International Conference on "Recent Trends in Technology and its Impact on Economy of India"
Guru Nanak College for Girls, Sri Mukstar Sahib, Punjab (India) (ICRTTIEI-17)
24th October 2017, www.conferenceworld.in ISBN: 978-93-86171-74-0

Study of Cytomorphological Variations in Genus *Poa* L. from District Kangra (Himachal Pradesh)

Harpreet Kaur[1]

[1]*Department of Botany, Guru Nanak College for Girls, Sri Muktsar Sahib (India)*

ABSTRACT

The present paper deals with population based meiotic studies for four different species of genus Poa belonging to tribe Poeae of family Poaceae from cytologically unexplored area of Western Himalayas i.e. district Kangra of Himachal Pradesh for the assessment of genetic diversity. One species, Poa setulosa (2n=28) has been cytologically worked out for the first time. B-chromosome and new tetraploid cytotype (2n=28+0-1B) has been recorded for Poa nepalensis. Additionally, variable number of B-chromosomes has been reported for species Poa annua (2n=28+1-3B). On India basis, diploid cytotype has been investigated for species Poa supina (2n=14). Total fifteen populations have been worked out for these species. Out of these, five populations show normal meiotic behavior and ten populations reveal meiotic abnormalities as cytomixis, unoriented bivalents, bridges and laggards which lead to the reduced pollen fertility and heterogenous sized pollen grains.

Keywords- Population, meiosis, chromosome numbers, cytotype, Kangra.

I. INTRODUCTION

Grasslands, which make up 20% of the world's vegetational cover, are primarily composed of the members of Poaceae [1]. There are about 898 genera and 10,300 species world-wide [2] which make Poaceae as the fourth largest flowering plant family preceeded by only 3 families surpassing grasses in the number of species as Composits (Asteraceae), Orchids (Orchidaceae) and Legumes (Leguminosae *sensu lato*). In India, it is represented by 240 genera covering about 1,200 species [3]. The family is cosmopolitan and distributed in all the continents and in all the climatic zones. Cytologically, the members of family Poaceae exhibit lots of variations due to hybridization, chromosome repatterning, intraspecific and intrageneric polyploidy and aneuploidy. Likely, *Poa* L. belonging to the tribe Poeae of family Poaceae, is taxonomically and genetically complex genus with many apomictic species which are highly variable for chromosome numbers [4]. It comprises 300 species on the world-wide basis embracing 45 species from India [5]. The cosmopolitan distribution of the genus is attributed to various types of reproductive systems [6] and high colonizing ability [7]. Many species of *Poa* are used as forage plants [8].

II. MATERIALS AND METHODS

For meiotic studies, young spikes were collected on population basis from various localities of district Kangra of Himachal Pradesh. Meiotic studies were carried out through standard smearing technique from 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 shrivelled and unstained pollen grains were counted as sterile. Pollen grain size was measured using occulomicrometer. Photomicrographs of pollen mother cells were made from freshly prepared slides using Nikon 80i eclipse Digital Imaging System. Voucher specimens are deposited in the Herbarium, Department of Botany, Punjabi University, Patiala (PUN).

III. RESULTS AND DISCUSSION

At present, detailed meiotic studies have been done for fifteen populations of four different species of genus *Poa* belonging to tribe Poeae of family Poaceae from different localities of district Kangra of Himachal Pradesh. The information about specific locality, altitude, accession number, meiotic chromosome number, figure number, ploidy level and course of meiosis is given in Table 1. Highly abnormal meiotic behaviour has been recorded in two species as shown in Table 2 and 3.

3.1. *P. annua* L.

The species commonly known as 'Winter grass', 'Goose grass', 'Annual meadow grass' or 'Annual bluegrass' is a grass of European origin which has spread over world-wide as a weed. The species occurs in most of the natural habitats as well as in cultivated fields. The species is characterized with all florets similar; all nerves of the lemma hairy; anthers 0.6-0.8mm long. Interestingly, it flowers and fruits throughout the year. Cytologically, the species is considered to be an allotetraploid (2n=28) derived from the diploids *P. supina* Schrader and *P. infirma* Kunth, adapting to variable habitats, therefore different morpho- and ecotypes have evolved within the species [9, 10].

During present studies, meiotic studies have been done for 11 populations out of which 7 reveal the presence of 14 bivalents in the PMCs at Diakinesis (Fig. 1A) and 14:14 chromosomal distribution at A-I (Fig. 1B). Remaining 4 populations show the PMCs with presence of variable range of B-chromosomes in different populations (Fig. 1C). The present report of tetraploid cytotype is in accordance with the large number of earlier reports from India and outside India. Previously, the species also showed the presence of various other chromosome numbers as 2n=14 from north India [11] and by various workers from outside India, 2n=24 from East Asia [12], 2n=42 from Pakistan [13] and China [14] and 2n=52 from the Western Himalayas [15]. Earlier reports also exhibited the presence of 1B-chromosome in the tetraploid cytotype (2n=28+1B) from north-eastern India [16], north India [17] and Punjab plains [18].

Out of 11 populations, 8 populations present highly abnormal meiotic behaviour (Table 2) in the form of chromatin stickiness at M-I as well as bridges and laggards at A-I/A-II to T-I/T-II (Figs. 1D, 1E). Besides these, the phenomenon of cytomixis has been observed in 2 populations at different stages of meiosis (Figs. 1F. 1G; Table 2), ultimately, variable sized pollen grains are formed with reduced pollen fertility (Figs. 1H, 1I). In all the populations studied here, variation in the bivalent size has also been observed, out of the 14 bivalents, 2 are quite large, whereas, the remaining ones are small to medium-sized.

The detailed studies of the above mentioned species have already been published in the form of research paper **"Meiotic Studies in *Poa annua* L. from different altitudinal ranges of North India. *Cytologia*, 75 (3): 313–318, 2010"**

3.2. *P. nepalensis* (Wall. ex Griseb.) Duthie

P. nepalensis is endemic to the Himalayas and occurs along its whole range. The species is found to be common in meadows on slopes and roadsides and at lower elevations in district Kangra. The plant has silvery panicled branches in pairs; lemma as long as wide, apex obtuse to acute; keels of palea ciliate.

PMCs of one population depict 2n=28+0-1B at different meiotic stages (Fig. 1J) and that of other population show the presence of quadrivalent configurations at M-I ($2n=12_{II}+1_{IV}$) (Fig. 1K). This chromosome count is reported here as new, additional tetraploid cytotype on the world-wide basis and the presence of B-chromosomes is also reported for the first time for the species. Earlier, only diploid cytotype (2n=14) for the species was reported from north India [11].

The course of meiosis is noted to be highly abnormal in both the populations (Table 3) with the presence of cytomixis witnessed at different stages of meiosis. Chromatin transfer occurs in the form of dark and thick chromatin balls at earlier stages and light stained threads to pinch out the chromatin during M-I/A-I/T-I (Figs. 1L, 1M). In addition to this, the other observations are the presence of unoriented bivalents at M-I and bridges and laggards at anaphases and telophases (Figs. 1N, 1O, 1P) are also seen. This lead to the formation of abnormal microsporogenesis, pollen grains of variable size (Fig. 1Q, Table 3) and lowered pollen fertility (48.89-51.20%).

3.3. *P. setulosa* Bor

The species is met within shady and moist places. It is an endemic species of the Western Himalayas and is characterized as perennial herb with ligules 2.5-3mm long; panicle narrow; spikelets wedge-shaped; lower glume longer than the lowest lemma; anthers 0.6-0.8mm long.

The species has been cytologically studied for the first time on the world-wide basis. Several PMCs of the species exhibit 2n=28+0-1B at Diakinesis (Figs. 1R, 1S) and equal distribution of 14:14 chromosomes at A-I (Fig. 1T). The bivalent size also varies within the PMCs. Out of 14 bivalents, 2 appear to be large-sized, 3 medium-sized and 9 small-sized (Fig. 1R).

Meiotic anomalies are there in the form of late disjunction of bivalents, formation of bridges (1-2 per PMC) and laggards (2-3 per PMC) at anaphases and telophases (Figs. 1U, 1V) along with chromatin stickiness also seen at different stages of meiosis. Thus, the pollen fertility is reduced to 56.00%.

3.4. *P. supina* Schrad.

It is called 'Creeping meadow-grass' (*P. supina* Schrad.) and is mostly found on slopes near damp places. It is characterized by spikelets crowded towards the ends of the panicle branches; anthers 1.2-1.6mm long.

During meiotic studies, many PMCs of the species depict 2n=14 in the form of 7 bivalents at M-I (Fig. 1W). The present report of diploid cytotype with 2n=14 conforms to the previous reports from the Kashmir

Himalayas in India [19] and other parts of the world. Earlier, tetraploid cytotype with 2n=28 has been reported by some Indian cytologists from north-eastern India [20], east India [21] and Kashmir [22]. Also, B-chromosome was reported at diploid level (2n=14+0-1B) from Europe [23] and at tetraploid level (2n=28+1B) from north India [11].

IV. ACKNOWLEDGEMENTS

The authors are grateful to the University Grants Commission, New Delhi for providing financial assistance under the DRS SAP III and FIST of DST as well as fellowship provided to H.K. under Maulana Azad National Fellowship Scheme. We are highly thankful to the Joint Director and Deputy Director, BSI and other staff of Herbarium of Dehra Dun for the help in the identification of the plant species.

REFERENCES

[1] C. Ture, and H. Bocuk, An investigatin on the diversity, distribution and conservation of Poaceae species growing naturally in Eskişehir province (Central Anatolia-Turkey), Pak. J. Bot., 39, 2007, 1055–1070.

[2] N.N. Tzvelev, The system of grasses (Poaceae) and their evolution, Bot. Rev., 55, 1989, 141–204.

[3] S.K. Murti, Flora of Cold Deserts of Western Himalaya. Vol. 1. (Monocotyledons). Botanical Survey of India, Calcutta, 2001.

[4] A.M. Kelley, P.G. Johnson, B.L. Waldron, and M.D. Peel, A Survey of Apomixis and Ploidy Levels among Poa L. (Poaceae) using Flow Cytometry, Crop Sci., 49, 2009, 1395–1402.

[5] H. Santapau, and A.N. Henry, A Dictionary of the Flowering Plants in India. C.S.I.R., New Delhi, 1973.

[6] P.G. Johnson, B.A. Rummele, P. Velguth, D.B. White, and P.D. Ascher, An overview of Poa annua L. reproductive biology, Int Turfgrass Soc. Res. J., 7, 1993, 798–804.

[7] Y. Frenot, J.C. Gloaguen, and P. Trehen, Climate change in Kerguelen Islands and colonization of recently deglaciated areas by Poa kerguelensis and P. annua, In Walton DWH (ed) Antarctic communities: species, structure and survivel. Cambridge University Press, Cambridge, 1997, 358–366.

[8] M. Ibrar, S. Hashim, and K.B. Marwat, Ethnobotanic study of the weeds of five crops in district Abbottabad, N-W Pakistan, Pak. J. Weed Sci. Res., 9, 2003, 229–240.

[9] H. Darmency, and J. Gasquez, Spontaneous hybridization of the putative ancestors of the allotetraploid Poa annua, New Phytol., 136, 1997, 497–501.

[10] R.J. Soreng, R.D. Bull, and L.J. Gillespie, Phylogeny and reticulation in Poa based on plastid trnTLF and nrITS sequences with attention to diploids, Pp. 619–643, In O. Seberg, G. Petersen, A.S. Barfod and J.I. Davis (eds.) Diversity, phylogeny, and evolution in the Monocotyledons, Aarhus University Press, Aarhus, 2010.

[11] P.N. Mehra, and S. Sunder, Cytological studies in the North Indian grasses. Part II, Res. Bull. Punjab. Univ., Sci., 20, 1969, 503–539.

[12] A.P. Sokolovskaya, and N.S. Probatova, A karyosystematic investigation of the Far-eastern species of Poa L., Bot. Zh. (Leningard), 53, 1968, 1737–1743.

[13] S. Khatoon, and S.I. Ali, Chromosome Atlas of the Angiosperms of Pakistan, Department of Botany, University of Karachi, Karachi, 1993.

[14] G.X. Yan, S.Z. Zheng, F.H. Xue, J.F. Yun, L.Y. Wang, and X.Q. Fu, The chromosome numbers of 35 forage species and their geographical distribution, Grassl. China, 1, 1995, 16–20.

[15] P.N. Mehra, and P. Remanandan, Cytological investigations on W. Himalayan Pooideae, Cytologia, 38, 1973, 237–258.

[16] P.N. Mehra, and B.L. Kohli, Cytological studies in North Indian grasses, Proc. 53rd Ind. Sci. Congr., Part 3, 1966, 277–278.

[17] P.N. Mehra, P.K. Khosla, B.L. Kohli, and J.S. Koonar, Cytogenetical studies in North-Indian grasses. I, Res Bull Panjab Univ. Sci. 19, 1968, 157–230.

[18] S.S. Bir, and M. Sahni, Chromosomal and morphological variations in grasses of Punjab, J. Cytol. Genet., 22, 1987, 12–22.

[19] H.K. Kaur, N. Mubarik, S. Kumari, and R.C. Gupta, In IOPB Chromosome Number Reports, Taxon, 60(4), 2011, 1221.

[20] O. Parkash, Cytological investigations in some grasses of northeastern India (Festucoids, Ischaemum L., Eulalia Kunth and Saccharum spontaneum L.), Ph.D. Thesis, Chandigarh, 1979.

[21] P.N. Mehra, Cytology of East-Indian grasses. Pramodh P. Kapur at Rajbandhu Industrial Company, New Delhi, 1982.

[22] K.K. Koul, and R.N. Gohil, SOCGI plant Chromosome number reports–VI, J. Cytol. Genet., 23, 1988, 38–52.

[23] M.M. Duckert-Henriod, and C. Favarger, Contribution à la cytotaxonomie et à la cytogéographie des Poa (Poaceae=Gramineae) de la Suisse, Denkschr. Schweiz. Naturf. Ges., 100, 1987, 1–130.

Table 1. Information about area, locality, altitude, accession number, meiotic chromosome number reports, ploidy level and meiotic course of four different species of genus *Poa* (Poaceae) from district Kangra of Himachal Pradesh.

Taxa	Locality, Altitude (m)	Accession numbers (PUN)	Meiotic chromosome number (n)	Ploidy level/ Meiotic course
***Poa annua* L.**	Tal-mata, 1,103m	52585	28	4x/N*
	Bhagsunaag, 1,650m	52587	28	4x/N
	Bada Gran, 3,500m	52583	28	4x/A*
	Dehra, 650m	52581	28	4x/A
	Andretta, 1,250m	55262	28	4x/A
	Swad, 2,800m	55263	28	4x/A
	Billing, 2,310m	56655	28	4x/N
	Rehlu, 950m	52592	28+0-1B	4x/A

	Dharamsala, 1,600m	52584	28+0-1B	4x/A
	Bandla, 1,266m	52586	28+2-3B■	4x/A
	Chhota Bhangal, 2,000m	52588	28+2-3B■	4x/A
P. nepalensis	Bandla, 1,266m	52590	28+0-1B■	4x/A
(Wall. Ex Griseb.)	Patti, 890m	52589	28^{++}	4x/A
Duthie				
***P. setulosa* Bor**	Bhagsunaag, 1,650m	52591	$28+0\text{-}B^{+}$■	4x/A
P. supina	Khaniara, 1,750m	56561	14	2x/N
Schrad.				

*__A__= Abnormal meiosis, *__N__=Normal meiosis.
+ Species cytologically worked out for the first time at world level.
++ Species with new intraspecific polyploid cytotypes at world level.
■ Species with B-chromosomes reported for the first time and /or for additional cytotypes.

Table 2: Data on cytomixis and abnormal meiotic course and occurrence of B-chromosomes in different populations of *Poa annua*.

	Cytomixis PMCs with			Meiotic course showing PMCs with		
Accession Number	%age of PMCs Laggards at Meiosis-I/ Meiosis-II (%age)	Number of PMCs involved B-chromosomes (%age)		Chromosomal stickiness at M-I (%age)	Unoriented bivalents at M-I (%age)	Bridges at Meiosis-I/ Meiosis-II (%age)
		1B	2B 3B			
52584	---- 23.97 (14/104)	---- 0.96	---- 13.46 ----	9.6 (12/125)	5.45 (6/110) (41/171)	---- (1/104)
52585	----- -----	----- -----		5.08 (6/118) ----	4.90 (5/102)	----- -----
52592	---- 4.25 (2/47)	---- 10.63 (5/47)		8.42 (8/95) ----	5.43 (5/92)	20.40 (20/98) -----
52586	----- 18.10 (21/116)	----- 14.65 (17/116)	10.34 (12/116)	3.07 (2/65)	2.17 (2/92)	22.34 (21/94) -----
52588	---- 6.00 (6/100)	----- 3.02 (3/100)	3.00 (3/100)	2.41 (3/124)	----	---- ----
52583	21.30 ---- (40/187)	4-8 ----		8.88 (12/135) ----	3.38 (4/118)	---- -----

ISBN: 978-93-86171-74-0

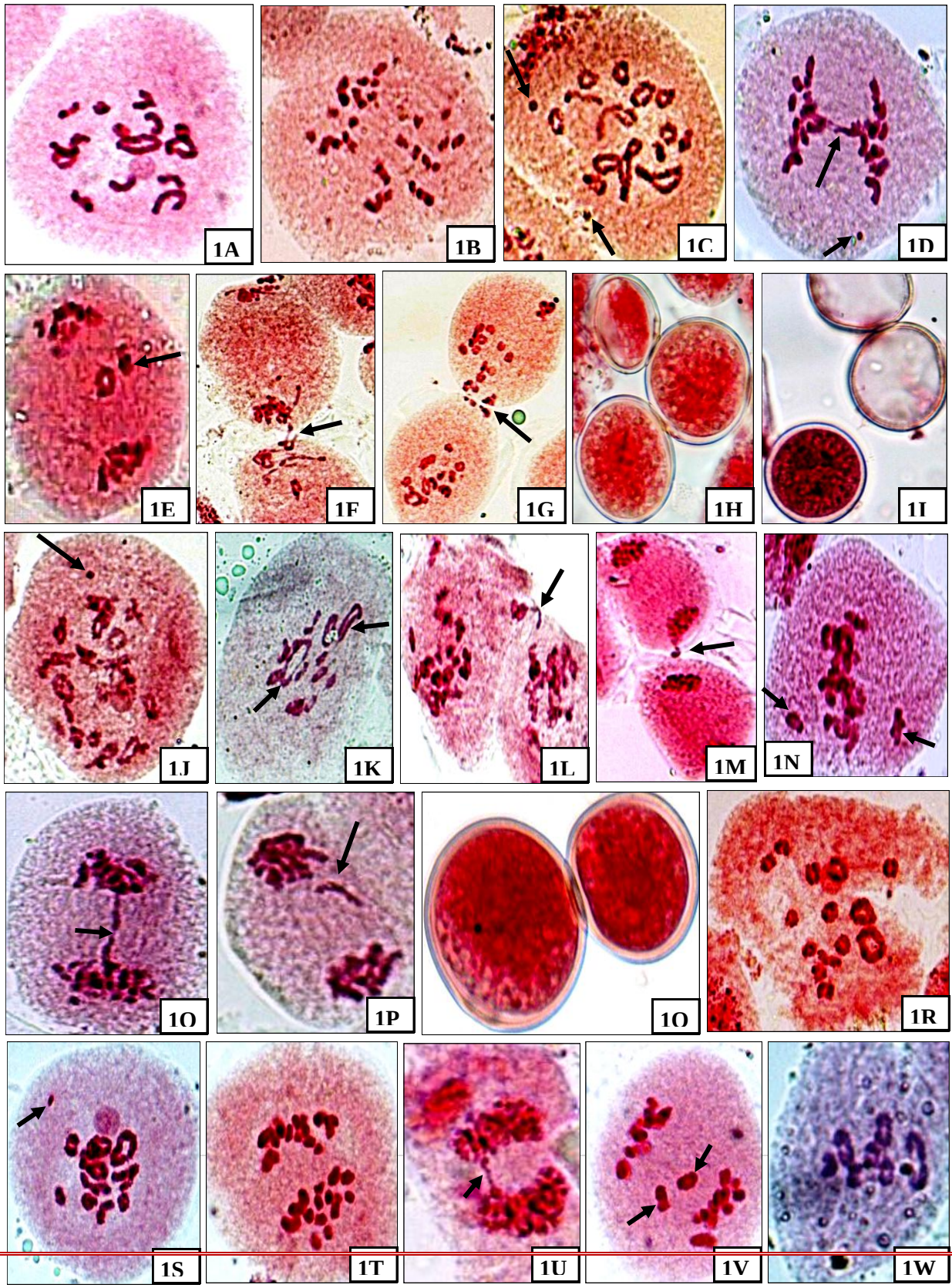
52581	(4/116)	---- ----	---- ----	---- ----	-----	9.67 (12/124)	3.44
55262	(6/112)	8.97 ---- (14/156)	3-5 ----	9.21 (14/152) ----	5.26 (8/152)	10.29 (14/136)	5.35
55263	(6/124)	---- ----	----- ----	2.5 (2/80) ----	1.16 (1/86)	6.71 (9/134)	4.83

Table 3. Data on abnormal meiotic course in populations of *Poa nepalensis*.

S.No.	Meiotic abnormalities	Frequency (%age) Ac. No. 52590	Ac. No. 52589
1.	Cytomixis observed	14.51 (18/124)	8.95 (12/134)
2.	Number of PMCs involved in cytomixis	4-9	4-6
3.	Chromatin stickiness at M-I	12.5 (16/128)	8.95 (12/134)
4.	Unoriented bivalents at M-I	5.64 (7/124)	----
5.	Bridges at Meiosis-I and Meiosis-II	6.25 (8/128)/ 4.90 (5/102)	11.94 (16/134)/ 7.05 (6/85)
6.	Laggards at Meiosis-I and Meiosis-II	8.77 (10/114)/ 7.69 (5/65)	12.67 (18/142)/ 8.16 (8/98)
7.	Diads – WMN^{+}/WM^{++}	5.26 (6/114)/ 2.77 (2/72)	4.08 (4/98)/ 2.56 (2/78)
8.	Triads – WMN/WM	4.31 (5/116)/ 1.47 (1/68)	3.15 (3/95)/ 1.81(2/110)
9.	Tetrads – WMN/WM	58.94 (56/95)/ 24.48 (24/98)	64.86 (48/74)/ 18.75 (12/64)

+ Without micronucleus ++ With micronucleus

International Conference on "Recent Trends in Technology and its Impact on Economy of India"
Guru Nanak College for Girls, Sri Mukstar Sahib, Punjab (India) (ICRTTIEI-17)
24th October 2017, www.conferenceworld.in ISBN: 978-93-86171-74-0



ISBN: 978-93-86171-74-0

Abbreviations: PMC= Pollen mother cells; M-I= Metaphase-I; A-I= Anaphase-I

Figure Plate 1: *Poa annua* : 1A- PMC at Diakinesis showing $14_{II.}$ **1B-** PMC at A-I with 14:14 distribution of chromosomes. **1C-** PMC at Diakinesis showing 2n=14+2B chromosomes. **1D-** PMC at A-I showing bridge and 1B chromosome. **1E-** PMC at A-I showing laggards. **1F-** PMC at M-I showing cytomixis. **1G-** Two PMCs showing cytomixis. **1H-** Heterogenous sized pollen grains. **1I-** Fertile and sterile pollen grains. ***P. nepalensis* : 1J-** PMC at Diakinesis showing 2n=28+1B. **1K-** PMC at M-I showing $12_{II}+1_{IV.}$ **1L-** Two PMCs showing cytomixis at M-I. **1M-** PMC at T-I showing cytomixis. **1N-** PMC at M-I showing unoriented bivalents. **1O-** PMC at T-I showing bridge. **1P-** PMC at T-I showing laggard. **1Q-** Variable sized pollen grains. ***P. setulosa* : 1R-** PMC at Diakinesis showing $14_{II.}$ **1S-** PMC at Diakinesis showing 2n=28+1B. **1T-** PMC at A-I with 14:14 distribution of chromosomes. **1U-** PMC at T-I showing bridge. **1V-** PMC at A-I showing laggards. ***P. supina* : 1W-** PMC at M-I showing $7_{II.}$
Scale=10µm