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STUDIES OF MEIOTIC BEHAVIORS IN SOME TAXA OF THE NEPALESE PAPILIONACEAE

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ABSTRACT

This paper reports studies of meiotic behaviors of seventeen species, sub-species and varieties in seven genera of the Nepalese Papilionaceae and new chromosome numbers for three species (*Crotalaria cytisoides* Roxb. ex DC (n=8), *Parochetus communis* Buch-Ham. ex D. Don (n=8) and *Piptanthus nepalensis* (Hook.) D. Don. (n=9, 18) are new number reports. Meiotic behaviors are often normal in all the species). However, a few aberrations like univalents, chromosome bridges, laggards, precocious chromosomes and/ or cytomixis have been observed in most of the taxa. Pollen fertility is high (more than 80%) indicating that the abnormalities had not reached mature stages.

Key words: haploid chromosome count, metaphase I, meiosis

INTRODUCTION

Papilionaceae consisting of 455 genera and 12,615 species (Brummit 1992, Judd *et al.* 1999) are cosmopoliton in distribution. About 80 genera and c. 290 species are reported from different regions of Nepal (Rajbhandari 2002-2003, Press *et. al.* 2000, Hara and Williams 1979, Ohashi 1975). Fourteen species of them are endemic to Nepal (Shrestha and Joshi 1996). These taxa are of great economic values because their roots exhibit peculiar tubercles containing bacterial organisms (*Rhizobium species*), are able to take up a lot of atmospheric nitrogen to enrich the soil. The plants have been used as fodder in the hilly regions of Nepal. They are good soil binders. They add beauty to the forests during their blooming periods. Taxa included in the present investigation are significant also for their uses in traditional medicines like tonic and to cure various diseases like coughs and convulsions etc. Some of them are used as forage, fibre and ornamental plants (Kirtikar and Basu 1935, Chopra *et al.* 1956, Tanaka 1976, Nair and Mohanan 1998).

MATERIALS AND METHODS

Plants were collected from different regions of the country and living plants with collection numbers (Coll. N.) were established in home garden for investigation. Flower buds from the plants of the pots were fixed in 1:3 acetic alcohol for 24 c. Proper anthers were chosen and squashed in 2% aceto-carmine. Cytological studies of the pollen mother cells of the taxa were done with the help of compound microscope. The meiotic behaviors were recorded and photographs of appropriate stages are used as illustrations. Pollen fertility was estimated by staining pollen grains in the Muntzing solution i.e. 1:1 acetocarmine and glycerine (Muntzing 1941). All the photomicrographs were taken under the uniform magnification of 1000x oil immersion.

RESULTS AND DISCUSSION

Alysicarpus rugosus (Willd.) DC. (Coll. N. 137)

Locality: Kirtipur road side (central Nepal) 1300 msl

Meiotic behaviour was mostly regular at diakinesis, metaphase I, anaphase I and telophase I. Eight bivalents at diakinesis were (Figure 1) rings and rods. Figure 2 shows dimorphic pollens. Pollen stainability was found to be 98.8%.

The chromosome number n=8 counted during the investigation for *Alysicarpus rugosus* tallies with the reports 2n=16 by Rao (1954), John *et al.* (1985), Peng and Chaw (1986), Kumari and Bir (1990), Sinha and Chandra (1991) and n=8 by Jahan *et al.* (1994). This species seems to be a diploid with the basic number x=8.

***Alysicarpus vaginalis* (L.) DC. (Coll. N. 138)**

Locality: Biratnagar (East Nepal) 72 msl

The haploid number in this taxa is n=16. Meiotic divisions were observed regular at various stages. Figure 3 is anaphase II with precocious movement of some chromosomes. Non-organised groupings and scattered chromosomes were observed at telophase II occasionally. Cytomixis has been recorded at different stages. Pollen stainability was found to be 99.7%. *A. vaginalis* was found to be with the haploid number n=16 during the present investigation. This haploid number n=16 is a new report for the species because it differs from the previous reports 2n=16 by Rao (1954), Frahm-Leliveld (1957), Bhatt (1974), Sanjappa and Bhatt (1976, 1978), John *et al.* (1985), Yeh *et al.* (1986), Sinha and Chandra (1991), 2n=20 by Gadella and Kliphius (1964), 2n=14 by Kapali and Patil (1987), n=8 by Bir and Sidhu (1966, 1967), Mitra and Datta (1967), Sareen and Singh (1976), Sanjappa and Bhatt (1978). The present species from Nepal seems to be a tetraploid one.

***Astragallus chlorostachys* Lindl. (Coll. N.133)**

Locality: Tadapani, Banthanti (West Nepal) 2650 msl growing on the moist place on the hill near road side

Chromosomes at metaphase I revealed 8 unequel sized bivalents. Figure 4 has evidenced sticky chromosomes at metaphase I. A few abnormalities like formation of univalents and multivalents, chromatin stickiness and agglutination, multipolar movements of chromosomes and cytomictic cells were developed in some cells. Pollen stainability was found to be 99.5%.

***Astragalis stipulatus* var. *phulchokiensis* H. Ohashi (Coll. N. 134)**

Locality: Phulchoki (C. Nepal) 2200 msl

At diakinesis chromosomes revealed 12 unequal sized bivalents (Figure 5). The irregularities during the divisions contained multivalents, stickiness, agglutination, non-synchronous movements. Pollen stainability was found to be 99.1%.

The haploid number of the genus *Astragalus* 6, 7, 8, 9, 11, 13, 15, 20 23 or polyploidy forms of these numbers (Chinnappa and Chmielewski 1987, Spellenberg and Rodrigues 1992, Pavlova 1995, Badr *et al.* 1996, Manandhar and Sakya 2004). The numbers found may be due to the development of hypoploids or hyperploids with basic number x=8 or of secondary origin. The haploid number n=8 of *A. chlorostachys* confirms the previous reports 2n=16 (Ledingham 1960, Ledingham and Rever 1963, Manandhar and Sakya 2004) and n= 8 (Asharf and Gohil 1989,

Kumari *et al.* 1989, Manandhar and Sakya 2004). This species is a diploid one with basic number x=8. The number n=12 of *A. stipulatus* var. *phulchokiensis* is different from previously reported number 2n=64 of *A. stipulatus* (Ledingham 1960). The number n=12 and 2n=24 is reported in this variety by Manandhar and Sakya (2004). The presently collected variety *phulchokiensis* of *A. stipulatus* is with the basic number 6. This suggests that both structural alteration as well as numerical mutations might have been occurred during the course of evolution in this genus.

***Crotalaria cytisoides* Roxb. ex DC. (Coll. N. 122)**

Locality: Nagarjun hill (Central Nepal) 1520 msl

Meiosis was regular. In metaphase I, bivalents and tetravalents with 1 to 3 B chromosomes was observed (Figure 6). Diads and pentads were also recorded. Pollen stainability was found to be 99.6%.

***Crotalaria pallida* Aiton (Coll. N. 147)**

Locality: Central Department of Botany garden, Kirtipur (Central Nepal) 1310 msl

Meiosis evidenced 8 unequal sized bivalents with one or two Bs. Bivalents at diakinesis were rings and rods. Figure 7 is metaphase II with 8 chromosomes at each pole. Some irregularities like stickiness, spindle fibres and chromatin bridges were prominent at different stages. Pollen stainability was found to be 99.8 %.

***Crotalaria sessiliflora* L. (Coll. N. 124)**

Locality:Bajrajogini area (C. Nepal) 1520 msl

Eight bivalents, in two chains with rods and one separate ring chromosome, are observed in Figure 8. Meiosis is fairly normal at different stages. Pollen stainability was found to be 99.8%.

The haploid number n=8 reported here in *Crotalaria cytisoides* tallies with the previous reports 2n=16 (Manandhar and Sakya 2003a, 2003b). The number n=8 reported presently in *C. pallida* confirms the previous reports n=8 by Bajracharya and Joshi (1980), Sarkar *et al.* (1982), Bairiganjan and Patnaik (1989) and 2n=16 by Bajracharya and Joshi (1979), Raina and Verma (1979), Kumari and Bir (1990), Oliviera and Aguiar-Percin (1989), Mangotra and Koul (1991a). The number n=8 *C. sessilflora* confirms 2n=16 by Kumari and Bir (1990), Gu and Sun (1996). It reveals that *Crotalaria* is a monobasic genus with x=8, structural changes might have played important role among the species of this genus during the course of evolution. However Mangottra and Koul (1991b) have encountered polyploidy in several species of the genus *Crotalaria* indicating numerical change in chromosomes also have some role in evolution.

***Desmodium concinnum* var*. concinnum* DC. (Coll. N. 109)**

Locality: Palung (Central Nepal) 2150 msl

Eleven bivalents were observed at diakinesis and metaphase I (Figure 9). Sometimes tetravalents were also observed. Chromosomes were towards peripheral regions. Second meiotic divisions were almost normal with some degree of stickiness. Figure 10 shows cytomixis of two cells at metaphase I. Normal decussate tetrads were observed. Pollen stainability was found to be 97.0%.

***Desmodium elegans* DC. ssp. *elegans* var. *elegans* (Coll. N. 111)**

Locality: Shivapuri hill (C. Nepal) 2000 msl

It showed 11 bivalents at diakinesis as rings and rods (Figure 11). Some mulivalents were frequently observed at metaphase I. The second meiotic divisions evidenced certain irregularities like exclusion of one or more chromosomes at metaphase II. Stickiness and chromatin bridges had been observed during anaphase II (Figure 12). Multicellular polyads were also seen. Pollen stainability was found to be 98.9 %.

***Desmodium laxiflorum* DC. (Coll. N.103)**

Locality: Nagarjun forest (Central Nepal) 1500 msl

Eleven bivalents were observed at diakinesis (Figure13). Rings and rods with few tetravalents were seen frequently. Exclusion and shifting of a group of chromosomes towards periphery had been observed. Sticky chromosomes, non-synchronous divisions, agglutination had been noticed. Pentads and polyads with 8 to 12 celled structures were also recorded. Pollen stainability was found to be 98.7 %.

***Desmodium multiflorum* DC. (Coll. N.110)**

Locality: Nagarjun forest (Central Nepal) 1510 msl

In metaphase I, chromosomes with both 11 and 22 bivalents were observed in different individuals of this species. Figure 14 is metaphase I of tetraploid species with 22 bivalents. Tetraploid plants were robust with with larger vegetative and floral parts compared to those of diploid ones. Both I and II meiotic divisions were normal with occasional occurrence of movement of chromosome towards periphery and cytomixis. Pollen stainability was found to be 95.5 %.

***Desmodium podocarpum* ssp *oxyphyllum* DC. var. *oxyphyllum* (Coll. N. 114)**

Locality: Phulchoki (C. Nepal) 2200 msl.

Eleven bivalents had been recorded at diakinesis (Figure 15) in rings and rods. Meiosis in different stages were recorded fairly normal. Pollen stainability was found to be 99.5 %

***Desmodium podocarpum* DC. ssp. *podocarpum* (Coll. N. 108)**

Locality: Namobuddha (Central Nepal) 1780 msl.

Ring and rod bivalents at diakinesis are common. Figure 16 shows sticky chromosomes at metaphase II. In most of the miocytes divisions were normal. Pollen stainability was found to be 99.1 %.

***Desmodium triflorum* (L.) DC. (Coll. N. 106)**

Locality: Dhulikhel (C. Nepal) 1420 msl.

At diakinesis 11 bivalent had been recorded with one or two bivalents attached to the nucleolus Stickiness of bivalents have evidenced to form rings (Figure 17). Occasional irregularities during the divisions had evidenced multipolar movement of chromosomes at metaphase II. Pollen stainability was found to be 95.8 %.

The chromosome number n=11 is confirmed in the present investigation for the genus *Desmodium*. This report tallies with the previous reports n=11 or/and 2n=22 by several authors (Ranjit 1978, Sanjappa and Bhatt 1985, Yeh *et al.* 1986, Bairiganjan and Patnaik 1989, Kumari and Bir 1990, Kumar and Kuriachan 1990, Jahan *et al.* 1994, Gao and Zou 1995, Manandhar and Sakya 2003a, 2003b). However, n=22, 2n=44 have been reported by Manandhar and Sakya (2003a) in *D. multiflorum* seems to be the tetraploid one.

***Parochetus communis* Buch.-Ham. ex D. Don (Coll. N. 132)**

Locality: Kharidhunga (C. Nepal) 2400 msl

Haploid chromosome number in this taxa is n= 8. Ring as well as rod tetravalents and bivalents were recorded at diakinesis (Figure 18). Meiosis was found to be fairly normal. Figure 19 was normal anaphase II. There were evidences of sticky chromosomes occasionally. Pollen stainability was found to be 95.6 %.

The present haploid number report n=8 in *Parochetus communis* tallies with the reports n=8 by Malakar (1978) and 2n=16 by Berger *et al.* (1958), Dhakhwa (1979 and Chatterjee *et al.* (1989). It could be that the taxa investigated here is a diploid one with basic number x=8.

***Piptanthus nepalensis* (Hook.) D. Don. (Coll. N.136)**

Locality: Kalinchok (C. Nepal) 3300 msl

Both n=9 and n=18 were observed in different individuals of this taxa. Meiosis evidenced certain irregularities. Stickiness is noted at diakinesis and metaphase I. Rod bivalents are more common. Non-oriented and lagging chromosomes are observed at different stages. Figure 20 shows diakinesis in a tetraploid cell. Cytomictic cells were also observed (Figure 21). Pollens were dimorphic. Pollen stainability was found to be 80.8 %. Haploid numbers n=9, 18 are new reports for *Piptanthus nepalensis.*

Piptanthus nepalensis reported with n=9 presently confirms the earlier reports n=8 by Malakar (1978) and 2n=18 by Berger *et al.* (1958) and Manandhar and Sakya (2003b). It could be that the taxa, reported in this investigation, is a diploid one.

***Smithia ciliata* Royle (Coll. N. 136)**

Locality:Panighat, 1700 msl.

Nineteen bivalents, all rod-like, have been observed at metaphase I (Figure 22). Sometimes disjointed bivalents are noted. In most of the cases, meiosis is quite normal. Pollen stainability was found to be 95.2 %.

Smithia ciliata with n=19 have been reported here that tallies with the previous report n=9, 2n=38 in this taxa (Tuladhar 1978, Manandhar and Sakya 2009). The haploid number n=19 in this species is a new chromosome number addition to the world flora. The chromosome counts 2n=38 in *S. bigemina* and *S. conferta* by Kumari and Bir (1990), Kumar and Kuriachan (1990) and haploid number n=19 in *S. sensitiva* by Mitra and Datta (1967) and in *S. racemosa* by Kumar and Kuriachan (1990) suggest that *Smithia* is a monobasic genus with the basic number n=19. It may be due to a genotypically controlled stability of the chromosome number in the genus *Smithia.*

The perusal of literature (Darlington and Janaki Amal 1945, Darlington and Wylie 1955, Fedorov 1969, Kumar and Subramanian 1986, Mangotra and Koul 1991a, Chen *et al*. 1993, Chen *et al*. 2003) and also the above findings, suggest that the deeply sealed basic number is, n= 8 in *Alysicarpus, Astragalus, Crotalaria* and *Parochetus,* n=11 in *Desmodium, n*=9 in *Piptanthus* and n=19 in *Smithia.*

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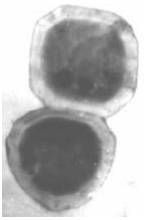
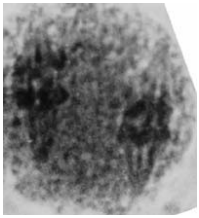
Legends for Figures:

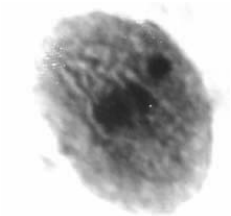
1. Alysicarpus rugosus (Metaphase I with unequal bivalents)



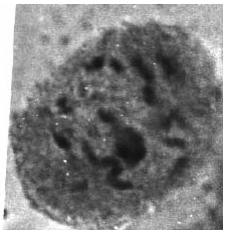
2. *Alysicarpus rugosus* (Tri and tetracolpate pollens)



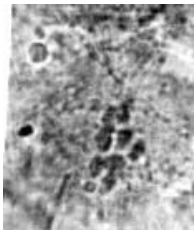
3. *Alysicarpus vaginalis* (Anaphase II with prcociouc movement of some chromosomes)



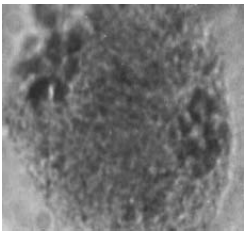
4. *Astragalus chlorostachys* (Sticky Metaphase I)



5. *Astragalus stipulatus* var. *phulchokiensis* (Diakinesis)

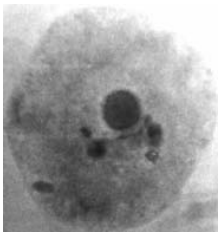


6. *Crotalaria cytisoides* (Metaphase I with bivalents and tetravalents)

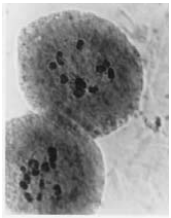


7. *Crotalaria pallida* (Sticky Metaphase II)

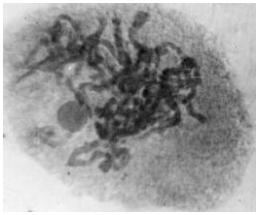
8. *Crotalaria sessiliflora* (Metaphase I with bivalents and tetravalents)



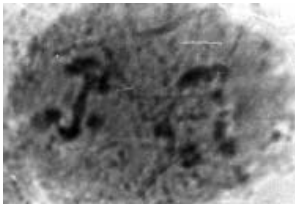
9. *Desmodium concinnum* var. *concinnum* (Diakinesis)



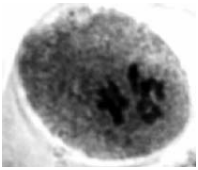
10. *Desmodium concinnum* var. *concinnum* (Metaphase I with cytomixis)



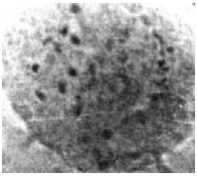
11. *Desmodium elegans* ssp. *elegans* var. *elegans* (Diakinesis)



12. *Desmodium elegans* ssp. *elegans* var. *elegans* (Anaphase II with bridge formation)

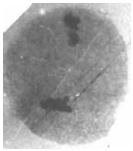


13. *Desmodium laxiflorum* (Metaphase II with sticky chromosomes)

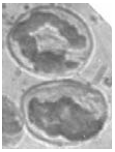


14. *Desmodium multiflorum* (Metaphase I-tetraploid)

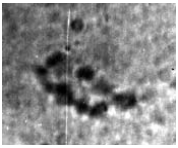
15. *Desmodium podocarpum* ssp. *oxyphyllum* var. *oxyphyllum* (Diakinesis)



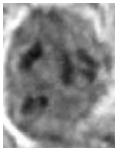
16. *Desmodium podocarpum* ssp. *podocarpum* (Sticky Metaphase II)



17. *Desmodium triflorum* (Sticky Metaphase I cells with chromosomes in chain)



18. *Parochetus communis* (8 Bivalents in a chain)



19. *Parochetus communis* (Anaphase II)

20. *Piptanthus nepalensis* (Metaphase I-tetraploid cell)

21. *Piptanthus nepalensis* (Anaphase I with cytomixis)

22. *Smithia ciliat*a (Bi and multivalents)

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