International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS | Volume 7 | Issue 4 | OCT-DEC| 2017 | 171-178
Research Article | Biological Sciences | Open Access | UGC Approved | MCI Approved Journal

INTRASPECIFIC EUPLOID (2*X*, 4*X*) CYTOTYPES (BASED ON *X* = 9) IN *BRACHYPODIUM SYLVATICUM* (HUDS.) P. BEAUV. FROM NORTH-WEST HIMALAYAS

V. Kumari* and V.K. Singhal
Department of Botany, Punjabi University, Patiala, 147002, Punjab, India

***Corresponding Author Email:** bits11285@gmail.com

ABSTRACT

On the basis of chromosome counts made through male meiosis in 12 wild accessions of Brachypodium sylvaticum from Kullu district, Himachal Pradesh in North-West Himalayas, we here report the existence of intraspecific diploid (2n = 18) and tetraploid (2n = 36) cytotypes based on x = 9. The presently studied tetraploid (4x) individuals grow much taller and robust in size and show gigantism in various vegetative and floral parts. These 4x plants showed increased cell size as reflected in the cases of stomata and pollen grains which are significantly larger compared to those in diploids. Diploid (2x) individuals showed regular meiotic course with 9 bivalents, regular distribution at poles, normal sporads and nearly 100% fertile and equal sized pollen grains. However, individuals in the accessions gathered from the Solang Nullah growing under the same climatic conditions showed meiotic aberrations in the form of non-synchronous disjunctions of bivalents, lagging chromosomes and sterile and variable sized fertile pollen grains. On the other hand, 4x plants showed perfectly normal meiosis, sporads and equal sized 100% fertile pollen grains.

KEY WORDS

Intaspecific Euploid Cytotypes, Brachypodium sylvaticum, Kullu district, Pollen grains, Basic numbers, North-West Himalayas.

INTRODUCTION

Brachypodium sylvaticum (Huds.) P. Beauv. (Family: Poaceae) referred as 'false-brome', 'slender false brome' or 'wood false brome', is a tall tufted out breeding perennial grass which is native to Europe, Asia and North Africa. The species has a broad native range stretching from North Africa to Eurasia. Species is listed as noxious invasive weed along the west coast of the United States, New Zealand and Australia along the west coast of United States invading parks and native reserves. It is left unchecked in the invaded range it eliminates other native species and forms a virtual monoculture. It is a shade tolerant species spreads rapidly by seeds. In India species is most commonly found in forests and woodlands between altitudes of 1600-3100 m, preferring the open areas, along rivers and streams. The grass has a broad tolerance of soil pH and is even able to grow in the limestone rich, rocky (quarries and screes) soils where it can be the first colonizer. The species can be recognized in the field by its rich green stem and leaves and drooping narrow long spike bearing sessile spikelets which appeared during the rainy months of July-August. Due to its fast-growing nature, high genetic diversity (numerous varieties are known based on pubescence), high reproductive potential (both sexual and asexual), adaptability to different environments, the species has been analyzed for chromosome counts by different cytologists both from India and outside of India. Existing chromosomal literature reveals that the species depicts an array of chromosome numbers and euploid chromosomal races with 2*n* = 14, 18, 28, 28, 42, 44, 56. As a part of the project to explore the germplasm of flowering plants from the phytogeographically different and cytologically unexplored regions of North-West Himalayas. The

present cytological studies were undertaken on *B. sylvaticum* on individual plant basis from the Kullu district, Himachal Pradesh. Consequent to intensive cytomorphological explorations, intraspecific 2*x* and 4*x* cytotypes have been detected in area. The aims of the study were to (i) work out the exact chromosome number on individual plant basis, (ii) work out in detail the male meiotic course, microsporogenesis and pollen fertility separately in the two taxa and (iii) segregate and pinpoint the characters to be used for identification of 2*x* and 4*x* individuals in the field.

MATERIALS AND METHODS

Materials for detailed cytomorphological studies were collected from the wild individuals growing in various localities between the altitudinal ranges of 1600-3100m in Kullu district in Himachal Pradesh. The sites of collection with GPS co-ordinates and voucher specimens deposited in the Herbarium, Department of Botany, Punjabi University, Patiala (PUN) were provided in Table 1. For chromosome counts and male meiosis, appropriate sized spikes/florets were fixed in Carnoy's fluid (absolute alcohol: chloroform: glacial acetic acid, 6:3:1). Meiocytes with meiotic chromosome numbers and sporads were prepared from the young and developing anthers from unopened florets squashed in 1% acetocarmine using standard acetocarmine technique. In each case, 400-500 meiocytes were observed under light microscope at different stages of meiosis for chromosome counts and detailed meiotic course. For microsporogenesis, 300-400 sporads were analyzed taken from different anthers/ florets. Pollen fertility was estimated through stainability tests by squashing the mature anthers in glycerol-acetocarmine mixture (1:1). Well filled pollen grains with fully stained cytoplasm and nuclei were scored as fertile while shrivelled and those with unstained /partially stained cytoplasm were counted as sterile. Photomicrographs of meiocytes with well spread chromosomes, meiotic aberrations, sporads and pollen grains were taken from the temporary preparations using Leica Qwin imaging system and Nikon 80i Eclipse microscope. For stomatal characters epidermal peels were obtained from the middle parts of mature leaves boiled in water along with KOH. Peels were subsequently stained in a drop of Safranin red and observed under microscope. Photographs of dried herbarium specimens and epidermal peels were taken separately for the 2*x* and 4*x* cytotypes.

RESULTS AND DISCUSSION

Plants of 12 wild accessions of *Brachypodium sylvaticum* growing in various localities of Kullu district were analyzed for detailed cytomorphological studies. The studied individuals depicted two different gametic chromosome counts of *n* = 9 and *n* = 18. The results covering male meiotic course including microsporogenesis, pollen fertility and pollen grain size for the 2*x* and 4*x* cytotypes were given separately. For comparison, the individuals of two cytotypes were also examined using various morphometric parameters.

Cytology

The diploid (n=9)

Majority of the accessions analyzed during the present study depicted a diploid gametic chromosome count of *n* = 9 as confirmed from the presence of 9 bivalents at diakinesis (Fig. 2A), M-I (Fig. 2B), equally distributed 9:9 chromosomes at A-I (Fig. 2C) and M-II (Fig. 2D). Majority of the diploid accessions showed perfectly regular meiotic course, normal sporads and uniform sized nearly 100% fertile pollen grains. However, in one of the accession (PUN 61359) from Solang Nullah, 44.63% of meiocytes showed non-synchronous disjunctions in 1-3 bivalents (Fig. 2E-G), irregular chromosome distribution of chromosomes (8:2:8) at A-I (Fig. 2H) and lagging chromosomes (Fig. 2I). In spite of having normal sporads formation (Fig. 2J), the plants showed reduced pollen fertility (2-11%) and sterile and heterogeneous sized pollen grains seems to be consequence of these meiotic aberrations (Fig. 2K).

The tetraploid (n=18)

Individuals of two accessions analyzed from Kothi (2500m) and Solang Village (2750m) depicted a tetraploid chromosome count of *n* = 18 as confirmed from the presence of 18 bivalents at M-I (Fig. 2L, M). Further, meiotic course including microsporogenesis were noticed to be regular leading to normal sporads (Fig. 2N) and equal sized fertile pollen grains (Fig. 2O).

Table 1. Cytologically worked out accessions of *B. sylvaticum* along with sites of collection, altitude, GPS co-ordinates, vouchers (PUN), gametic chromosome number, ploidy level and pollen fertility (%).

S. No.	Sites of collection (altitude in meters)	GPS co-ordinates	Vouchers (PUN)	Gametic chromosome number (*n*)	Ploidy level (x)	Pollen fertility (%)
1.	Kasol (1640m)	32°00'38" N, 77°19'00" E	60308	9	2x	98
2.	Sarari (1750m)	31° 94' 05" N 77°18' 10" E	61355	9	2x	100
3.	Old Manali (2242m)	32° 15' 11" N 77° 10' 50" E	61357	9	2x	90
4.	Kanial Village (2280m)	32° 13' 26" N 77° 10' 30" E	61356	9	2x	100
5.	Tosh Village (2400m)	32° 03' 08" N 77° 26' 58" E	61361	9	2x	98
6.	Palchan (2475m)	32° 18' 35" N 77° 10' 31" E	61362	9	2x	100
7.	Chauki (2500m)	32° 00' 16"N 77°14' 36"E	61276	9	2x	92
8.	Malana (2652m)	32° 03 '45" N 77° 15' 38"E	61360	9	2x	100
9.	Solang Nullah (2700m)	32° 18 ' 13" N 77° 07' 31"E	61359	9	2x	89
10.	Jibhi (3100m)	31° 35 ' 14" N 77° 21' 22"E	61363	9	2x	90
11.	Kothi (2500m)	32º 18' 52" N 77º 11' 24" E	61358	18	4x	88
12.	Solang Village (2750m)	32° 18 ' 47" N 77° 09' 48"E	61202	18	4x	92

Table 2. Comparision of macro-and microscopic characters of the 2*x* and 4*x* cytotypes of *Brachypodium sylvaticum* (Huds.) P. Beauv.

		Cytotypes	
S.No.	**Characters**	**Diploid (*n*=9)**	**Tetrapolid (*n*=18)**
1.	Distribution	Quite common	As isolated individuals
2.	Habit	Perennial	Perennial
3.	Habitat	Along roadsides	On slopes
4.	Plant height (cm)	45-60	75-90
5.	Stem		
(i)	Number of internodes	5-7	12-16
(ii)	Length of uppermost internode (cm)	10-12	20-26
(iii)	Length of 2nd internode from the top (cm)	5-7	15-17
6.	Leaf		
(i)	Length of sheath (cm)	4.5-7.5	8.5-10.5
(ii)	Length of lamina(cm)	7.5-12.5	14.5-17.5
(iii)	Width of lamina(mm)	4-5	5-7
7.	Inflorescence		
(i)	Length of a spike (cm)	7-14	9-18
(ii)	Length of spikelet (cm)	1.5-3.7	2-5
(iii)	Lemma(mm)	6-9	8-11
(iv)	Palea (mm)	6-7	7-8
8.	Pollen size (µm)	16.40-22.36 × 16.40-22.36	22.36-25.35 × 22.36-23.85
9.	Stomatal size (µm)	10.09 × 6.95	20.66×10.93

Morphometric Analysis

The individuals of two cytotypes were also compared morphometrically covering various macro-and microscopic characters (Table 2). Analysis revealed that the individuals of 4*x* cytotypes grow much taller (75-90 cm) compared to diploids (45-60 cm; Fig. 1A, C). The 4*x* individuals also showed increase in size of various vegetative and floral parts such as internode length, leaf size, size of spike and spikelets, lemma and palea (Table 2). The two cytotypes also differ in micro-characters like stomata (Fig. 1 B, D) and pollen grains (Fig. 2K, O) which are significantly larger in the 4*x* compared to the diploid (Table 2).

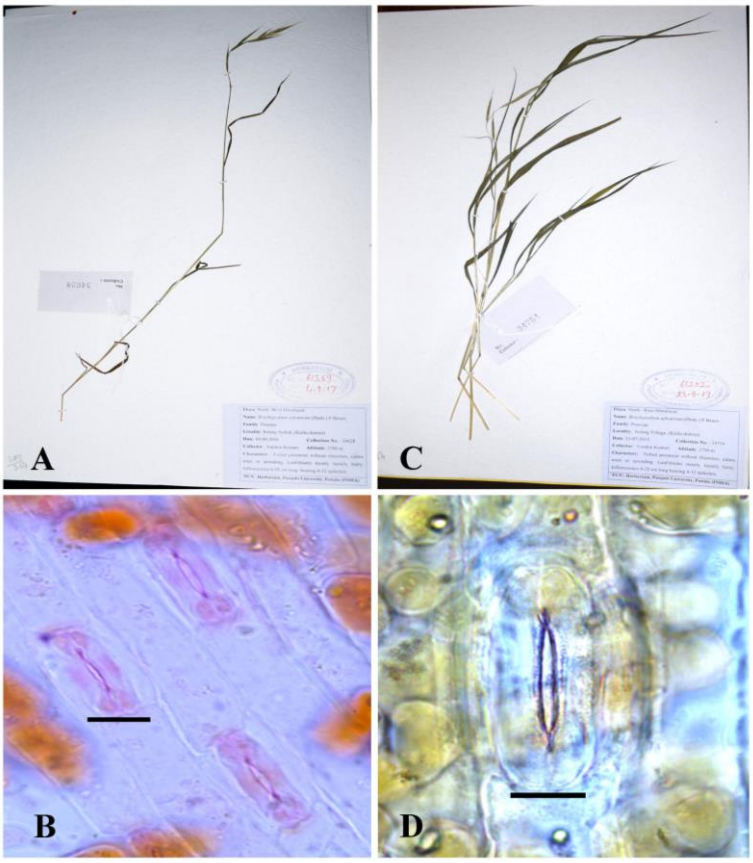

Fig. 1. A) Herbarium specimen of 2*x* cytotype of *Brachypodium sylvaticum.* B) Stomata of 2*x* cytotype. C) Herbarium specimen of 4*x* cytotype. D) Stomata of 4*x* cytotype. Scale bar = 10 µm.

The species has been worked out quite extensively both from India and outside of India. Existing chromosome literature reveals that species depicts an array of chromosome numbers involving intraspecific euploidy and dysploidy. Majority of the cytologists from India and other parts of the world reported a diploid chromosome count of 2*n* = 18 [1-24]. Intraspecific euploid cytotypes (2*x*, 6*x*) based on *x*=7 have been reported for the first time from India [25]. Subsequently, some researchers from outside India have also reported the presence of intraspecific euploid cytotypes (4*x*, 8*x*) based on *x* = 7 in the individuals analyzed from the Bulgaria [26]. A year later, tetraploid chromosome count of with 2*n* = 28 in the plants scored from the from Khasia and Jainitia hills, Shillong in Eastern Himalayas [27]. The presence of B-chromosomes have also has been reported in the tetraploid (2*n* = 28) individuals analyzed from Khasia hills in Eastern Himalayas [28]. Intensive cytomorphological study carried out presently from the wild accessions collected from Kullu district, we here report for the first time the existence of 2*x* and 4*x* cytotypes from the North-West Himalayas with a gametic chromosome count of *n* = 9 and *n* = 18. It thus indicates that the species which is very widely distributed in India and outside of India depicted a considerable amount of chromosomal variability involving the role of dysploidy and euploidy. Perusal of literature reveals that there are reports of other species of *Brachypodium* which also exhibit such chromosomal heterogeneity depicting polyploid races involving two

different basic numbers (*viz. B. distachyon,* 2*x*, 4*x* based on *x*=7; 2*x*, 4*x*, 6*x* based on *x*=5 and *B. pinnatum,* 2*x*, 4*x* based on *x*=7; 2*x*, 4*x* based on *x*=9).

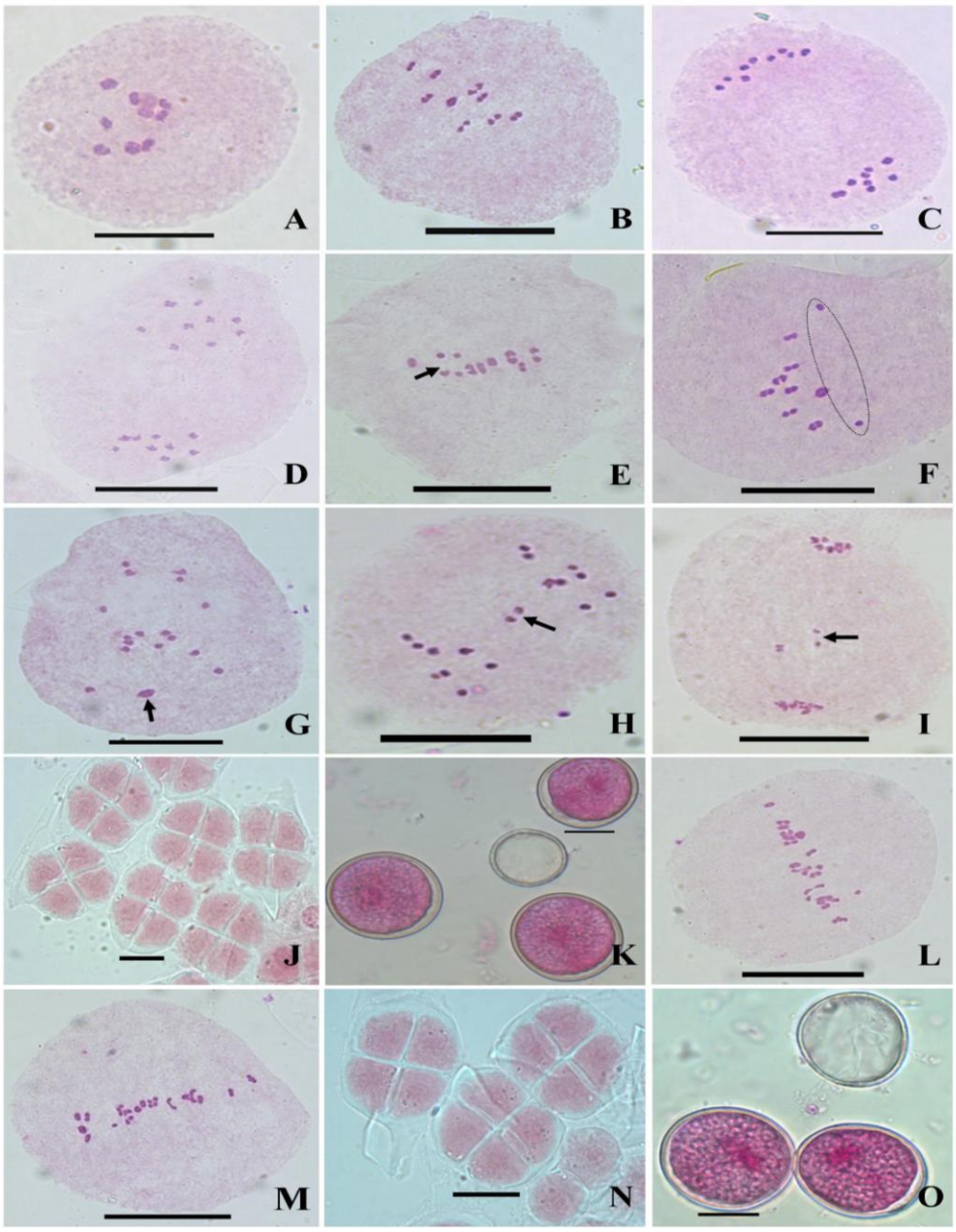

Fig. 2. Meiosis in 2*x* cytotype, A) A PMC showing 9 bivalents at diakinesis. B) A PMC showing 9 bivalents at metaphase-I (M-I). C) A PMC showing 9:9 equal distribution of chromosomes at anaphase-I (A-I). D) A PMC showing 9:9 equal distributions of chromosomes at metaphase-II. E) A PMC showing early disjunction of bivalents at M-I (arrowed). F) A PMC showing early disjunction of a bivalent at M-I (encircled). G) A PMC showing late disjunction of a bivalent at A-I (arrowed). H) A PMC showing 8:2:8 chromosomes at A-I (arrowed). I) A PMC showing laggards at A-I (arrowed). J) Normal sporads. K) Sterile and heterogeneous sized pollen grains. Meiosis in 4*x* cytotype, L-M) PMCs showing 18 bivalents at M-I. N) Normal sporads. O) Sterile and fertile pollen grains. Scale bar= 10 µm.

Polyploidy is the process of acquiescing more than two sets of gametes in the same nucleus by means of intraspecific genome duplication (autoplyploidy) or through hybridization among two distinct species followed by gemome duplication (alloployploidy) potentially has played an important role in the evolution and ecological adoption of plants [29-31]. Scientists have estimated that half of two-thirds of plants are polyploids including more than 99% of the ferns and 80% of the species in grass family [32-33]. Polyploidization as a process of evolution in grasses seems to have favored well as majority of species have vegetative modes of propagation which helped in the success of newly originated polyploids. Polyploidy is also known to alter plant morphology, phenology, physiology and/or ecological adaptation and invasion process of different species [29, 31]. The immediate effect of polyploidy is change in cell size with polyploids having larger size cells than their diploid progenitors [34, 35]. Some workers have suggested that changes in morphological characters due to increase in cell size among polyploids are often associated with plant vigour and ability to grow in different environments [31, 36]. Correlated with a larger cell size lead to alternation in plant morphology and polyploids becomes much taller and robust [34, 37, 38]. Such larger sized polyploids might also have an advantage during invasion due to increased competiveness and vigour [39-41]. In *B. sylvaticum* increased size and robust nature of tetraploids compared to the diploids also seems to have favored in successful adaptation to the varied and harsh climatic conditions and acquiring invasive nature. Such an increase in size of individual vegetative and floral parts and overall robust nature in response to polyploidy has been reported earlier in *Terminalia chebula* [42], *Syzigium cumini* [43], *Centaurea stoebe* [44], *Ranunculus hirtellus* [45], *Dactylis glomerata* [46], *Agrimonia eupatoria* [47], *Silene vulgaris* [48], *Inula grandiflora* [49] and *Physochlaina praelata* [50].

CONCLUSION

Present work concludes the existence of intraspecific euploidy (2*x*, 4*x*) for the very first time in the species. Also the tetraploid chromosome count of 2*n*=36 adds a new cytotype for the species at world level. Both the cytotype differ greatly in terms of their morphological characters and microscopic characters. Diploid cytotype show some meiotic irregularities in form of non-synchronous disjunctions, irregular chromosomal distribution and laggards with reduced pollen fertility, while tetraploid cytotype showed normal segregation and high pollen fertility. Meiotic studies in the presently analyzed individuals will help the plant breeders for future breeding programmes.

ACKNOWLEDGMENTS

The authors are indebted to the University Grants Commission (UGC), New Delhi, for providing financial support under the DRS SAP I, II & III, ASIST programme, DBT-IPLS (DBT Interdisciplinary Programme of Life Sciences for advanced research and education) Project funded by the Department of Biotechnology, Govt. of India and DST for providing financial assistance to Vandna Kumari under the Women Scientist Scheme [award letter no.SR/WOS-A/LS-145/2014/(G)]. Thanks, are also due to the Head, Department of Botany, Punjabi University, Patiala for necessary laboratory, Herbarium (PUN) and library facilities.

REFERENCES

1. Tischler G., Die Bedeutung der Polyploidie für die Verbreitung der Angiospermen, erläutert a den Arten Schleswig-Holsteins, mit Ausblicken auf andere Florengebiete. Botanische Jahrbücher, (1934)
2. Tateoka T., Karyotaxonomic studies in Poaceae. I. Natl Inst Genet Japan, Ann Rep, 4: 45–47, 1953.
3. Tateoka T., Karyotaxonomic studies in Poaceae. III. Natl. Inst. Genet. (Japan), Ann Rep, 6: 73–74, (1956)
4. Hubbard C.E., Grasses: Penguin Books, Middlesex: England, UK, (1954)
5. Matsumura S., Sakamoto S., *Agropyron* and related genera collected in Nepal. Natl. Inst. Genet. (Japan), Ann Rep, 6: 50, (1956)
6. Larsen K., Cytological and experimental studies on the flowering plants of the Canary Islands. Biol Skr Dan Vid Selsk, 11: 1–60, (1960)
7. Hedberg I., Hedberg O., Chromosome counts in British vascular plants. Bot. Not, 114: 11397–399, (1961)
8. Mehra P.N., Khosla P.K., Kohli, B.L., Koonar J.S., Cytological studies in North Indian grasses. I. Res Bull Panjab Uni Sci, 19: 157–230, (1968)
9. Májovský J. et al., Index of chromosome numbers of Slovakian flora Part 2. - Acta Fac Rerum Nat Univ Comen Bot, 18: 45–60, (1970)
10. Saxena B.K, Gupta P.K., in: Love, A. (Ed.), IOPB Chromosome number reports XXVII. Taxon, 19: 437–442, (1970)

11. Mehra P.N., Remanandan P., Cytological investigations on W.I. Himalayan Pooideae. Cytologia, 38: 237–258, (1973)
12. Mehra P.N., Sharma M.L., Cytological studies in some Central and Eastern Himalayan grasses. III. The Agrostideae, Aveneae, Brachypodieae, Bromeae, Festuceae, Phalarideae and Triticeae. Cytologia, 40: 441–452, (1975)
13. Mehra P.N., Sharma M.L. Cytological studies on some grasses of Kashmir. Cytologia, 42: 111–123, (1977)
14. Strid A., Franzen R., in: Löve, A. (Ed.). IOPB Chromosome number reports LXXIII. Taxon, 30: 829–842, (1981)
15. Mehra P.N., Cytology of East Indian grasses. New Delhi. (1982)
16. Guzik M.B., Khromosomnye chisla nekotorykh dikorastushchikh zlakov Predural'ja. Ekologija Opylenija Rastenij. Mezhvuzovskij Sbornik Nauchnykh Trudov. Perm. 82–86, (1984)
17. Luque T., Zarco C.R., Devesa J.A., Números 321-330. In Números cromosómicos para la flora española. 300-364. Lagascalia, 12: 286–290, (1984).
18. Devesa J.A., Ruiz T., Ortega A., Carrasco J.P., Viera M.C., Torno R., Pastor J., Contribución al conocimiento cariológico de las Poaceae en Extremadura (Espana)-I. Bol Soc Brot sér 2, 63: 29–66, (1990)
19. Baltisberger M., Leuchtmann A., Investigations on some Gramineae from Albania and Greece (chromosome numbers and endophyte infection). Ber Geobot Inst ETH Stiftung Rübel, 57: 182–192, (1991)
20. Shi Y., Draper J., Stace C., Ribosomal DNA variation and its phylogenetic implication in the genus *Brachypodium* (Poaceae). Plant Syst Evol, 188: 125–138, (1993)
21. Dobes Ch., Hahn B., Morawetz W., Chromosome numbers for the vascular plant flora of Austria. Linzer Biol Contributions, 29: 5–43, (1997)
22. Lövkvist B., Hultgård, U.M., Chromosome numbers in the South Swedish vascular plants. Opera Bot, 137: 1–42, (1999)
23. Gupta R.C., Chauhan H.S., Saggoo M.I.S., Kaur N., Cytomorphology of some grasses (Poaceae) from Lahaul-Spiti (Himachal Pradesh), India. Biolife, 2: 1234–1247, (2014)
24. Singhal V.K., Kumari V., Kumar P., Cytomorphological diversity in some selected members of Poaceae from Parvati Valley in Kullu district of Himachal Pradesh, India. Plant Syst Evol, 300:1385–1408, (2014)
25. Mehra P.N., Sunder S., Cytological studies in the North Indian grasses. Part II. Res Bull Panjab Uni Sci, 20: 503–539, (1970)
26. Kozuharov S.I., Petrova A.V., in: Love, A. (Ed.), IOPB chromosome number reports XL. Taxon, 22: 28–291, (1973)
27. Mehra P.N., Sood O.P., in: Love, A. (Ed.), IOPB chromosome number reports XLVI. Taxon, 23: 801–812, (1974)
28. Mehra P.N., Sood O.P., in: Love, A. (Ed.), IOPB chromosome number reports XLIX. Taxon, 24: 512, (1975)
29. Leitch A.R., Leitch, I.J., Genomic plasticity and the diversity of polyploidy in plants. Science, 320: 481–483, (2008)
30. Van de Peer Y, Maere S., Meyer A., The evolutionary significance of ancient genome duplications. Nature Reviews. Genetics, 10: 725–732, (2009)
31. te Beest M., Le Roux J.J., Richardson D.M., Brysling A.K., Suda J., Kubešová M., Pyšek P., The more the better? The role of polyploidy in facilitating plant invasions. Ann Bot, 109: 19–45, (2012)
32. Stebbins G.L., Variation and evolution in plants. Columbia University Press, New York 1950.
33. Masterson J., Stomatal size in fossil plants: evidence for polyploidy in majority of angiosperms. Science, 264: 421–424, (1994)
34. Müntzing A., The evolutionary significance of autopolyploidy. Hereditas 21: 363–378 (1936)
35. Stebbins G.L., Chromosomal evolution in higher plants. Edward Arnold Publishers, London, UK 1971.
36. Ramsey J., Ramsey T.S., Ecological studies of polyploidy in the 100 years following its discovery. Phil Trans R Soc, 369 (2014)
37. Garbutt K., Bazzaz F.A., Leaf demography, flower production and biomass of diploid and tetraploid populations of *Phlox drummondii* Hook. on a soil moisture gradient. New Phytol, 93: 129–141, (1983)
38. Bretagnolle F., Thompson J.D., Tansley Review No. 78. Gametes with the somatic chromosome number: mechanisms of their formation and role in the evolution of autopolyploid plants. New Phytol, 129: 1–22, (1995)
39. Blossey B., Nötzold R., Evolution of increased competitive ability in invasive nonindigenous plants: a hypothesis. J Ecol, 83: 887–889, (1995)
40. Jakobs G., Weber E., Edwards P.J., Introduced plants of the invasive *Solidago gigantea* (Asteraceae) are larger and grow denser than conspecifics in the native range. Divers Distributions, 10: 11–19 (2004)
41. Stastny M, Schaffner U., Elle E., Do vigour of introduced populations and escape from specialist herbivores contribute to invasiveness? J Ecol, 93: 27–37, (2005)
42. Gill B.S., Bir S.S., Singhal V.K., Cytogenetics of some timber species of *Terminalia* Linn. (Combretaceae). Indian nat Sci Acad 48B:779–790, (1982)
43. Gill B.S., Singhal V.K., Intraspecific variability in *Syzygium cumini.* J Tree Sci, 10: 23-26, (1990)
44. Mráz P., Barchier P.S, Treier U.A., Schafner U., Müller-Schärer H., Polyploidy phenotypic space and invasion

context: a morphometric study of *Centauera stoebe*. Int J Plant Sci, 172: 386–402, (2011)

45. Kumar P., Singhal, V.K., Male meiosis, morphometric analysis and distribution pattern of 2x and 4x cytotypes of *Ranunculus hirtellus* Royle (Ranunculaceae) from the cold regions of northwest Himalayas (India). Comp Cytogenet, 5:143–161, (2011)
46. Maceira N.O., Jacquard P., Lumaret R., Competition between diploid and derivative autotetraploid *Dactylis glomerata* L. from Galicia. Implications for the establishment of novel polyploid populations. New Phytol, 124: 321-328 (1993)
47. Kumar P., Rana P.K., Himshikha, Singhal V.K., Gupta R.C., Chromosome numbers, characterization of chromosomal pairing during meiosis, origin and natural propagation in polyploid cytotypes (4*x*, 5*x* and 6*x*) of *Agrimonia eupatoria* L. (Rosaceae) in northwest Himalayas (India). Protoplasma, 251:781–795, (2014)
48. Kumar P., Rana P.K., Himshikha, Singhal V.K., Gupta R.C., Cytogeography and phenomenon of cytomixis in *Silene vulgaris* from cold regions of Northwest Himalayas (India). Plant Syst Evol, 300:831–842, (2014)
49. Himshikha, Gupta R.C., Kumar R., Singhal V.K., Cytomixis and Intraspecific Polyploidy (2*x*, 4*x*) in *Inula grandiflora* Willd. from Malana Valley, Kullu District, Himachal Pradesh. Cytologia, 82: 273–278, (2017)
50. Singhal V.K.,Tantray Y.R., Kaur D., Gupta R.C., First report of intraspecific polyploidy (2*x*, 4*x*) in *Physochlaina praelata* (Decne.) Miers. (Family: Solanaceae). Cytologia, 82: 245–250, (2017)

***Corresponding Author:**
V. Kumari*
Email: bits11285@gmail.com