and Wylie (1955) suggested $x=15$, 16 and 18 for *Verbascum*. Perusal of the literature reveals that *V. adriano-politanum* and *V. glanduligerum* both with $2n=24$ (Boryana Stefanova 1974) represent the lowest chromosome number for the genus. The basic nature of this number cannot be authentically supported since the meiotic behaviour is not known in these species. On the other hand the predominant occurrence of the chromosome numbers $2n=24$, 32 , 40 , 48 , 64 (cf. Fedorov 1969, Moore 1973) is tempting enough to consider $x=8$ as basic for *Verbascum*. Other chromosome numbers ($2n=26$, 28 , 30 , 34 , 66 ; Fedorov, l.c.; Moore, l.c.) reported for the genus may have either originated as a consequence of aneuploid loss or gain at different ploidy levels on $x=8$ or represent different basic sets. Thus both the presently investigated *V. celsioides* and *V. thapsus* showing $n=18$ might be tetraploids on $x=9$ or hypertetraploids on $x=8$. It is of interest to note that *V. phoeniceum* exists as two cytotypes with $2n=32$ and 36 (cf. Fedorov, l.c.; Moore, l.c.) indicating thereby,

Figs. 1–15. 1, *Verbascum thapsus*, $n=18$, note secondary associations of similar sized bivalents. 2, *V. celsioides*, $n=18$. 3, *Kickxia ramosissima*, 9 bivalents and a nucleolus at diakinesis. 4, *Antirrhinum orontium*, $n=8$ at diakinesis. 5–7, *A. majus*. 5, diakinesis showing 8 bivalents and a nucleolus. 6, two IV and Four II at M-I. 7, A-I showing laggard. 8, *Veronica anagallis*, $n=27$ at diakinesis. 9, *V. agrestis*, $n=14$ at late M-I. 10, *Mazus rugosus*, diakinesis showing

n=20. 11, *Lindenbergia, urticaefolia*, n=25 at M-I. 12, *Striga euphrasioides*, M-I with 20 bivalents. 13-14. *Vandellia crustacea*. 13, cytomorphotype 'A' showing n=7. 14, cytomorphotype 'B' with n=14. 15, *Centranthera hispida*, n=15 at diakinesis.

the derivation of both these numbers from one another.

Kickxia ramosissima Janchen ($n=9$; Fig. 3), tallies with the earlier report for the species (Verma and Dhillon, l.c.). Literature studies reveals that all the so far cytologically known species of the genus, are uniformly diploid on $x=9$. Earlier *Kickxia* was treated under the genus *Linaria* which is based on $x=7$ (Darlington and Wylie, l.c.).

Antirrhinum L.

Besides *A. orontium* L. which occurs as a common weed, *A. majus* L. is a common horticultural species in the North-Indian parts. Both these uniformly showed $n=8$ (Figs. 4, 5, 6) and are diploid on $x=8$ like most of the species of the genus (Darlington and Wylie, l.c.). Meiosis was normal in *A. orontium*, while variable chromosomal configurations in the form of multiple associations, bivalents and univalents were often observed at diakinesis and M-I in the pollen mother cells in *A. majus* (Table 2; Figs. 5, 6). In such cells lagging chromatin material (Fig. 7) resulted in abnormal anaphases. Pollen sterility was measured at 40%. The polyploid like meiotic behaviour in the formation of multivalents etc. in this taxon ($2x$) suggests its interchange heterozygous nature. It is worthwhile to mention here that due to its horticultural values, the species is, constantly under selection pressure.

Table 2. Chromosomal configurations at diakinesis and M-I as observed in *A. majus*

No. of cells studied	Types of associations							
	I	II	III	IV	V	VI	VII	VIII
2	×	2	×	1	×	×	×	1
2	×	4	×	2	×	×	×	×
1	×	5	×	×	×	1	×	×
1	1	6	1	×	×	×	×	×
2	×	2	×	×	×	2	×	×
1	×	6	×	1	×	×	×	×
1	×	2	×	3	×	×	×	×
2	1	3	1	×	×	1	×	×
8	×	8	×	×	×	×	×	×
20								

Intra-specific aneuploidy in *A. orontium* ($n=8$: Authors; Heitz 1926, 1927, Larsen 1967, Verma and Dhillon l.c.; Gill 1971, Vanloon *et al.* 1971, Gadella and Kliphuis 1971, $n=7$: Sobti and Singh 1968) and polyploidy in *A. majus* ($n=8$: Authors; $2n=16$: Tischler 1920, Heitz 1927, Propach 1935, Rieger 1957, 1958; Stubbe 1966, Verma and Dhillon, l.c.; Bose and Panigrahi 1969, Gill 1971, $n=16$, $2n=32$: Bamford and Winkles 1941, Ruttle and Nebel 1934, Straub 1941, Morrison and Rajhathy 1960), is evident.

Veronica L.

Two species, *V. anagallis* L. and *V. agrestis* L. were studied from the polybasic genus *Veronica* ($x=7, 8, 9$; $x_2=13, 17, 26$; Darlington and Wylie l.c.). *V. agrestis*

($n=14$, Fig. 9) is in line with the earlier identical findings for the species (Rohweder 1937, Yamashita 1937, Wulff 1937, Lehmann *et al.* 1954, Fischer 1969) and is tetraploid on $x=7$. Yamashita (l.c.) also recorded a diploid cytotype ($2n=14$) in this species. The present and the earlier available cytological data for *V. anagallis* points towards its existence at different ploidy levels, i.e. diploid ($n=9$: Fischer 1969), tetraploid ($n=18$, $2n=36$: Löve and Löve 1956, Khoshoo and Khushu 1966) and hexaploid (Fig. 8, $n=27$: Authors; Khoshoo and Khushu, l.c.; Verma and Dhillon, l.c.).

Mazus rugosus Lour. The morphologically variable species, grows in the moist and shady places in the North Indian planes and Himalayan ranges. All the presently studied morphologically variable taxa uniformly revealed $n=20$ (Fig. 10) in line with the earlier records for the species (Verma and Dhillon, l.c.). Discordant chromosome numbers $2n=24$, 38, 40 and 104 (cf. Fedorov, l.c., Moore, l.c.) make it difficult to ascertain the basic set for *Mazus*.

Lindenbergia urticaefolia Link and Otto. ($n=25$, Fig. 11) confirms the previous records for the species (Mehra and Vasudevan 1972). Though $n=10$ is the lowest gametic number known in *Lindenbergia* ($n=10$, 14, 25 cf. Mehra and Vasudevan, l.c.), $n=25$ in *L. urticaefolia* with normal meiotic sequence is suggestive of either a low basic set ($x=5$?) or its secondary origin.

Torenia cordifolia Roxb. ($n=16$) in accordance with the earlier findings by Mehra and Vasudevan (l.c.), is tetraploid on $x=8$. The other base number known for the genus is $x=9$ (Darlington and Wylie l.c.).

Striga euphrasioides Benth. Cytological studies revealed $n=20$ (Fig. 12) in agreement with the previous reports for this species (Kumar and Abraham 1941, Verma and Dhillon, l.c.).

Kumar and Abraham (l.c.) suggested $x=8$ for *Striga* ($n=20$, $2n=40$) on the basis of the occurrence of secondary associations in *S. densiflora*, *S. euphrasioides*, *S. lutea* and *S. orobanchoides*, whereas, Darlington and Wylie (l.c.) considered $x=10$ for the genus. According to Mehra and Vij (1972), the complete reliance upon the secondary associations which generally vary from cell to cell in most of the cases is not justified for the deduction of base number. In light of the fact that no such associations were observed in the presently studied *S. euphrasioides*, Darlington and Wylie's contention ($x=10$) is supported.

Centranthera hispida R. Br. ($n=15$, Fig. 15) represents the first cytological document for the genus. Though high $x=15$ may be taken as basic for *Centranthera*.

Vandellia crustacea Benth. A common weed met throughout the Indian plains and hills, ascending upto 1,200 m. Flowers: October-November.

A number of collections made from different localities in the area under survey and conforming to the broad description of the species as given by Hooker (l.c.), revealed morphological variations, both in the vegetative and floral characters. Two morphotypes, "A" and "B" (Figs. 16, 17, 18) and differing in their chromosome numbers were distinguished. The morphotype "A" was common in the hills (Mandi, 1,500 m.) and rarely descended below 500 m. It was diploid ($n=7$). The morphotype "B" was very common in the plain country and was never observed ascending beyond 500 m. It was tetraploid ($n=14$). Both the cytomorpha-

types dwelled along the water channels and/in moist situations.

Morphology

The polyploidy, in general was associated with the quantitative increase in the morphological features.

Plant size and branching pattern: The plants ranged in size between 3.00–5.00 cms. in diploids and 3.50–8.00 cms. in the tetraploids. However, both the cytotypes differed in their branching pattern. The diploids were less branched and more or less erect whereas the tetraploids were diffusely branched, with both prostrate and erect branches. The branches were more or less hairy in diploids in contrast to tetraploids where these were smooth.

Leaves: The diploids differed from the tetraploids in the number, size and margin of the leaves. In the former the leaves numbering from 5–20, measured 1.0×0.7 cm. These were shortly petioled and more or less dentate in outline. On the other hand, the tetraploids possessed 10–30 leaves, which measured from 1.5×1.1 cms. It was interesting to note that the lower leaves in the tetraploid were petioled with more or less smooth margins in comparison with the upper leaves which were sessile and markedly dentate. The stomata were smaller in diploid ($19.98 \times 13.32 \mu$) than the tetraploid ($23.31 \times 16.65 \mu$).

Flowers: Like the vegetative parts, the floral parts also showed a progressive increase in dimensions with the increase in the level of ploidy. The bluish white flowers were smaller in size in the diploid than the tetraploid, and were characterised by pink spots.

Pollen grains: In the diploids, pollen grains were comparatively smaller ($14.98 \times 11.65 \mu$) in size than tetraploids ($19.98 \times 16.65 \mu$).

Fruits: Ovoid capsules with persistent calyx (0.5 cm; diploid; 0.6–0.7 cm, tetraploid) were characteristic of both the cytotypes.

Seeds: Seeds were rectangular in both the cytotypes but varied in size (120×162 – $114 \times 150 \mu$ in the diploids and 96×174 – $120 \times 144 \mu$ in the tetraploids).

The comparative morphological features of the two cytotypes are given in Table 3.

Cytology

Meiotic studies in the cytotype 'A' revealed $n=7$. The bivalents were more or less uniform in size (about 1.85μ) and these underwent normal segregation at anaphases. Fig. 13 shows 7:7 distribution of the chromosomes at A-I. Pollen fertility was measured at 100%.

In the cytotype 'B' the cytological studies revealed $n=14$. Fig. 14 shows fourteen bivalents which could be classed into two size categories of 7 comparatively larger (1.80 – 3.01μ) and 7 smaller (1.2 – 1.7μ) bivalents. Two of these were nucleolar. Normal meiosis led to 100% fertile pollen.

Figs. 16–18. *Vandellia crustacea*. 16, general morphology. A: Diploid ($n=7$), B: Tetraploid ($n=14$). 17, diploid: a, leaves, $\times 8$. b, stomata, $\times 810$. c, seeds, $\times 200$. d, pollen grains, $\times 810$.

18, tetraploid: a, leaves, $\times 8$. b, stomata, $\times 810$. c, seeds, $\times 200$. d, pollen grains, $\times 810$.

Table 3. Comparative morphological account of diploid and tetraploid taxa of *Vandellia crustacea* Benth.

Characters	Diploid 'A'	Tetraploid 'B'
Habit and habitat	Comparatively less branched, more or less erect taxon, growing in moist shady places upto 1,500 m (Fig. 16a)	Diffusely branched with both prostrate and erect branches. Grows in mat formation in moist and shady situations within 300 m (Fig. 16b)
Area of collection	Kalka, 500 m Mandi (H.P.), 1,500 m	Chandigarh, 300 m
Flowering period	October–November	October–November
Plant size	3.0–5.0 cm	3.5–8.0 cm
Leaves	(Fig. 17a)	(Fig. 18a)
Number	5–20	10–30
Size		
petiole	0.3–0.5 cm	0.3–0.7 cm
lamina	1.0 × 0.7 cm	1.5 × 1.0 cm
Shape, apex and margin	Oblong, more or less dentate in outline	Oblong, margin of lower leaves more or less smooth and upper leaves sessile and markedly dentate
Stomata		
frequency	25.0%	27.3%
size	19.98 × 13.32 μ (Fig. 17b)	23.31 × 16.65 μ (Fig. 18b)
Inflorescence	Flowers small, mostly single on axillary stalk and may form small racemes at the end of branches. Flowers bluish white with pink spots	Flowers small, mostly single on axillary stalk and forming small racemes. Flowers bluish white
Total no. of flowers	3–8	8–15
Size of the flower	0.6 × 0.3 cm	0.9 × 0.3 cm
Size of the pedicel	0.6–1.4 cm	0.5–1 cm
Size of pollen grain	14.98 × 11.65 μ (Fig. 17d)	19.98 × 16.65 μ (Fig. 18d)
Fruit	More or less ovoid with persistent calyx	Oblong to ovoid with persistent calyx
Size	0.45 cm	0.5–0.6 cm
Size range of seeds	120 × 162–114 × 150 μ (Fig. 17c)	96 × 174–120 × 144 μ (Fig. 18c)
Chromosome no.	n=7	n=14

The present observations coupled with cytological diversities in literature are indicative of cytomorphological complexity of the species. Both the cytotypes (n=7, 14) reported presently were earlier uninvestigated. Raghavan and Srinivasan (1940) reported n=21, 2n=42 from South India (Annamalainagar) for this species while Bhattacharyya (1968) investigated the species as *Lindernia crustacea* (L.) Mueller from Eastern India and reported the existence of two discordant chromosome numbers n=16 and n=21.

Darlington and Wylie (l.c.) considered x=7 for *Vandellia*. *V. crustacea* thus seems to exist at diploid (n=7), tetraploid (n=14), hypertetraploid (n=16) and hexaploid (n=21) levels. *V. nummularifolia* (n=12: Gill 1971) is the only other cytologically known species of the genus.

Normal diploid like meiotic behaviour in the tetraploid and two size sets of 7 bivalents each in it suggest its allopolyploid nature. Its diploid counterpart shows

equal sized bivalents. Unequal sized chromosomes are also evident in the hexaploid taxon in this species (cf. Raghavan and Srinivasan, l.c.; p. 45, Fig. 35). The significant morphological differences such as the leaf character, hairyness and branching pattern etc., between the diploids and tetraploids presently investigated, are an added pointer towards the allopolyploid constitution of the tetraploid taxon.

Much can not be said with certainty about the probable ancestry of the polyploid taxa in *V. crustacea*. It is, however, worthwhile to point out that the branching pattern and occasionally the leaf shapes in the tetraploid taxon (present observations) are more or less intermediate between *V. crustacea* (2x) and *V. multiflora* (see Hooker l.c.). Though, the chromosomal constitution (number and shape) is not known in the latter species, its sympatric distribution with *V. crustacea* (2x) in N. Indian hills possibly speaks of its being the other parent involved in the evolution of the tetraploid taxon. It may be added that both *V. crustacea* (2x) and *V. multiflora* differ mainly in their branching habit, leaf and fruit characteristics.

Taking into consideration the present observations together with the earlier records on the species, it seems evident that the different cytotypes of *V. crustacea* complex are distributed from the colder to the warmer climates in India. The pattern of distribution is indicative of the fact that while the diploids prefer colder Northern areas (Common in the hills between 500–1,800 m), the tetraploids are met in the less colder areas in the North and East Indian plains, whereas the hexaploids are adapted for the warmer regions extending down into the South India. The different cytotypes in *V. crustacea* thus seem to be geographically isolated and possibly deserve sub-specific ranks atleast. Almost similar state of affairs have earlier been noted in regards to the nature of ploidy and distribution pattern in *Veronica anagallis* by Khoshoo and Khushu (1966).

Summary

Cytological studies were carried out in 14 N. Indian taxa. *Centranthera hispida* (n=15) and *Verbascum celsioides* (n=18) represent the first chromosome counts for the genus and the species respectively. Two cytotypes with n=7 and n=14 in *Vandellia crustacea* were also unknown earlier. For the remaining taxa one or the other earlier chromosome reports are confirmed. Meiosis was normal in most of the taxa. Secondary associations were observed in *Verbascum thapsus* and *V. celsioides*. Multiple associations in *Antirrhinum majus* (2x) represents interchange heterozygosity. Intraspecific aneuploidy in *Verbascum thapsus* (2n=34, 36), *Antirrhinum orontium* (2n=14, 16), intraspecific polyploidy in *Antirrhinum majus* (2n=16, 32), *Veronica anagallis* (2n=18, 36, 54) and both polyploidy and aneuploidy are evident in *Vandellia crustacea* (2n=14, 28, 32, 42). Origin and evolution of the tetraploid taxon in the last species is discussed.

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