18-33 J. Nat. Hist. Mus. Vol. 28, 2014

CHROMOSOME NUMBERS OF SOME NEPALESE FLORA

BUDDHA LAXMI VAIDYA[1] AND LAXMI MANANDHAR[1]✉
[1]Botany Instruction Committee,Tri-chandra Multiple Campus,
Tribhuvan University, Kathmandu, Nepal
cyberword427370@gmail

ABSTRACT

Previous and present chromosome counts of 10 Nepalese taxa within 7 families viz. Amaryllidaceae, Asteraceae, Caricaceae, Leguminosae, Nyctaginaceae, Passifloraceae and Scrophulariaceae are reported here. Diploid or haploid chromosome numbers of the taxa collected from the local gardens of Kathmandu are n=15 in *Agapanthus africanus* (L.) Hoffmanns (Amaryllidaceae); 2n=48+3B in *Allium tuberosum* Rottler ex Spreng. (Amaryllidaceae); 2n=18 in *Artemisia indica* Willd. (Asteraceae); 2n=27 in *Carica papaya* L. (Caricaceae); 2n=16 in *Cicer arietinum* L., 2n=14, 21 in *Pisum sativum* L., 2n=12 in *Vicia faba* L. (Leguminosae); 2n=28 in *Bougainvillea glabra* Choisy (Nyctaginaceae); 2n=18 in *Passiflora edulis* Sims. (Passifloraceae) and 2n= 34 in *Bacopa monnieri* (L.) Pennel (Scrophulariaceae) in the present research. Of these, the chromosome count of *Bougainvillea glabra* in this research is perhaps the new report. The reports of chromosome number in *Artemisia indica*, *Carica papaya* and *Bacopa monnieri* in the present investigation are confirmed to be different from the previously reported numbers for these taxa. The chromosome number of *Agapanthus africanus, Allium tuberosum, Cicer arietinum, Passiflora edulis, Pisum sativum, Vicia faba* in the present research tally with the previous reports. The present counts in *Bacopa monnieri, Carica papaya* and *Passiflora edulis* are new records for Nepal.

Keywords: Nepalese flora, genetic diversity, chromosome counts, mitosis

INTRODUCTION

Nepal occupies the central part of the highest Himalayas. Its flora is exceptionally rich. The flora has been noted as a prestigious heritage in the world. The country is known for its wide range of habitats from the plains to the mountains, with elevation varying from 125msl to more than 8839msl within as less as 4° width of latitude. The country occupies only 0.1 % of the total land of the earth, however it contains as large as over 7, 000 diverse floral vegetation within ca. 200 families (Manandhar *et al.*, 2010; 2011).

In the eastern parts of the country Sino-Japanese flora are dominant whereas in its western parts the Mediterranean elements are more dominant. The southern Terai region possesses north Indian elements, while in the northern Trans-Himalayan arid zone, the vegetation is similar to that of Tibet. The country can therefore be regarded as an area of transition or the merging point of the flora (Nepal Biodiversity Strategy, 2002). It is also noteworthy that the Himalayas and immediate adjacent areas contain 1223 plant species of which 975 (79.7 %) are endemic or limited to the adjacent areas (Ohba, 1997). This diversity in flora harbors within it a huge genetic diversity.

Kumar & Subramanian (1986) have estimated that the risk of extinction of the existing floral diversity in the near future, due to global climate change and habitat loss, is as high as 25 percent. The cytologically known flowering plants are only about 25 % of 2, 50,000 on earth and the Himalayan flora are much less investigated in the cytological field (Wakabayashi, 1988; Dhar, 2002).

The literature (Hara & Williams, 1979; Hara *et al.,* 1982; Press *et al.,* 2000; Rajbhandari, 2002-2003) indicates that the genera represented in Nepal are 5 in Amaryllidaceae, 111 in Asteraceae, 1 in Caricaceae, 80 in Leguminosae, 3 in Nyctaginaceae, 1 in Passifloraceae and 37 in Scrophulariaceae. The presently researched genera of the above mentioned families may be a valuable addition document to give recognition of the plant genetic heritage resources of the country to scientific world.

MATERIALS AND METHODS

The somatic chromosome counts in the present investigation were obtained from the root tips (mitosis). The haploid count was done from the microsporogenesis in flower buds (meiosis). The mitotic studies were made from fixed excised healthy root tip cells. To ensure full turgidity, plants were sufficiently watered two hours before the excision of the root tips for pretreatment. The root tips were taken in between 9. 00 AM and 11. 00 AM. The root tips were cleaned with the help of a fine camel hair brush before pretreatment. The materials were pretreated in aqueous solution of super saturated solution of para-dichlorobenzine for 3 hrs at room temperature before fixing them. The fixative used for roots as well as floral buds was acetic alcohol (glacial acetic acid and ethyl alcohol in 1:3 ratios). The root tip cells were made soft by treating root tips with 1N HCl for about 3 hours (Cota & Philbrick, 1994).

The terminology of Sakya (1999) was used for chromosome size: small < 1 μ m., medium 1 to < 2.5 μ m. and large above 2.5 μ m.

The meiotic behaviors of pollen mother cells were observed from appropriate anthers of fixed and preserved flower buds. The desired stages of both mitosis and meiosis were photographed under the microscope with 1000 magnifications.

At least five slides were observed to confirm the results of both mitosis and meiosis. Best slides were made permanent by using acetic acid n-butyl alcohol series of three grades viz. the frist grade was of acetic acid and n- butyl alcohol solution in 1: 1 ratio, in the second grade acetic acid was 1 and n- butyl alcohol was 3 in ratio and the third grade was of absolute n- butyl alcohol (Celarier, 1956).

RESULTS

TABLE 1. List of voucher number (V. N.) of the presently studied taxa. Place of collection and chromosome number.

VN	Taxa	Place of collection (msl)	Chromosome number
54	*Agapanthus africanus* (L.) Hoffmanns	Kuleswor, 1250	n=15
53	*Allium tuberosum* Rottler ex Spreng.	Kuleswor, 1250	2n=48+3B
112	*Artemisia indica* Willd.	Swontha, Lalitpur, 1250	2n=18
302	*Bacopa monnieri* (L.) Pennel	Kuleswor, 1250	2n=34
303	*Bougainvillea glabra* Choisy	Kuleswor, 1250	2n=28
304	*Carica papaya* L.	Kuleswor, 1250	2n=27
120	*Cicer arietinum* L.	Lalitpur, 1250	2n=16
305	*Passiflora edulis* Sims	Lalitpur, 1250	2n=18
121	*Pisum sativum* L.	Lalitpur, 1250	2n=14, 21
122	*Vicia faba L.*	Kuleswor, 1250	2n=12

Countable metaphase photographs of the presently researched taxa are given in fig. 1-12. The reports of present and previous counts for the presently studied taxa are in table 2.

Amaryllidaceae

Agapanthus africanus (L.) Hoffmanns. . (V. N. 54), n=15

Both rod and ring bivalents are seen during diakinesis (fig. 1 & 2). Different phases in meiotic divisions have revealed both normal and irregular stages. Irregularities like chromatin bridges at anaphase I, unequal distribution of chromosomes at telophase I, telophase II and cytomixis between cells were evidenced occasionally. Pentad were noted occasionally.

Allium tuberosum Rottler ex Spreng. (V. N. 53), 2n=48+3B

Mitotic divisions encountered 48 symmetrical as well as asymmetrical graded types of chromosomes (fig. 3). All the chromosomes with centromere at median, sub-median and sub-terminal regions are large sized. Individuals with a few Bs are evidenced frequently. Abnormal separations of chromosomes are evidenced during telophase in some cases.

Asteraceae

Artemisia indica Willd. (V. N. 112), 2n=18

Mitotic divisions comprise 18 chromosomes with centromeres at median and sub-median regions (fig. 4). All the chromosomes are large sized.

Caricaceae

Carica papaya L. (V. N. 304), 2n=27

Mitotic division evidenced 27 chromosomes mostly having centromere at median and sub-median regions (fig. 7), but a few of them were sub-terminal ones. Most of the chromosomes were large sized.

Leguminosae

Cicer arietinum L. (V. N. 120), 2n=16

Mitotic divisions revealed 16 chromosomes mostly with centromeres at median and sub-median regions (fig. 8). All the chromosomes were large sized.

Pisum sativum L. (V. N. 121), 2n=14, 21

Fourteen chromosomes having centromere at median and sub-median encountered frequently (fig. 10), but triploid individuals with graded chromosomes having centromere at median, sub-median and sub-terminal regions were also observed occasionally (fig. 11).

Vicia faba L. (V. N. 122), 2n=12

Twelve large and graded chromosomes with centromere at median, sub-median and sub-terminal regions were evidenced during countable metaphase. A few B-chromosomes were encountered occasionally (fig. 12).

Nyctaginaceae

Bougainvillea glabra Choisy (V. N. 303), 2n=28

All 28 chromosomes revealed centromere at median and sub-median regions. The chromosomes were all large sized (fig. 6).

Passifloraceae

Passiflora edulis Sims (V. N.305), 2n=18

Mitotic metaphase encountered 18 chromosomes. All the chromosomes were with centromere at median and sub-median regions (fig. 9).

Scrophulariaceae

Bacopa monnieri (L.) Pennel (V. N. 302), 2n=34

Mitotic division encountered 34 chromosomes with centromere at median and sub-median regions (fig. 5). The chromosomes were small, medium as well as large sized. The chromosomes were of graded types with centromere at median, sub-median and sub-terminal regions.

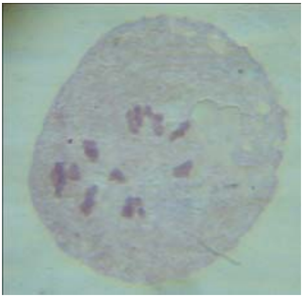

FIG. 1.

FIG. 2.

FIG. 3.

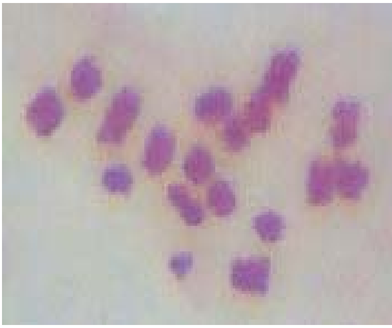

FIG. 4.

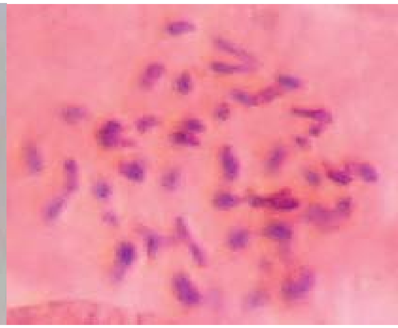

FIG. 5.

FIG. 6.

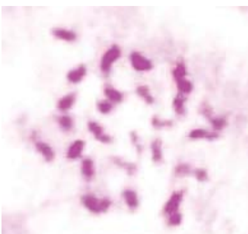

FIG. 7.

FIG. 8.

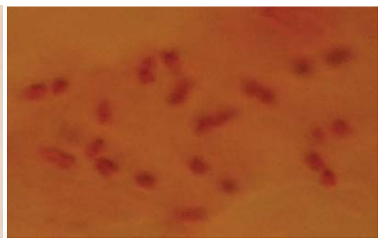

FIG. 9.

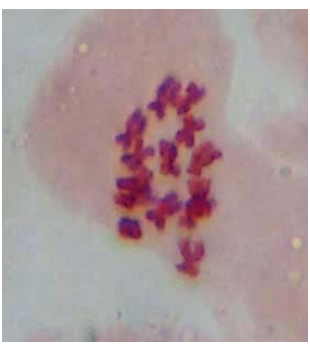

FIG. 10

FIG. 11

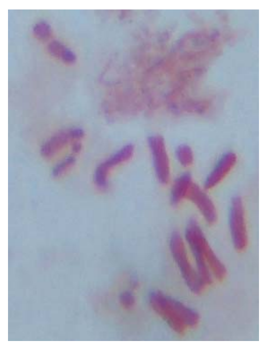

FIG.12

Legends for Figures

FIG. 1. *Agapanthus africanus* (L.) Hoffmanns. n=15. Fig. 2. *Agapanthus africanus* (L.) Hoffmanns. (meiotic bivalents in a row) Fig. 3. *Allium tuberosum* Rottler ex Spreng. 2n=48+3B. Fig. 4. *Artemisia indica* Willd. 2n=18. Fig. 5. *Bacopa monnieri* (L.) Pennel 2n=34. Fig. 6. *Bougainvillea glabra* Choisy 2n=28. Fig. 7. *Carica papaya* L. 2n=27. Fig. 8. *Cicer arietinum* L. 2n=16. Fig. 9. *Passiflora edulis* Sims 2n=18. Fig. 10. *Pisum sativum* L. 2n=14. Fig. 11. *Pisum sativum* L. 2n=21. Fig.12. *Vicia faba* L. 2n=12.

DISCUSSION

Agapanthus africanus has been reported with chromosome number 2n=30 (Prajapati, 2000; Sakya *et al.,* 2001). This taxa is with n=15 in the present investigation. It can be suggested that the taxa maybe having basic number x=15.

Different species of *Allium* have been reported with the haploid number n=8 (c. 82 % Federov, 1969). It can be suggested that this genus is with the basic number x=8 and may be unibasic (Manandhar *et al.*, 2011). *A. tuberosum* is reported with 2n=32, 48+3B (Banerjee, 1980; Xu *et al.,* 1985; Nanuscyan & Polyakov, 1989; Li, 1989; Ohi, 1990; Shang *et al.,* 1997; Zhang, 1998; Yan *et al.,*1999; Talukder & Sen, 2000, Ohri & Pistrick, 2001; Manandhar *et al.*, 2011). Previous reports support that *A. tuberosum* is a tetraploid one with the basic number x=8 in the taxa. However *A. tuberosum* has also been reported with the irregular numbers viz. n=8IV - 32I, 2n=9, 11, 24, 32, 31-33, n=8-10, 8-14, 31 (irr), 32 (irr)etc 2n=31, 32, 33, 62 (Seo, 1977; Gohil & Koul, 1983; Roy, 1980), n=32, 2n=64 Kojima *et al.* (1991). 2n=24 Huang *et al.* (1985). This shows that polyploids and aneuploids have been occurring frequently in this taxa. It may be suggested that 2n=32 and 2n= 64 are tetraploid and hexaploid individuals respectfully, where as 2n=24 should be triploid. In the present report 2n=48 may be due to duplication of chromosomes in triploid individuals.

According to Torrel *et al.* (2001) the basic number for the genus *Artemisia* are x=8 or 9 and plolyploidy has played significant role in the genus during evolution. Chromosome numbers for *Artemisia indica* are 2n=34 (Joshi & Joshi, 2001) and 2n=32 (Manandhar *et al.*, 2011; Karna Mallick *et al.*, 2011) in the previous reports. Present research with 2n=18 suggests that the basic number for this taxa may be n=9. The previously reported individuals may be due to the loss of a pair or two pairs of chromosomes in tetraploid individuals with basic number x=9 or may be due to the duplication of a pair of chromosome (2n-34) with the basic number x=8.

Joshi & Ranjekar (1982) and Chen (1993) have reported 2n=18 in *Carica papaya* whereas Fernández Casas (1981) has confirmed n=18 in this taxa. It indicates that haploid number n=18 in this taxa may be of tetraploid individual. The previous report 2n=18 should be a diploid one. The presently counted number 2n=27 maybe of triploid one.

Perusal of literature (Bairiganjan & Patnaik, 1989; Yan *et al.*, 1989; Mannan *et al.,* 1991; Venora *et al.,* 1995; Nazarova, 1997; Kabir & Singh, 1991; Ahmad, 1993; Jahan *et al.,* 1994; Ahmad & Chen, 2000; Manandhar, 2012) has suggested that *Cicer arietinum* is with the basic number x=8. The present research with 2n=16 also suggests that this taxa is unibasic.

Several authors (Marks & Davies, 1979; Mercy Kutty & Kumar, 1983; Bairiganjan & Patnaik, 1989) have reported n=7, 2n=14 in *Pisum sativum*. Present report 2n=14, 21 confirms that the taxa contains both diploid and triploid individuals.

Present report for *Vicia faba* 2n=12 tallies with the perusal of literature (Langer & Koul, 1982; Rost, 1982; Tanaka & Ohta, 1982; Anis *et al.,* 1998 ; Zhang, 1998; Kamel, 1999; Koul *et al.,* 1999).The reports n=6 by Kesavacharyulu *et al.* (1982) and Jahan *et al.* (1994) have indicated that this taxa is with the basic number x=6. The irregular number n=4, 5, 6, 7 reported by Wang & Zheng (1985) maybe due to disploidy.

Bougainvillea glabra reported here is with 2n=28. This is perhaps the first report for this taxa.

Passiflora edulis has been reported with 2n=18 (Guerra, 1986). There is no haploid number report for this taxa. Present report 2n=18 maybe a diploid individual that tallies with the earlier report.

There is no haploid number report for the taxa *Bacopa monnieri.* This taxa has been reported with 2n=68 (Chandran & Bhavanandan, 1981) previously. Present report 2n=34 suggests that basic number for the taxa may be x=17. The previously reported individual of this taxa may be a tetraploid one.

With the exception of the genus *Artemisia,* all the presently studied taxa may be of unibasic nature. It is noteworthy that all the investigated genera, in this research, are with some kind of polyploids. Polyploidy is considered to be one of the characteristics of advancement in the process of evolution and such cases lead to speciation.

TABLE 2. Present and previous chromosome counts for the presently studied taxa.

Taxa	**Chromosome count**	**Author and year**	**Distribution (msl)**
Agapanthus africanus (L.) Hoffmanns.	2n=30	Prajapati (2000)	Botany garden, Kirtipur, 1300
A. africanus (L.) Hoffmanns.	2n=30	Sakya *et al*. (2001)	Botany garden, Kirtipur, 1300
A. africanus (L.) Hoffmanns.	n=15	**Present report**	Kuleswor, 1250
Allium tuberosum Rottler ex Spreng.	2n=32, 48+3B	Banerjee (1980), Xu *et al.* (1985), Nanuscyan & Polyakov (1989) Li (1989), Ohi (1990), Shang *et al.* (1997), Zhang (1998), Yan *et al.* (1999), Talukder & Sen (2000), Ohri & Pistrick (2001)	Cultivated, 1200
A. tuberosum Rottler ex Spreng.	n=32II, 2n=64	Kojima *et al.* (1991)	Cultivated, 1200
A. tuberosum Rottler ex Spreng.	2n=24	Huang *et al.* (1985)	Cultivated, 1200
A. tuberosum Rottler ex Spreng.	n=8, 2n= 32	Li *et al.* (1985)	Cultivated, 1200

A. tuberosum Rottler ex Spreng.	n=4IV+8I, 2n= 32	Rao *et al.* (1992)	Cultivated, 1200
A. tuberosum Rottler ex Spreng.	n=8-10,8-14, 31(irr), 32(irr) Etc, 2n=31,32, 33,62	Seo (1977)	Cultivated, 1200
A. tuberosum Rottler ex Spreng.	n=8IV - 32I, 2n=9,11,24,321983;	Roy (1980)	Cultivated, 1200
A. tuberosum Rottler ex Spreng.	n=8-10, 8-14, 31, 32 (irr) etc 2n=31,32, 33, 62	Gohil & Koul (1983)	Cultivated, 1200
A. tuberosum Rottler ex Spreng.	n=16, 2n=32	Zou & Jia (1985)	Cultivated, 1200
A. tuberosum Rottler ex Spreng.	2n=16, 32	Yang *et al.* (1998)	Cultivated, 1200
A. tuberosum Rottler ex Spreng.	2n=31-33	Mehra & Pandita (1979), Pandita (1981)	Cultivated, 1200
A. tuberosum Rottler ex Spreng.	2n=21-32	Gohil & Koul (1978)	Cultivated, 1200
A. tuberosum Rottler ex Spreng.	n=8, 2n=16	Pradhan (1980)	Kuleswor, 1250
A. tuberosum Rottler ex Spreng.	2n=32	Saiju (1982), Adhikari (1998), Manandhar *et al.* (2011)	Kuleswor, 1250
A. tuberosum Rottler ex Spreng.	2n=48+3B	**Present report**	Kuleswor, 1250
Artemisia indica Willd.	2n=34	Joshi & Joshi (2001)	CE 300-2400
A. indica Willd.	2n=32	Manandhar *et al.* (2011)	CE 300-2400
A. indica Willd.	2n=18	**Present report**	Lalitpur, 1250
Bacopa monnieri (L.) Pennel	2n=68	Chandran & Bhavanandan (1981)	WC, 700-900
B. monnieri (L.) Pennel	2n=34	**Present report**	Kuleswor, 1250

Bougainvillea glabra Choisy	2n=28	**Present report**	Lalitpur, 1250
Carica papaya L.	2n=18	Joshi & Ranjekar (1982), Chen (1993)	C**.** 500
C. papaya L.	n=18	Fernández Casas (1981)	C. 500
C. papaya L.	2n=27	**Present report**	Kuleswor, 1250
Cicer arietinum L.	n=8	Kumar (1976)	WCE, 150-1300. Botanical Garden (CDB), Kirtipur, 1300
C. arietinum L.	2n=16	Fukuda (1984)	Kathmandu market, 1250
C. arietinum L.	n=8, 2n=16	Sarbhoy & Sinha (1978)	WCE, 150-1300
C. arietinum L.	2n=16	Phadnis (1971), Farook & Nizam (1979), Astanova (1981), Lavania & Lavania (1982, 1983), Sharma & Gupta (1982), Kutarekar & Wanjari (1983), Yeh *et al.* (1986), Mukhopadhyay (1986), Bairiganjan & Patnaik (1989),Yan *et al.*(1989), Mannan *et al.* (1991), Venora *et al.* (1995), Nazarova (1997)	WCE, 150-1300
C. arietinum L.	n=8	Kabir & Singh (1991), Ahmad (1993), Jahan *et al.* (1994), Ahmad & Chen (2000)	WCE, 150-1300
C. arietinum L	2n=16	**Present report**	Lalitpur, 1250

Passiflora edulis Sims	2n=18	Guerra (1986)	E, 1300-1700
P. edulis Sims	2n=18	**Present report**	Lalitpur, 1250
Pisum sativum L.	n=7, 2n=14	Kumar (1976)	WCE, 1200-4000 Botanical Garden (CDB), Kirtipur, 1300
P. sativum L.	2n=14	Shrestha (1979)	Kirtipur, 1300
P. sativum L.	2n=14	Lavania. & Lavania (1982, 1983), Rost (1982), Sharma & Gupta (1982), Kodama & Mitchell (1982), Li & Du (1984), Therman & Murashige (1984), Zhang (1986) Yeh *et al.* (1986), Mukhopadhyay (1986), Li (1989), Kodama (1989), Kar & Sen (1991), Mannan *et al.* (1991), Koul & Nirmala (1993), Nirmala & Kaul (1993), Zhang *et al.* (1993), Baranyi & Greilhuber (1995), Zhang (1998).	WCE, 1200-4000
P. sativum L.	2n=14, 21	**Present report**	Lalitpur, 1250
Vicia faba L.	n=6, 2n=12	Kumar (1976)	Godawari, Lalitpur 1360
V. faba L.	n=6, 7	Malakar (1978)	Botanical Garden (CDB), Kirtipur, 1300
V. faba L.	2n=12	Fukuda (1984)	Kathmandu, 1250
V. faba L.	n=6, 2n=12	Bairiganjan & Patnaik (1989).	
V. faba L.	n=6	Kesavacharyulu *et al.* (1982), Jahan *et al.* (1994).	

V. faba L.	n=4, 5, 6, 7	Wang & Zheng (1985).	
V. faba L.	2n=12	Langer & Koul (1982), Rost (1982), Tanaka & Ohta (1982), Zhang *et al.* (1982), Langer & Koul (1984), Zhang (1986), Yuan (1986), Yeh *et al.* (1986), Sato (1988), Li (1989), Chen (1989), Rizzoni *et al.* (1989), Matsuda & Muramatsu (1989), Schubert & Rieger (1990), Hizume (1992, 1993), Unnikrishna Pillai & Verma (1992), Schifino-Wittmann *et al.* (1994), Bisht *et al.* (1998), Anis *et al.* (1998), Zhang (1998), Kamel (1999), Koul *et al.* (1999).	
V. faba L.	2n=12	**Present report**	Kuleswor, 1250

ACKNOWLEDGMENTS

This research article is the continuation of the previous project works by the second author that was sponsored by the University Grants Commission of Nepal. Present chromosome counts for the taxa by the authors were done in the Central Department of Botany, Kirtipur and in the Botany Department, Trichandra Multiple Campus, Kathmandu.

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