Further Studies on the Cytology of Central and East Himalayan Grasses

P. N. Mehra and M. L. Sharma

Department of Botany, Panjab University, Chandigarh, India

Received February 21, 1976

This is in continuation of our previous work on the cytology of Central and East Himalayan grasses, under which data on the cytology of 173 taxa belonging to 141 species of grasses have already been presented ((Mehra and Sharma 1975 a-f). In the present paper, the results of cytological studies in 20 more taxa belonging to 13 species from the hills of Darjeeling (E. Himalayas) are being reported.

Material and methods

The material for meiotic studies was procured from natural populations growing in and around Darjeeling. The methods employed for meiotic studies were the same as discussed in our earlier papers (see Mehra and Sharma 1975). For somatic chromosome studies of bamboos, the rhizomes were wrapped in wet moss material and placed in polythene bags for 7-8 days for obtaining actively growing root-tips. These were pretreated with 0.004 M, 8-hydroxyquinoline for 4-5 hours at room temperature (15°C-20°C), fixed in 1:3 acetic-alcohol for about 1 hour and kept overnight in 2% aceto-lacmoid. The hydrolysis was carried out in N. HCl for 10-15 minutes and the squashing accomplished in 2% aceto-lacmoid. The slides were passed through acetic acid-n-butyl alcohol grades and mounted in Euparal. The voucher specimens have been deposited in the Herbarium of the Botany Department, Panjab University Chandigarh. Pollen fertility refers to morphologically normal pollen which stained with carmine. All the photomicrographs are at a uniform magnification of $\times 1500$.

Results and discussion

Table 1 summarises the results of cytological observations on 20 taxa belonging to 13 species. In the succeeding pages, only the interesting features in some of the species are given.

Agrostis stolonifera Linn.

This grass forms an important part of the alpine pastures in the hills of India and is a welcome fodder.

The populations growing on the border of Senchal Lake in Darjeeling revealed a haploid set of 14 chromosomes along with 3B-chromosomes of comparatively small size. The 3 B's were associated to form a trivalent in some of the PMC's (Fig. 3) whereas in others, two of the B's formed a bivalent and the third one could be seen as

Figs. 1-6. 1, *Agrostis micrantha* Steud., diakinesis, $n=14$, 2, *A. myriantha* Hook. f. diakinesis, $n=14$. 3-5, *A. stolonifera* Linn. 3, M_1 , $n=14+3B$'s forming a trivalent (arrow). 4, $n=14+3B$. The $2B$'s forming a bivalent (arrow) and the third univalent B . 5, A_1 , showing 16 chromosomes ($14+2B$) at one pole and 15 chromosomes ($14+1B$) at the other (B -chromosomes are at arrows). 6, *Bromus japonicus* Thunb. var. *japonicus*, M_1 , showing secondary associations among the bivalents and a configuration of 4 bivalents.

Table 1.

Species	Collection number	Locality	Chromosome number	Fig. no.	Previous reports
Tribe: Agrostideae <i>Agrostis micrantha</i> Steud.	2850	Jalapahar, Darjeeling 2200 m	n=14	1	n=14: Mehra and Sood (1974)
<i>A. myriantha</i> Hook. f.	2849	Senchal, Darjeeling 1800 m	n=14	2	n=14: Mehra and Sood (1974)
<i>A. stolonifera</i> Linn.	2853	Senchal, Darjeeling 1800 m	n=14+3B	3-5	n=7: Mehra and Sunder (1970) 2n=28: Löve and Löve (1948, 1956), Juhl (1952), Björkman (1954), Hubbard (1954), Jones C. (1956), Heitz (1967) Delay (1969) Leveque and Gorenflot (1969), Widen and Britton (1971) 2n=30, 32, 42, 44: Juhl (1952) 2n=35: Björkman (1954) Jones C. (1956) 2n=42: Müntzing (1937) Björkman (1954) Jones C. (1956) Carlborn (1969) Fernandes and Queiros (1969) 2n=46: Juhl (1956)
Tribe: Bromeae <i>Bromus japonicus</i> Thunb.	2855	Jalapahar,	n=14	6, 7	2n=14: Avdulov (1928), Nielson and

Table 1. (Cont'd)

Species	Collection number	Locality	Chromosome number	Fig. no.	Previous reports
<i>var. japonicus</i>					
	2846	Darjeeling 2200 m	n = 14		Humphrey (1937), Tateoka (1953), Barnett (1955) Schulz-Schaeffer (1956, 1960) Walters (1936), Gould (1968) Ovadiahu-yavin (1969) n = 7, 14: Schulz-Schaeffer (1956) n = 7, 21: Mehra and Remanandan (1973a) n = 7, 21: Mehra and Sunder (1970) n = 7: Mehra and Sharma (1977 for <i>var. falconeri</i>)
Tribe: Festuceae					
* <i>Colpodium nutans</i> Griseb.	2856	Gandhi road, Darjeeling 2000 m	n = 7	8	
<i>Dactylis glomerata</i> Linn.	2857	Jalapahar, Darjeeling 2200 m	n = 7		+2n = 14: Borrell and Lindner (1971) 2n = 14, 14 + 1 - 3B: Zohary and Ashken (1958), Shah (1963) 2n = 14, 14 + 1B: Borrell and Carroll (1969) 2n = 14, 14 + 1 - 4B, 28, 28 + 1 - 3B, 42: Jones <i>et al.</i> (1961) 2n = 14, 28: Doroszevska (1963) 2n = 27 - 31: Müntzing (1933), 1937) 2n = 27, 28 + 0 - 2B, 29: Borrell and Carroll (1969) 2n = 28: Leveque and Gorenflot (1969) n = 7, 14: Mehra and Remanandan (1973a)

Poa annua Linn.

2852	Senchal, Darjeeling 1800 m	n = 14	n = 7: Mehra and Sharma (1977) +n = 7: Elias <i>et al.</i> (1970) Gould (1970), Mehra and Sunder (1970)
2848	Ghoom, Darjeeling 1600 m	n = 14	n = 14 + 1B: Mehra <i>et al.</i> (1968) 2n = 24-26: Sokolovskaya and Probatova (1968) 2n = 28: Mulligan and Porsild (1969) 2n = 52: Mehra and Ramanandan (1973a) n = 14: Mehra and Sharma (1975c)

P. trivialis Linn.

2841	Lloyd Bot. Garden, Darjeeling 1800 m	n = 7	+2n = 14: Fernandes and Queiros (1970) Pohl and Davidse (1971) 2n = 14, 14 + 1-4B: Rosemark (1957) 2n = 14, 15: Akerberg (1942) 2n = 14, 28: Guinochet (1943) 2n = 28: Tateoka (1954)
2844	Observatory hill, Darjeeling 2000 m	n = 7	
2847	Tiger hill Darjeeling	n = 7	9 10

Table 1. (Cont'd)

Species	Collection number	Locality	Chromosome number	Fig. no.	Previous reports
Tribe: Eragrosteae		2200 m			
* <i>Tripogon jacquemontii</i> Stapf	2861	Gandhi road, Darjeeling 2000 m	n=10	11	
Tribe: Sporoboleae 3					
<i>Sporobolus diander</i> (Retz.) P. Beauv.	2842	Peshoke, Darjeeling 1500 m	n=12		n=12: Gupta and Yashvir (1971) n=27: Mehra <i>et al.</i> (1968) 2n=24: Larsen (1963) 2n=36: Avdulov (1928) n=12: Mehra and Sharma (1975d)
Tribe: Glycerieae					
<i>Glyceria tongolensis</i> C. B. Clarke	2854	Tiger hill, Darjeeling 2200 m	n=10		n=20: Mehra and Sunder (1970) n=10: Mehra and Sharma (1975f)
Tribe: Bambuseae					
* <i>Phyllostachys kumasaca</i> Munro	2859	Lloyd Bot. Garden Darjeeling 1800 m	2n=48	12	
Tribe: Paniceae					
* <i>Opilsmenus compositus</i> (L.) P. Beauv. × <i>O. burmannii</i> (Retz.) P. Beauv.	2851	Lloyd Bot. Garden, Darjeeling 1800 m	2n=54	13-16	

* Species investigated for the first time.

† New report for the species (new cytotype).

Literature cited in Table 1 according to Fedorov (ed.) 1969; Moore (ed.) 1973; IOPB chromosome number reports 1971-74, Taxon 20-23; Mehra, P. N. and collaborators 1968-1977. The mark (†) before the previous reports for the species indicates that the species has been extensively investigated by various workers and only the recent reports have been mentioned.

a univalent (Fig. 4). At anaphase I, the B's were distributed at the two poles in the ratio of 2:1 so that the PMC's at this stage showed 16 chromosomes (14+2B) at one pole and 15 chromosomes (14+1B) at the other (Fig. 5). Meiosis was quite normal as evidenced by nearly 100% pollen fertility.

Bromus japonicus Thunb. var. *japonicus*

It is one of the most common among the good fodder grasses in the N. W. Himalayas and occurs usually on abandoned cultivation.

Meiotic studies revealed the present taxon to be tetraploid with $n=14$. The PMC's at diakinesis and M_1 showed nonhomologous bivalent associations with a tendency among some of the bivalents to form multivalent configurations (Fig. 6). Thus, an exact chromosome count was made difficult at these stages. At A_1 too, a similar tendency was noticed but here, in some of the PMC's, 14 chromosomes could be counted at each pole. About 92% pollen fertility was recorded.

Colpodium nutans Griseb

This grass grows at very high altitudes of North-West India and is not much valued as a fodder.

The present collections were made from populations of only 4-5 individuals growing on the road-side near B. T. College, Darjeeling and was not observed to grow in any other locality. This species, which has been investigated for the first time, turned out to be diploid with $n=7$ (Fig. 8). A quadrivalent was invariably noticed in the PMC's at diakinesis and metaphase I. Anaphase I and further stages of meiosis were, however, normal and about 96% pollen were fertile.

Poa trivialis Linn.

This is a very good fodder grass which can also be made into hay.

Collections for meiotic studies were made from various localities of Darjeeling and nearby suburbs. In each case, a meiotic count of $n=7$ was observed (Fig. 9). The course of meiosis was normal except in the individuals from Tiger hill which showed nonhomologous bivalent associations at diakinesis and metaphase I (Fig. 10). Anaphase I was normal with 7:7 distribution of chromosomes. Pollen fertility was about 96%.

Phyllostachys kumasaca Munro

This is a dwarf bamboo, upto 1 metre tall, and is cultivated in the Lloyd Botanical Gardens, Darjeeling.

The root tip preparations revealed a diploid chromosome number $2n=48$ (Fig. 12). The karyotype could not be analysed due to very small size of the chromosomes. The genus *Phyllostachys* seems to be based on $x=6$ as suggested by us earlier (Mehra and Sharma 1975e). The lowest haploid number, $n=12$, in bamboos is reported in this genus in *P. makinoi* by Hsu (1967), whereas all the other reports are of higher ploidies.

Oplismenus compositus (L.) P. Beauv. $\times$ *O. burmannii* (Retz.) P. Beauv.

This grass collected from the Lloyd Botanical Gardens was found to form a

turf under the shade of trees and resembled *O. burmanii* in appearance. However, on close examination of the inflorescence characters, it was found to match *O. compositus*. The inflorescence is a panicle of racemes arranged alternately on the axis

Figs. 7-11. 7, *Bromus japonicus* Thunb. var. *japonicus*, A_1 showing 14 chromosomes at each pole. 8, *Colpodium nutans* Griseb, diakinesis, $n=7$ (note the quadrivalent). 9-10, *Poa trivialis* Linn. (Tiger hill population), diakinesis and M_1 showing secondary associations among the bivalents, $n=7$. 11, *Tripogon jacquemontii* Stapf, M_1 , $n=10$.

and the awns of the spikelets are reddish, and stiff. These are the characters of *O. compositus*. But the habit of growth, general appearance, hairiness of leaves and spikelets and the barbellate awns are like *O. burmannii*. The plant size is much smaller than either of them.

Figs. 12-16. 12, *Phyllostachys kumasaca* Munro, root-tip preparation showing $2n=48$ chromosomes. 13-16, *Oplismenus compositus* $\times$ *O. burmannii*, $2n=54$, 13, M_1 showing univalents, bivalents and trivalents. 14, M_1 showing noncongression of univalents at the metaphase plate. 15, mixed A_1 , $2n=54$. 16, late A_1 showing lagging chromosomes.

Meiotic studies revealed an equally interesting situation. Meiosis was highly disturbed resulting in the production of 70% sterile pollen. The following were the chief aberrations noticed: i) nonhomologous bivalent associations, ii) loose pairing of chromosomes at diakinesis, iii) nonorientation of univalents, bivalents and trivalents at M_1 and lagging of chromosomes at A_1 (Figs. 13–16). At mixed A_1 , 54 chromosomes could be counted in some of the mother cells (Fig. 15). All the species of *Oplismenus* so far known cytologically, are based on $x=9$, as concluded in our earlier paper (Mehra and Sharma 1975b). The cytological evidence for the present population suggests a hybrid origin, as do the morphological features discussed above. The meiotic breakdown is due to the unbalanced genomic constitution. The two species, *O. compositus* and *O. burmannii* grow sympatrically in the locality from where the present collections were made. It seems that they represent hybrid population of *O. compositus* $\times$ *O. burmannii*.

Summary

Cytological investigations on 20 taxa belonging to 13 species of grasses have been carried out from the hills of Darjeeling (E. Himalayas). Three species, *Colpodium nutans* ($n=7$), *Tripogon jacquemontii* ($n=10$) and *Phyllostachys kumasaca* ($2n=48$) were investigated for the first time. A putative hybrid *O. compositus* $\times$ *O. burmannii* was discovered from the area. It showed highly aberrant meiosis. *Agrostis stolonifera* has been found to possess 3B-chromosomes.

Acknowledgements

Thanks are due to the PL 480 authorities of U. S. A. for the financial aid under grant FG-In-482 (A7-CR-441) to the senior author.

Literature cited

- Fedorov, An. A. (ed.) 1969. Chromosome Numbers of Flowering Plants. Komarov Botanical Institute USSR, 926 pp.
- Hsu, C. C. 1967. Preliminary chromosome studies on the vascular plants of Taiwan (I). Taiwania No. 13: 117–130.
- Mehra, P. N., Khosla, P. K., Kohli, B. L. and Koonar, J. S. 1968. Cytological studies in the North-Indian grasses (Part I), Res. Bull. Panjab Univ. 19: 157–230.
- and Remanandan, P. 1973a. Cytological investigations on W. Himalayan Pooideae. Cytologia 38: 237–258.
- and — 1973b. Cytological investigations on W. Himalayan Panicoideae. Cytologia 38: 259–270.
- and Sharma, M. L. 1975a. Cytological studies in some Central and Eastern Himalayan grasses I. The Andropogoneae. Cytologia 40: 61–74.
- and — 1975b. Cytological studies in some Central and Eastern Himalayan grasses II. The Paniceae. Cytologia 40: 75–89.
- and — 1975c. Cytological studies in some Central and Eastern Himalayan grasses III. The Agrostideae, Aveneae, Brachypodieae, Bromae, Festuceae, Phalarideae, and Triticeae. Cytologia 40: 441–452.
- and — 1975d. Cytological studies in some Central and Eastern Himalayan grasses IV. The

- Arundinelleae, Eragrosteae, Isachneae, Chlorideae, Sporoboleae, Meliceae, Stipeae, Arundineae and Garnotieae. *Cytologia* **40**: 453–462.
- and — 1975e. Cytological studies in some Central and Eastern Himalayan grasses V. The Bambuseae. *Cytologia* **40**: 463–467.
- and — 1975f. In IOPB chromosome number reports XLIX. *Taxon* **24** (4): 501–516.
- and — 1977. Cytological studies on some grasses of Kashmir. *Cytologia* **42**: 111–123.
- and Sunder, Shyam 1970. Cytological studies in the North-Indian grasses (Part II). *Res. Bull. Panjab Univ.* **20**: 503–539.
- and Sood, O. P. 1974. In IOPB chromosome number reports XLV. *Taxon* **23**(4): 619–624.
- Moore, R. J. (ed). 1973. *Index to Plant Chromosome Numbers 1967–1971*. Published by Oosthoek's Uitge versmaatschappij B. V., Domstraat 5–13, Utrecht, Netherlands.
-