

CYTOLOGICAL STUDIES ON SOME MEMBERS OF COMMELINACEAE MIRB. FROM KANGRA VALLEY (HIMACHAL PRADESH) WITH A SHORT SUMMARY OF KARYOLOGICAL DATA ON THE ANALYZED GENERA

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The present work deals with population-based meiotic studies on eight species belonging to four genera of the family Commelinaceae from different regions of Kangra Valley which is well known for its rich floristic diversity. At the world level, different cytotypes for four species such as *Commelina hasskarlii* ($2n = 22, 60$), *C. kurzii* ($2n = 60$), *Murdannia nudiflora* ($2n = 24$) and *M. spirata* ($2n = 24$) have been recorded for the first time at various ploidy levels. Additionally, from India, the new chromosome count for *Tradescantia pallida* ($2n = 24$) has been reported at the tetraploid level. The course of meiosis has been found to be normal in all the populations of *Commelina benghalensis*, *C. paludosa*, *Murdannia nudiflora* and *M. spirata* while four species, *Commelina hasskarlii*, *C. kurzii*, *Cyanotis cristata* and *Tradescantia pallida* have shown a normal to abnormal meiotic course in different populations. These meiotic abnormalities have revealed a clear effect on the pollen size and pollen fertility.

Keywords: Chromosome numbers, *Commelinaceae*, Kangra Valley, meiosis, population

INTRODUCTION

‘The Spiderwort family’ (Commelinaceae Mirb.) is a group of herbaceous Monocots with about 600 species belonging to 40 genera (Mabberley, 2008). The family is represented by 14 genera and 84 species in India (Karthikeyan et al., 1989) of which 4 genera and 5 species are present in Kangra district of H.P. (Chowdhery and Wadhwa, 1984). The members of the family are mainly distributed in the tropical, subtropical and subtemperate regions of the Northern Hemisphere. The family is distinguished by several features including: stem – erect, ascending or decumbent; leaves – flat or trough-like; inflorescence – mostly a condensed cyme subtended by involucre bracts or cymbiform spathe; trimerous flowers – mostly white, blue or pinkish; perianth – biseriata; outer 3 segments – free, green to white; inner 3 – colored, one often larger than the two; stamens – 6 in 2 whorls, sometimes 2–4, reduced to staminodes; ovary – superior,

3-loculed (rarely 2); fruit – a capsule. Floral dimorphism is common in the family as the occurrence of cleistogamous as well as chasmogamous flowers is known in the species of *Commelina* and *Murdannia*. Economically, several species of *Commelina*, *Floscopa*, *Tradescantia*, *Rhoeo*, *Zebrina* and *Cyanotis* are cultivated due to their ornamental value and for medicinal purposes for having antiinflammatory (Tag et al., 2007) and antimicrobial properties (Sharma and Sharma, 2010) and in the cases of bone fractures, chronic cough, cold, stimulating blood circulation and as a muscle relaxant (Dash, 2010).

Faden and Hunt (1991) recognized 2 subfamilies of the family Commelinaceae, the first one – Cartonematoideae – containing the tribes Cartonemateae and Triceratelleae, each comprising a single genus and the second one – Commelinoideae – covering the rest of the 38 genera arranged in 2 tribes, Tradescantieae and Commelineae, the former of which is divided into 7 subtribes. Recent-

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ly, Evans et al. (2000) conducted a cladistic analysis of the family using both morphological and molecular characters. Bhattacharya (1975) divided it into different groups of plants on the basis of basic chromosome numbers such as *Amischophacelus* ($x = 13$); *Aneilema* ($x = 9, 10, 13, 15$); *Belosynapsis* ($x = 10$); *Commelina* ($x = 11, 14, 15$); *Cyanotis* ($x = 12$); *Floscopa* ($x = 6$ or 12); *Murdannia* ($x = 6, 10, 11$) and *Zebrina* ($x = 6$).

Various cytologists have studied the cytology of the members of the family Commelinaceae from India (Kammathy and Rao, 1965; Rao et al., 1972; Bhattacharya, 1975; Mehra and Sachdeva, 1976; Sidhu and Bir, 1983; Renugadevi and Sampathkumar, 1986; Vyas and Verma, 1992) and also from other parts of the world (Jones and Jopling, 1972; Pitrez, 2001; Yang and Kang, 2003). The chromosome patterns and evolutionary trends were studied by Rao et al. (1972). Further details of several genera and evolutionary trends in the karyotypes within the family were discussed by Faden and Suda (1980). It is one exceptional family marked with the presence of asymmetrical to symmetrical type of the karyotype evolution (Jones and Jopling, 1972). The different genera of the family always remain in a controversial position for their morphological and cytological characters.

MATERIALS AND METHODS

For meiotic studies, young spikes were collected on the population basis from various localities in Kangra Valley of Himachal Pradesh. The meiotic studies were carried out using the standard smearing technique on young spikes fixed in Carnoy's fixative. Pollen fertility was estimated by mounting mature pollen grains in glycerol-acetocarmine (1:1) mixture. Well-filled pollen grains with stained nuclei were taken as apparently fertile while shriveled and unstained pollen grains were counted as sterile ones. Photomicrographs of pollen mother cells were made of freshly prepared slides using Nikon 80i eclipse Digital Imaging System. Voucher specimens are deposited in the Herbarium, Department of Botany, Punjabi University, Patiala (PUN).

RESULTS

The detailed information regarding the area, locality, altitude, accession number, meiotic chromosome number reports, ploidy level and meiotic course of different species of the family Commelinaceae from Kangra Valley of Himachal Pradesh is provided in Table 1.

COMMELINA BENGHALENSIS L.

During the meiotic studies, the species exhibited $2n = 22$ in Pollen Mother Cells (PMCs) at metaphase-I (M-I) (Fig. 1a) which is found to be in accordance with most of the earlier reports from India (Koul et al., 1976; Mehra and Sachdeva, 1976) and outside India (Pitrez, 2001; Yang and Kang, 2003). In addition to the present report, the species was previously known to exhibit $2n = 28, 30, 44, 48, 56, 66$ and 68 from different parts of the world from outside India along with a single report of the presence of B chromosomes in diploid cytotype, $2n = 22 + 1-4B$ by Vyas and Verma (1992) from India.

C. HASSKARLII C. B. CLARKE
 (= *C. CAROLINIANA* WALTER)

Out of 3 populations, 2 are found to have PMCs with $2n = 22$ at diakinesis (Fig. 1b) and the remaining third population shows $2n = 60$ again at diakinesis (Fig. 1c). One cytotype is at the diploid level ($2n = 22$) based on $x = 11$ and the other one at the tetraploid level ($2n = 4x$) based on $x = 15$. Both cytotypes make new chromosome records on a world-wide basis. The species was previously known to exhibit $2n = 30$ at the diploid level (Patwary et al., 1987) from Bangladesh, $2n = 90$ at the hexaploid level (Kammathy and Rao, 1965; Mehra and Sachdeva, 1976) from India based on $x = 15$ along with $2n = 84$ (Fotedar and Roy, 1969) from India based on another basic number $x = 12$.

Regarding meiotic behaviour, only the tetraploid accession shows abnormal meiosis (Table 1) with the formation of chromatin stickiness at metaphases, bridges and laggards at anaphases and telophases (Figs. 1d-f) and thus, the pollen fertility is reduced to 78.02%.

C. KURZII C. B. CLARKE (= *C. UNDULATA* R.BR.)

The presently worked out accession of the species clearly shows the chromosomal count of $2n = 60$ at anaphase-I (A-I) (Fig. 1g) which makes a new record for the species at $4x$ level. The species has already been reported from India to have $2n = 90$ and $2n = 120$ (Kammathy and Rao, 1961). Thus, the species shows intraspecific polyploid races ($4x, 6x, 8x$) based on $x = 15$ from India.

The meiotic course for the species is found to be abnormal with late disjunction of 3-4 bivalents per PMC at A-I (Fig. 1h). As a result, the pollen fertility is reduced to 82.08%.

C. PALUDOSA BLUME

During meiosis, chromosomal count of $2n = 120$ is clearly seen in the PMCs at diakinesis (Fig. 1i). This octoploid cytotype is found to be in conformity

TABLE 1. Information about the area, locality, altitude, accession number, meiotic chromosome number, ploidy level and meiotic course of different species of the family Commelinaceae from Kangra Valley of Himachal Pradesh.

Taxa	Locality, Altitude (m)	Accession numbers (PUN)	Meiotic chromosome number (2n)	Ploidy level/ Meiotic course
<i>Commelina benghalensis</i> L.	Dehra, 650 m	53569	22	2x/N*
<i>C. hasskarlii</i> C.B.Clarke (= <i>C. caroliniana</i> Walter)	Chhota Bhangal, 2,000 m	55354	22	2x/N
	Suliali, 500 m	55356	22	2x/N
	Bada Gran, 3,500 m	55355	60	4x/A**
<i>C.kurzii</i> C.B.Clarke (= <i>C.undulata</i> R.Br.)	Dharamsala, 1,600 m	56398	60	4x/A
<i>C. paludosa</i> Blume	Chandpur, 1,676 m	56608	120	8x/N
	Bandla, 1,266 m	56519	120	8x/N
	Khaniara, 1,750 m	56524	120	8x/N
<i>Cyanotis cristata</i> (L.) D.Don	Dehra, 650 m	53518	24	2x/N
	Nagrota Surian, 527 m	55359	24	2x/N
	Bhanala, 800 m	55353	24	2x/A
<i>Murdannia nudiflora</i> (L.) Brenan	Chhota Bhangal, 2,000 m	53570	24	4x/N
	Ranehar, 850 m	53571	24	4x/N
	Dharamsala, 1,600 m	56577	24	4x/N
<i>M. spirata</i> G. Brückn.	Dharamsala, 1,600 m	56540	24	4x/N
	Rehlu, 950 m	56564	24	4x/N
	Bandla, 1,266 m	56565	24	4x/N
<i>Tradescantia pallida</i> (Rose) D.R.Hunt	Tal-Mata, 1,103 m	56526	24	4x/A

* N = normal meiosis, ** A = abnormal meiosis

with the previous reports by Raghavan and Rao (1961) and Bhattacharya (1975) from India. In addition, the species is already known to have $2n = 60$ (Kammathy and Rao, 1965; Renugadevi and Sampathkumar, 1986; Patwary et al., 1987) from India along with a single report of $2n = 75$ available from Pakistan (Baquar and Saeed, 1977).

CYANOTIS CRISTATA (L.) D. DON

At present, 3 populations have been cytologically studied and all the populations are found to be strictly diploid with $2n = 24$ (Fig. 1j) through equal distribution of 12 : 12 chromosomes at A-I (Fig. 1k). The present chromosome report of $2n = 24$ conforms to the previous reports by various cytologists from India (Kammathy and Rao, 1961; Mehra and Sachdeva, 1976) as well as from outside India (Islam and Baten, 1952). Some other reports such as $2n = 26, 30$ are also known from outside India along with a single report of the presence of supernumerary chromosomes ($2n = 24 + 1B$) by Islam and Baten (1952) from Bangladesh.

One population collected from Bhanala (800m) reveals abnormal meiosis with the presence of

interbivalent connections at diakinesis/ M-I (Fig. 1l). Heterogenous sized pollen grains (Fig. 1m) as well as low pollen fertility (88.70%) are also noted.

MURDANNIA NUDIFLORA (L.) BRENNAN

The study of PMCs clearly depicts the presence of 12 bivalents at diakinesis (Fig. 1n) making an additional chromosomal record for the species on a world-wide basis as the species has been previously reported to exhibit $2n = 20$ – from India (Bhattacharya, 1975; Renugadevi and Sampathkumar, 1986) and from outside India (Jones and Jopling, 1972).

M. SPIRATA G. BRÜCKN.

The meiotic studies on PMCs of all the 3 populations exhibit $2n = 24$ through equal distribution of 12 : 12 chromosomes at A-I (Fig. 1o). This tetraploid cytotype makes a new chromosomal record for the species. The species has already been reported from India to have other cytotypes with $2n = 18$ (Raghavan and Rao, 1961) and $2n = 20$ (Rao et al., 1970).

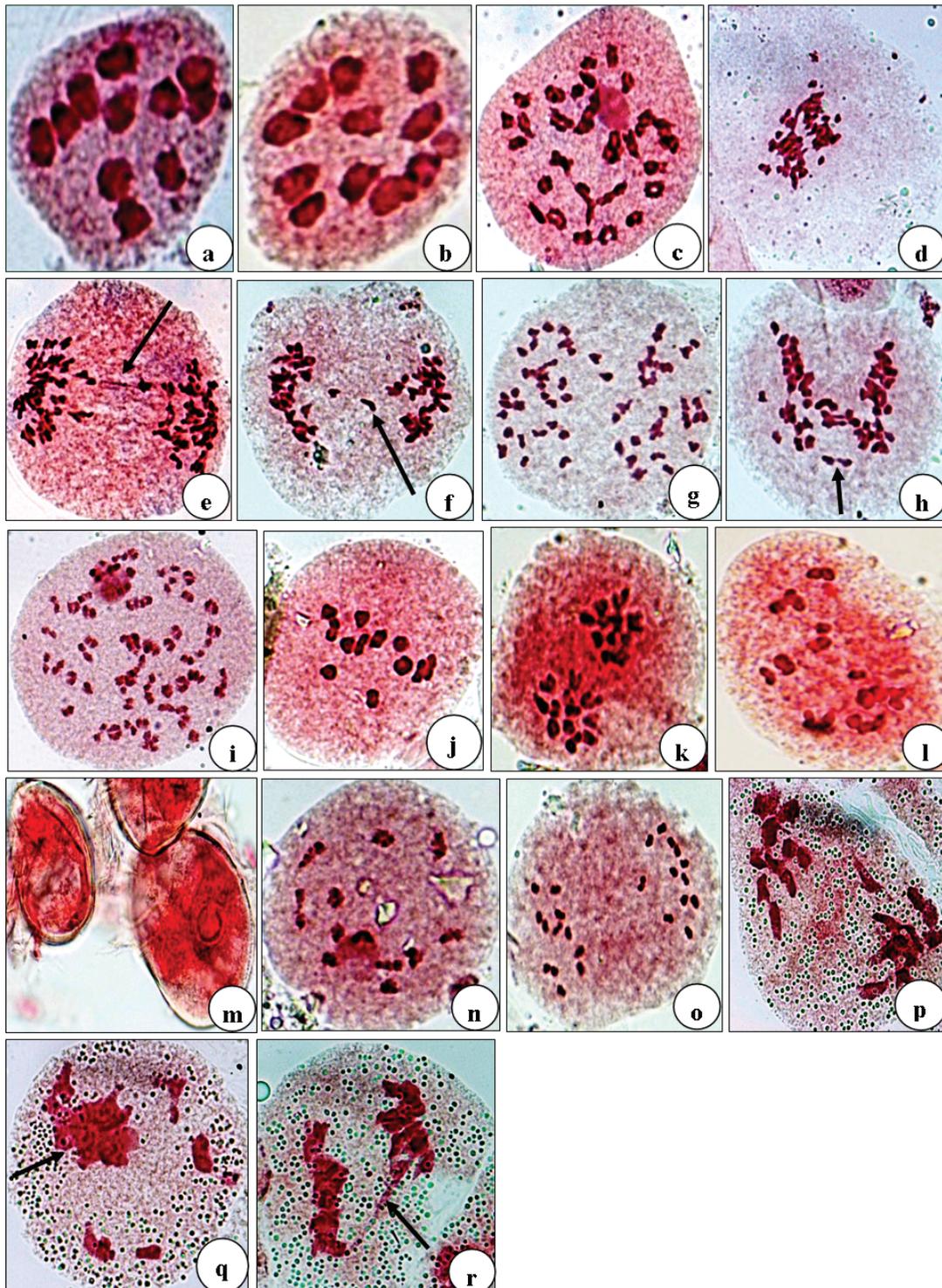


Fig. 1. (a) *Commelina benghalensis* ($2n = 22$), PMC at metaphase I. (b) *C. hasskarlii* ($2n = 22$), PMC at diakinesis. (c) *C. hasskarlii* ($2n = 60$), PMC at diakinesis. (d) Chromatin stickiness at metaphase I. (e) Chromatin bridge at anaphase I. (f) Laggards at anaphase I. (g) *C. kurzii* ($2n = 60$), PMC at anaphase I. (h) Late disjunction of bivalents at anaphase I. (i) *C. paludosa* ($2n = 120$), PMC at diakinesis. (j) *Cyanotis cristata* ($2n = 24$), PMC at metaphase I. (k) *Cyanotis cristata* ($2n = 24$), PMC at anaphase I. (l) Interbivalent connections at metaphase I. (m) Heterogenous sized fertile pollen grains. (n) *Murdannia nudiflora* ($2n = 24$), PMC at diakinesis. (o) *M. spirata* ($2n = 24$), PMC at anaphase I. (p) *Tradescantia pal-lida* ($2n = 24$), PMC at anaphase I. (q) Chromatin stickiness at metaphase I. (r) Chromatin bridge at telophase I.

TRADESCANTIA PALLIDA (ROSE) D. R. HUNT

During meiotic studies, the PMCs of the species exhibited $2n = 24$ (Fig. 1p) in conformity with the previous reports by Sobhan et al. (1991) and Garcia-Velazquez (1998) from outside India, but make a new chromosomal count from India. Earlier, the species was also reported to have $2n = 18$ by Garcia-Velazquez (1998) from outside India.

The species is found to have abnormal meiotic behaviour with the presence of chromatin stickiness at M-I (Fig. 1q) and formation of chromatin bridges at anaphases and telophases (Fig. 1r). Still, high pollen fertility is observed (98.05%).

DISCUSSION

In the present research, eighteen populations of eight species belonging to four different genera of the family Commelinaceae from Kangra Valley of Himachal Pradesh have been cytologically worked out for the first time. Out of these, four species reveal five new different cytotypes on a world-wide basis and one species has been recorded with a new chromosome count in India. This chromosomal data can be further used for various genetic programmes.

In order to understand the status of the cytologically investigated four genera belonging to the family Commelinaceae and calculation of basic chromosome numbers, the chromosome number details have been compiled for each genus in Table 2 – at the world as well as at India level by using various parameters like the number of cytologically worked out species out of the total number of taxonomically known species, including the number of diploids as well as frequency and level of polyploids, updated number of various chromosomal races including present reports for various species for each genus and information on the number of species per genus marked with inter- and intraspecific euploid and aneuploid variability.

Eighty-five species of the genus *Commelina* have been cytologically worked out by now, of which 52 species are found to be polyploid on the world level and the chromosome numbers range from $2n = 16$ to $2n = 180$ (Table 2), whereas, in India out of 35 investigated species, 22 species show polyploid nature. The genus is found to be polybasic with $x = 8, 9, 10, 11, 12, 13, 14, 15$ ($x = 15$ being the most common followed by $x = 14$). Different cytologists proposed different basic numbers for the genus *Commelina* such as $x = 11, 12, 15$ (Darlington and Wylie, 1955), $x = 14$ (Morton, 1956) and $x = 4$ (Sharma and Sharma,

1958). A few years later, Fedorov (1969) stated that 90% species of this genus correspond to the basic chromosome number $x = 15$ and the other basic numbers such as $x = 10, 11, 12, 13$ and 14 are confined to a few species only. The same was further supported by Patwary et al. (1987). Thus, the present results also support Fedorov's (1969) observation of $x = 15$ being the common basic number.

The cytological data compiled for the genus *Cyanotis* (Table 2) show that 34 species out of total 50 world-wide known species are found to exist in the form of 51 cytotypes. The genus is polybasic with $x = 8, 10, 11, 12, 13$ ($x = 12$ being common) and $x = 14, 15$ and 17 are to be taken with caution as these numbers do not exist independently. Previously, Lewis (1964) suggested $x = 6$ from which $x = 12$ and other basic numbers have evolved through hypo- and hyper-aneuploidy and euploidy. Mehra and Sachdeva (1976) recorded $x = 12$ as the most frequently occurring basic number in the genus.

For the genus *Murdannia*, it is noticed that at the world-level, 56% of the species are cytologically worked out with 60.71% polyploidy reaching up to $8x$ level. In India, of all the taxonomically known species, 95.83% species are cytologically known with 65.21% polyploid species (Table 2). The genus is polybasic in nature with $x = 6, 7, 9, 10$ and 11 with $x = 6$ and 10 remaining to be the most common numbers. Rao et al. (1968) stated that the evolutionary trend in *Murdannia* seems to follow 2 different paths, one with the basic chromosome number $x = 10$ and the other with $x = 6$, both arising from a common extinct ancestor with $x = 5$, and both polyploidy and aneuploidy being quite common.

The cumulative chromosomal data for the genus *Tradescantia* (Table 2) show that 98.33% species are cytologically worked out, of which 67.79% species are polyploid reaching upto $24x$ level. The chromosome numbers show a lot of chromosomal variations ranging from $2n = 12$ to $2n = 144$. The genus is tribasic with $x = 6, 7, 8$ ($x = 6$ being common) and possesses 22 species showing intraspecific euploidy and 7 species with intraspecific aneuploidy. In India, 12% species are cytologically worked out with 83.33% polyploidy.

Bhattacharya (1975) performed cytological studies on 5 genera of the family Commelinaceae covering 35 taxa, mainly from West Bengal, and a few species from the eastern part of the Himalayas and Khasia hills, observing two chromosomal lines: one based on $x = 4$ or 5 (*Murdannia*, *Aneilema*, *Commelina*) with medium-sized to short acrocentric chromosomes and the other with $x = 6$ (*Tradescantia*, *Zebrina*, *Setcreasea*) with mostly large-sized metacentric

TABLE 2. Cytological information about the investigated genera of the family Commelinaceae on the basis of complete information including previous as well as present chromosome number reports.

Genus	World/ India	Number of taxo- nically known species	Number of cyto- logically worked out spe- cies/ (%)	Number of diploids	Polyploids		Various ploidy levels	Total number of cytotypes (Chromo- somal races)	Known 2n [*] chromosome numbers (in parenthesis number of species/ taxa)	Number of		
					Number Frequency (%)	Number				species with intraspecific eu ploidy (Respective basic numbers in parenthesis)	Number of species with intra- specific aneuploid cytotypes	Common basic numbers**
I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Family: Commelinaceae Mirb. (x=6, 10, 12, 14, 15)												
<i>Commelina</i> L.	World	170	85 (50.00)	33	52	61.17	2x, 3x, 4x, 5x, 6x, 8x, 10x, 12x, 13x, 14x	174	16(1), 18(1), 20(3), 22(5), 24(5), 26(5), 28(16), 30(26), 32(1), 35(1), 36(1), 40(1), 42(5), 44(4), 45(2), 46(1), 48(4), 52(5), 53(1), 56(12), 58(3), 60(25), 61(1), 62(2), 64(1), 66(1), 68(1), 72(2), 75(2), 84(2), 86(2), 88(2), 90(17), 104(2), 110(1), 112(1), 120(6), 150(2), 180(1)	1(8), 1(9), 3(11), 1(12), 1(13), 5(14), 14(15)	24	8, 9, 10, 11, 12, 13, <u>14, 15</u>
	India	24(35)	35 (100)	13	22	62.85	2x, 3x, 4x, 5x, 6x, 8x, 10x, 13x, 14x	63	20(2), 22(2), 24(5), 28(3), 30(9), 35(1), 42(1), 44(1), 45(1), 48(2), 56(2), 60(11), 62(1), 64(1), 72(1), 75(1), 90(10), 104(1), 110(1), 112(1), 120(4), 150(2)	1(12), 1(14), 9(15)	7	10, 11, 12, <u>14, 15</u>
<i>Cyanotis</i> D. Don	World	50	34 (68.00)	27	7	20.58	2x, 4x, 6x	51	16(2), 20(3), 22(4), 24(25), 26(6), 28(1), 30(1), 34(1), 48(4), 52(1), 72(3)	3(12)	9	8, 10, 11, 12, 13, (14), (15), 17
	India	16(25)	25 (100)	20	5	20.00	2x, 6x	32	16(1), 20(2), 22(1), 24(20), 26(2), 48(3), 72(3)	2(12)	3	8, 10, 11, <u>12, 13</u>

<i>Murdannia</i> Royle	World	50	28 (56.00)	11	17	60.71	2x, 4x, 6x, 8x	47	12(1), 14(1), 18(5), 20(9), 22(1), 24(5), 36(1), 39(1), 40(12), 42(3), 44(1), 60(3), 64(2), 80(2)	4(10), I(11)	8	<u>6</u> , 7, 9, <u>10</u> , 11
	India	24	23 (95.83)	8	15	65.21	2x, 4x, 6x, 8x	40	12(1), 14(1), 18(5), 20(9), 22(1), 24(4), 36(1), 40(9), 42(1), 44(1), 60(3), 64(2), 80(2)	4(10), I(11)	6	<u>6</u> , 7, 9, <u>10</u> , 11
<i>Tradescantia</i> L.	World	+60	59 (98.33)	19	40	67.79	2x, 3x, 4x, 5x, 6x, 8x, 10x, 12x, 15x, 18x, 19x, 20x, 22x, 24x	115	12(28), 14(3), 15(1), 16(7), 18(5), 22(1), 23(1), 24(30), 26(2), 28(1), 30(2), 32(3), 36(2), 38(1), 40(1), 42(1), 48(2), 50(2), 60(2), 64(2), 67(1), 70(2), 72(3), 74(1), 76(2), 90(1), 92(1), 108(1), 109(1), 110(1), 114(1), 132(1), 140(1), 144(1)	20(6), I(7), I(8)	7	<u>6</u> , 7, 8
	India	+50	6 (12.00)	1	5	83.33	2x, 4x, 10x	7	12(1), 24(5), 60(1)	1(6)	----	6

+ World and Indian data for the genera is taken from the latest journals. The higher number of cytologically known species than the taxonomically recorded species is shown in parenthesis and the disparity might be due to the taxonomic revisions because of recognition of some hybrids, exotics, cultivars, etc. assigned the status of species.

* Compiled from Chromosome Atlas of Flowering Plants (Darlington and Wylie, 1955), Chromosome Numbers of Flowering Plants (Fedorov, 1974) and Subramaniam Vol. II. (1986), Index to Plant Chromosome Number Reports from 1968 onwards, various journals, proceedings volumes and internet. The chromosome numbers are recorded as mitotic numbers as such or converted from meiotic numbers.

** Common basic numbers are underlined and doubtful ones to be taken with caution are given in parenthesis

chromosomes as well as the third possible line with $x = 16$ observed in *Pollia*. Thus, cytological data also show the presence of 2 major groups represented by *Tradescantia* and *Commelina* types, in the Commelinaceous genera.

Various meiotic irregularities such as chromosomal stickiness, unoriented bivalents, early and late disjunction of bivalents, formation of laggards and bridges among the presently studied species at the population level indicate the existence of intraspecific genetic diversity. Such results were also recorded for different plant species by Singhal and Kumar (2008), Sheidai et al. (2003), Gupta et al. (2009), Kumar (2011), Kumar et al. (2012), etc. As a result of these meiotic abnormalities, variable sized pollen grains are formed with low pollen fertility. Similar observations were also made earlier in many angiospermic plants by various researchers (Sheidai et al., 2008; Kumar et al., 2008a, b). The formation of the heterogenous sized pollen grains may lead to the formation of unreduced $2n$ pollen grains (Sheidai et al., 2008; Fadaie et al., 2010; Guan et al., 2012).

CONCLUSION

All the species of the family Commelinaceae from Kangra Valley have been cytologically studied for the first time, which is a great contribution to the Indian chromosomal database. Among 8 species, 5 new cytotypes, which do not show any variation at the morphological level, have been recorded for the world as well as for India. The critical evaluation of the chromosomal database shows that all the studied genera of the family Commelinaceae are polybasic in nature and polyploidy is also very common at a high level in certain genera. Cytologically, many meiotic irregularities reveal the intraspecific genetic diversity. Thus, there is still need to explore this highly variable geographical area on the population basis so that genetic diversity could be used to raise new ecotypes.

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