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Chromosomal Diversity in Some Species of *Plantago* (Plantaginaceae) from North India

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Abstract: The present study deals with the detailed cytomorphological analysis and meiotic chromosome behavior in three species (14 accessions) of genus *Plantago*, *P. depressa* (2n = 24, 24+0-1B, 36), *P. major* (2n = 12, 24) and *P. lanceolata* (2n = 12) from North India. Chromosome number n = 12 and 18 for *P. depressa* are worked out for the first time from India. The hexaploid cytotype with n = 18 is a new report to the world. These are observed with various meiotic abnormalities during microsporogenesis. Different accessions of all the species show meiotic irregularities in the form of chromatin stickiness, univalent and multivalent formation, early as well as late disjunction of bivalents, laggards, chromatin bridges, abnormal microsporogenesis, pollen sterlity and variable pollen size. A significant association of bivalents is observed in all the accessions of *P. depressa* and in tetraploid accession of *P. major*.

Key words: Cytotypes, B-chromosome, meiotic abnormalities, morphovariants, *P. depressa, P. lanceolata, P. major*, secondary associations

INTRODUCTION

The genus *Plantago* L. (Plantaginaceae) has approximately 483 species (Tutel *et al*., 2005) of small, inconspicuous plants commonly called plantains. Most of its species show wide range of distribution with maximum concentration in the temperate regions and grow as cosmopolitan weeds, frequently seen along roadsides.

The genus *Plantago* is tribasic; x = 4, 5 and 6 (Dhar and Sharma, 1999) with x = 6 as the primary base number and others as the secondary derivatives of it. The genus represents unique group of plants whose phylogenetic relationships are still under investigations. Polyploidy is one of the prominent phenomenons found in *Plantago* species (Vamosi *et al.,* 2007).

Since, prehistoric times the species of the genus have been cultivated for their various medicinal uses. Phenylethanoid glycosides were isolated from aerial parts of *Plantago* herbs (Atta-ur-Rahman, 2006) and therefore, known for various medicinal purposes (Blumenthal *et al*., 2000). *P. lanceolata* and *P. major* have been used as anti-inflammatory agents (Beara *et al*., 2010). *P. major* contains biologically active compounds such as polysaccharides, lipids, caffeic acid derivatives, flavonoids, iridoid glycosides and terpenoids (Samuelsen, 2000). *P. major* is known to possess hepatoprotective and anti-inflammatory (Turel *et al*., 2009) and hematopoietic activity (Velasco-Lezama *et al*., 2006). *P. lanceolata* is known to show antimicrobial and nematicidal activity (Biere *et al*., 2004; Blumenthal *et al*., 2000). Sorbitol (20) is the characteristic carbohydrate in *Plantago* (Ronsted *et al*., 2000, 2003). Research on different aspects of *Plantago* species is made by various workers (Hammad, 2002; Koocheki *et al*., 2007; Prakash *et al*., 2011; Rifat-uz-Zaman *et al*., 2006; Vahabi *et al*., 2008).

Contributions on cytology of *Plantago* species of different phytogeographic regions have been made by various workers from within India (Dhar *et al*., 2006; Singh *et al*., 2009) and outside (Mohsenzadeh *et al*., 2008). However, there is no report on the cytology of the genus from Kangra area of Western Himalaya. Keeping in view the economic importance and the species and genetic diversity in the genus *Plantago*, the present study is made on the species of the genus on accession basis from Himachal Pradesh and Kashmir of Western Himalayas, as there is no cytological report on the genus from these areas.

MATERIALS AND METHODS

Plant materials of wild taxa of *Plantago* were collected throughout the year in 2007-2009 from different localities of Kashmir and Himachal Pradesh of North India (Table 1). Voucher specimens are deposited in Herbarium, Department of Botany, Punjabi University Patiala (PUN). Appropriate sized flower buds were fixed in Carnoy's fixative, as given by Carnoy in 1887 (6:3:1 = absolute alcohol: chloroform: glacial acetic acid v/v) for 24 h and preserved in 70% alcohol at 4°C. For meiotic studies the

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Table 1: Data on locality, altitude, accession number, chromosome number, ploidy level, pollen fertility and pollen size of presently investigated taxa

Taxa Locality with altitude (m)	Accession number (PUN)	Chromosome number (2n)	Ploidy level	Pollen fertility (%)	Pollen size (µm) ±Standard deviation
***Plantagodepressa* Willd.**					
Ac no.1 (Dharamshala, Himachal Pradesh, 1500)	52448	36	6x*	82	18.37×18.75±0.29
Ac no.2 (Bir; Himachal Pradesh, 1300)	52449	24	4x	90	18.75×24.37±1.59
Ac no.3 (Palampur; Himachal Pradesh, 1219)	52450	24+0-1B**	4x	81	19.50×22.50±2.12
***P. major* Linn.**					
Ac no.4 (Shahpur; Himachal Pradesh, 780)	52451	12	2x	95	15.00×15.37±0.26
Ac no.5 (Badagaon; Chhota banghal, Himachal Pradesh, 3300)	52452	12	2x	96	7.80×15.37±5.35
Ac no.6 (Multhan; Chhota banghal, Himachal Pradesh, 2000)	52453	12	2x	97	15.37×15.37±0.00
Ac no.7 (Garden cultivation; Punjabi University; Patiala; Punjab, 250)	52430	24	4x	86	15.37×15.37±0.00
Ac no.8 (Kulgam; Kashmir, 2000)	52459	12	2x	95	15.00×15.00±0.00
Ac no.9 (Pulwana ; Kashmir, 1850)	52458	12	2x	95	15.37×15.37±0.00
***P. lanceolata* Linn.**					
Ac no.10 (Mcleodganj; Himachal Pradesh, 1780)	52454	12	2x	92	11.25×20.62±3.92
Ac no.11 (Palampur; Himachal Pradesh, 1219)	52455	12	2x	91	15.37×16.12±0.51
Ac no.12 (Multhan; Chhota banghal; Himachal Pradesh, 2000)	52456	12	2x	100	19.12×22.50±1.46
Ac no.13 (Badagaon; Chhota banghal; Himachal Pradesh, 3300)	52457	12	2x	80	15.00×22.50±3.87
Ac no.14 (Kasauli; Himachal Pradesh, 1925)	52431	12	2x	100	19.12×22.50±2.38

*x = Base number, **0-1 B-chromosome is reported besides 24 chromosomes

appropriate sized anthers were squashed in 1% acetocarmine. Pollen fertility was estimated by mounting mature pollen grains in glycero-acetocarmine (1:1). Normal well filled and deeply stained pollens were taken as fertile while shriveled up and unstained pollens as sterile. Measurements of pollen size of fertile pollens were done using ocular micrometer. For stomata studies, mature leaves were treated with KOH, then epidermal peels stained with saffarnin. Photomicrographs of chromosome counts were made from freshly prepared slides using Nikon Microscope Eclipse 80i system. For morphological analysis of plants, various morphological parameters were carefully examined. The statistical tool involves the standard deviation, calculated for the number of observations for a particular character.

RESULTS

Detailed analysis of cytomorphological studies on different accessions of three species of *Plantago* have been done presently. Data on locality, altitude, accession number, chromosome number, ploidy level, pollen fertility and pollen size of presently investigated taxa is provided in Table 1. One new cytotype (6x) is recorded in *P. depressa* for the first time all over the world and this species has been worked out cytologically for the first time from India with tetraploid and hexaploid cytotypes. All the accessions of *P. lanceolata* were diploid (n = 6) whereas in *P. major*, one accession was with tetraploid cytotype (n = 12) and others were diploid (n = 6).

***P. depressa* willd. syn. *P. tibetica* hook f. and thomas morphological variabilities:** *P. depressa* was very variable in its morphological characters and forms a polyploid complex based on basic chromosome number (x = 6). Cytologically, these accessions were found to be on different ploidy levels (4x, 6x), the tetraploid cytotype having more number of leaves/plant as compared to the hexaploid (6x) accession. Leaf surface was hairy, tip acute and lamina was less broad in tetraploid cytotype whereas hexaploid cytotype leaves were glaucous, tip rounded and comparatively has broader lamina. Stomata size and frequency/mm^2 were found to be more in tetraploid cytotype as compared to the hexaploid cytotype (Table 2).

Cytological studies: The present meiotic studies made on three accessions of the species revealed the occurrence of n = 18 in Dharamshala accession and n = 12 in both Bir and Palampur accessions. In Dharamshala accession, the diakinesis showed n = 18 (Fig. 1a) and balanced segregation of 18:18 chromosomes at anaphase-I (Fig. 1b). The bivalents have the tendency of association to form groups either in the form of secondary association of (2-4) bivalents or (2-4) quadrivalents (Fig. 1c,d). The secondary association of bivalents was found in 55% of PMCs at

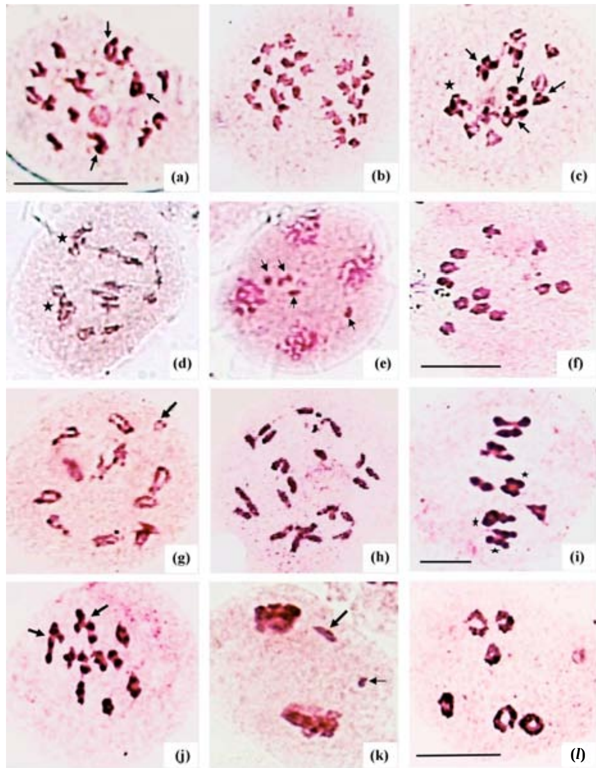

Fig. 1: Meiotic analysis. Scale bar = 10 μm. (a-l). *Plantago depressa* (a) metaphase I with 12 II + 3 IV (→) (b) anaphase I with 2n = 36 (c) metaphase I with 8 II+5 IV (→) and secondary association of bivalents (*) (d) secondary association of bivalents (*) (e) telophase II with laggards (f) metaphase I with 12 II (g) diakinesis with 12 II+1 B (h) metaphase I with 5 II+14 I (i) secondary association of bivalents (*) (j) metaphase I with 8 II+2 IV (→) and interbivalent connections (k) telophase I with laggards, *Plantago major* (*l*) metaphase I with 6 II

Table 2: Comparison of morphological characters of *P. depressa* cytotypes

Character	Tetraploid (2n = 4x = 24)	Hexaploid (2n = 6x = 36)
Plant height (cm)	23±4.24	15±14.6
Number of leaves/plant	26±2.82	9±1.414
Petiole length (cm)	3.5±1.95	3.75±0.25
Leaf size (cm)	5.05±1.12×1.85±0.38	4.6±0.90×2.75±0.873
Leaf tip	Acute	Somewhat rounded
Leaf lamina	Less broad	More broad
Leaf surface	Hairy on both sides	No hairs
Number of spikes/plant	6±1	6±1.414
Peduncle length (cm)	11.25±2.29	7.25±0.353
Spike length (cm)	87.35±1.070	8.35±0.05
Stomata size (μm)		
Upper epidermis	30.00-31.87±0.35× 22.87-23.25±0.1	26.63-27.30±0.41× 15.75-16.13±0.07
Lower epidermis	28.13-30±0.76× 20.6-22.5±0.32	28.13±0.00× 18.75±0.00
Frequency/mm²		
Upper epidermis	14	12
Lower epidermis	13	11
Stomatal Index		
Upper epidermis	30.43-31.12±0.5	30.76-32.43±0.41
Lower epidermis	28.89-30.23±1.42	29.73-31.42±1.42

Values are as Mean±SD

diakinesis/metaphase I. Data on detailed analysis of various chromosomal configurations at diakinesis/ metaphase I stage of meiosis in three accessions of *P. depressa* are compiled in Table 3. Further meiotic course was found to be abnormal (Table 4, Fig. 1e).

The Bir and Palampur accessions were found to exist at tetraploid level with the presence of n = 12 at metaphase I, besides this in some PMCs, there was a presence of one B- chromosome which was reported for the first time in the species. Besides bivalents, some univalents were also observed (Fig. 1f-h). Though, there was presence of 12 II in most of the PMCs but some PMCs have (1-4) quadrivalents (Table 3, Fig. 1i, j). The occurrence of associations among bivalents (2-6) is found in 16% PMCs in Bir accession and 48.34% in Palampur accession. Further meiotic course was found to be abnormal with the formation of laggards and bridges, etc. (Table 4, Fig. 1k). Pollen fertility percentage varies in three accessions whereas there was not much variation in the pollen size.

***P. major* Linn.**

Morphological variabilities: *P. major* also showed variation in its morphological characters and forms a polyploid complex based on x = 6. Cytologically, these accessions were found to be on different ploidy levels (2x), having more number of leaves and spikes/plant as compared to tetraploid (4x) populations. During present investigation great amount of morpho-variability is found within the diploid cytotypes from different altitudinal ranges (Table 5). Some characters like peduncle and spike length, spike diameter and leaf color were distinct features between low altitude accession from Shahpur as compared to other high altitude accessions. Stomata size was more in diploid than in the tetraploid cytotype but the frequency of stomata was more in the tetraploid cytotype. Data on morphological characters and stomata analysis of both the cytotypes was compiled in Table 6.

Table 3: Chromosomal associations at diakinesis/metaphase I in three accessions of *Plantago depressa*

		PMCs observed		Configurations	
Accession	2n	No.	%	IV	II
Ac No. 1	36	18	60.00	0.00	18.00
		5	25.00	1.00	16.00
		1	5.00	2.00	14.00
		1	5.00	4.00	10.00
		1	5.00	5.00	8.00
Total		26	100.00	16.00	436.00
Average frequency/PMC				0.61	16.76
% of chromosomes				6.77	93.11
Ac No. 2	24	14	82.35	0.00	12.00
		3	17.64	2.00	8.00
Total		17	99.99	6.00	192.00
Average frequency/PMC				0.35	11.29
% of chromosomes				5.84	94.08
Ac No. 3	24	12	37.50	0.00	12.00
		2	6.25	0.00	12.00
		2	6.25	1.00	10.00
		5	15.62	2.00	8.00
		7	21.87	3.00	6.00
		4	12.50	4.00	4.00
Total		32	99.99	49.00	142.00
Average frequency/PMC				1.53	4.43
% of chromosomes				25.50	36.92

Cytological studies: The present meiotic studies made on six accessions of the species revealed the occurrence of n = 6 and 12 number. Five populations revealed the occurrence of n = 6 at metaphase-I. Diploid accessions showed abnormal meiotic behavior in the form of various irregularities. Phenomenon of transfer of chromatin material was found to be quite prominent in Badagaon and garden cultivated population at Patiala. As a result of this in some PMCs aneuploid (hypo and hyperploid) number was observed (Table 4, Fig. 1*l*, 2a, b). Pollen fertility

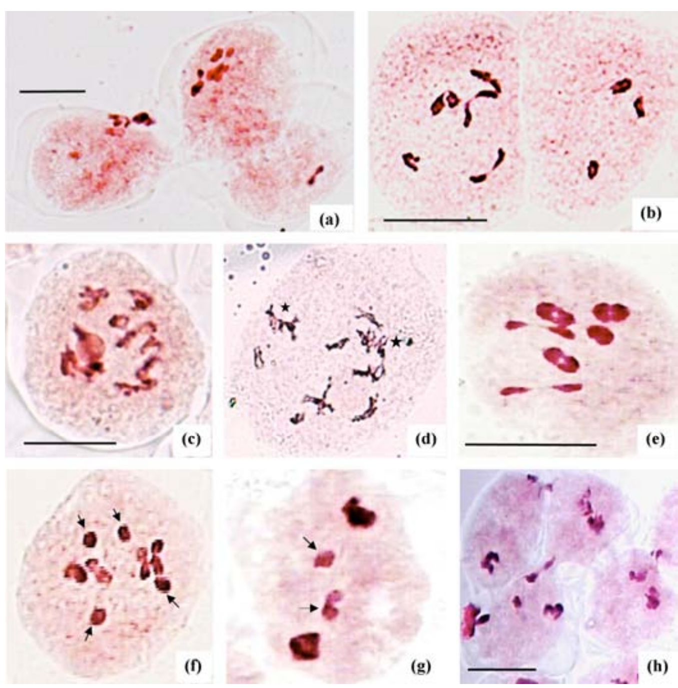

Fig. 2: Meiotic analysis. Scale bar = 10 µm. (a-h) *Plantago major* (a) Three PMCs involved in cytomixis (b) PMCs with aneuploid (n = 5 and 3) number (c) diakinesis with n = 12 (d) metaphase I showing secondary association of bivalents (*), *Plantago lanceolata* (e) metaphase I with 6 II (f) metaphase I with early disjunction of 2 bivalents (g) telophase I showing late disjunction of 2 bivalents (h) PMCs involved in cytomixis

Table 4: Frequency of meiotic abnormalities in different accessions of presently investigated taxa

Accession No.	Cytomixis: Meiotic stages/s	Cytomixis: No. of PMCs involved	Cytomixis: % age of PMCs involved	PMCs with laggards (%) A-I/T-I	PMCs with laggards (%) A-II/T-II	PMCs with bridges (%) A-I/T-I	PMCs with bridges (%) A-II/T-II	PMCs with late disjunction of bivalents (%) at A-I/T-I	Abnormal microsporogenesis
1	-	00.00	00.00	16.90	15.00	10.18	9.24	00.00	25.95
2	-	00.00	00.00	13.34	12.00	10.80	9.80	00.00	00.00
3	P-I, M-1	2-6	23.40	1.50	1.00	8.14	8.00	14.00	00.00
4	-	00.00	00.00	18.00	17.42	13.14	12.80	00.00	4.71
5	M-I	2-6	2.72	1.72	1.50	2.10	2.00	00.00	00.00
6	-	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00
7	P-I, A-1	2-6	40.27	1.50	1.40	0.70	0.60	00.00	12.60
8	-	00.00	00.00	1.50	1.20	0.50	0.50	00.00	00.00
9	-	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00
10	M-1, A-1	2-4	29.10	7.76	7.10	9.91	9.00	8.90	00.00
11	-	00.00	00.00	1.40	1.22	0.80	0.50	1.63	4.54
12	-	00.00	00.00	7.00	6.50	3.00	2.82	00.00	00.00
13	P-I, M-1	2-6	22-29	10.00	9.34	2.42	2.50	4.34	20.66
14	-	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00

For meiotic analysis, a minimum of 50 PMCs were observed at each stage of meiosis. Abbreviations: Prophase-1 (P-I), Metaphase-I (M-I), Anaphase-I/II (A-I/II), Telophase-I/II (T-I/II), means no cytomixis is observed at any meiotic stage

Table 5: Data on morphological characters of different accessions of *P. major* (n=12)

Character*	Ac No. 4	Ac No. 5	Ac No. 6
Plant height (cm)	36	45	37
Number of leaves/plant	8	6	6
Leaf size (cm)	9.5×4.3	9.5×4.6	9.5×4.8
Petiole length (cm)	5.5	3.5	5.5
Number of spikes/ plant	3	4	4
Spike length (cm)	11	28	27
Peduncle length (cm)	23	16.5	20
Spike diameter (cm)	0.45	0.10	0.10
Leaf color	dark green	green	green

*Based on minimum of eight readings

Table 6: Comparison of morphological characters of *P. major* cytotypes

Character	Diploid (2n = 2x = 12)	Tetraploid (2n = 4x = 24)
Plant height (cm)	36.5±6.80	37.5±3.50
Number of leaves/plant	7.5±2.79	28±2.82
Petiole length (cm)	5.1±1.10	5±1.41
Leaf size (cm)	10.16±0.41×4.7±0.36	10.15±1.20×7.3±1.55
Leaf tip	Acute	Rounded to acute
Leaf color	Green to dark green	Green
Number of spikes/plant	4.5±0.57	23.5±4.12
Peduncle length (cm)	17.45±5.05	23±1.41
Spike length (cm)	22.5±11.09	17±1.414
Spike diameter (cm)	0.275±0.22	0.10±0.00
Stomata size(μm)		
Upper epidermis	30.00-33.75±0.5× 18.75-21.75±0.42	20.63-24.37±0.513× 15.00-19.13±0.60
Lower epidermis	28.13-30±0.35× 18.75-19.13±0.07	22.50-24.37±0.28× 18.75-22.50±0.51
Frequency/mm²		
Upper epidermis	9	12
Lower epidermis	9	20
Stomatal Index		
Upper epidermis	21.95-22.50±0.5	25.32-25.00±0.70
Lower epidermis	23.07-23.68±0.5	22.22-23.07±1.42

percentage varied between 95-97 and pollen size is 15.00×15.37 μm in all diploids but Badagaon accession showed a wide range of difference in pollen size (7.8-15.00) μm.

The garden cultivated accession of the species is found to exist at tetraploid level with n = 12 (Fig. 2c). Besides, the presence of bivalents, some quadrivalents

Table 7: Chromosomal associations at diakinesis/ metaphase I in tetraploid accession of *P. major*

Accession	2n	PMCs observed No.	PMCs observed %	Configurations IV	Configurations II	Configurations I
Ac No. 7	24	28	66.67	0	12.0	0.0
		1	2.38	0	11.0	4.0
		2	4.76	0	8.0	8.0
		1	2.38	1	7.0	6.0
		1	2.38	1	8.0	4.0
		2	4.76	1	10.0	0.0
		1	2.38	2	6.0	4.0
		2	4.76	2	7.0	2.0
		1	2.38	2	8.0	0.0
		1	2.38	3	6.0	0.0
		2	4.76	4	4.0	0.0
Total		42	99.99	23	440.0	62.0
Average frequency/PMC				0.54	10.47	1.47
% of chromosomes involved				0.09	87.25	6.13

(1-4) and univalents (2-8) were also found (Fig. 2d). The bivalents (2-4) join end to end to form associations which is reported in 28.57% PMCs at diakinesis/metaphase I. Data on detailed analysis of various chromosomal configurations at diakinesis/ metaphase I in tetraploid accession was compiled in Table 7. Chromosomal stickiness was also observed in 38.43% of PMCs. Further meiotic course was abnormal (Table 4). Pollen fertility is reduced to 86%.

P. lanceolata Linn.

Morhological variabilities: Presently studied five accessions of *P. lanceolata* showed a great morphological variability within diploid cytotypes from different altitudinal ranges. There was a clear distinction in leaf color and wide range of difference in petiole length, peduncle length and plant height. Accessions from lower ranges show dark green colored leaves, comparatively shorter plant height, petiole and peduncle length than those from higher altitudes (Table 8).

Table 8: Data on morphological characters of different accessions of *P. lanceolata* (2n = 12)

Character*	A. No. 10	A. No. 14	A. No. 11	A. No. 12	A. No. 13
Plant height (cm)	17	16	47	67	45
Number of leaves/plant	19	18	29	13	9
Leaf size (cm)	5.5×1.5	5.3×1.4	6.9×1.4	15×2.3	11×1.5
Petiole length (cm)	0.7	0.6	3	13	8
Number of spikes/plant	8	7	12	16	6
Spike length (cm)	4.1	4	5	8	3
Peduncle length (cm)	13	11	39	55	37
Leaf color	dark green	dark green	dark green	green	green

*Based on minimum of eight readings

Cytological studies: All the five accessions were found to exist at diploid level with n = 6 at metaphase I (Fig. 2e). Meiotic studies revealed the presence of highly abnormal meiotic course starting from early prophase I up to tetrad phase of meiosis. Interbivalent connections in 2-3 bivalents were observed at metaphase I. In Palampur accession some PMCs showed chromatin stickiness. Phenomenon of transfer of chromatin material was found to be quite prominent in Mecleodganj and Badagaon accession. As a result of this in some PMCs hypoploid and hyperploid numbers are observed. Further course of meiosis was found to be abnormal with early as well as late disjunction of 1-2 bivalents (Table 4, Fig. 2f-h). Pollen fertility varied between 80-100 per cent and there was wide range of pollen size (11.25-22.50 μm) in different accessions.

DISCUSSION

Two new cytotypes (4x, 6x) for *Plantago depressa* are new reports to India and hexaploid cytotype with n = 18 for the species is reported for the first time from world with only earlier reports of n = 6 (Huss, 1981; Khatoon and Ali, 1993) and n = 12 (McCullagh, 1934). The presence of 1B- chromosome, secondary associations of bivalents and quadrivalents are observed for first time in the species.

Chromosome number n = 6 and 12 for *P. major* is in conformity with the previous report by Sharma *et al.* (1992). Besides the diploid cytotype, a tetraploid cytotype is also known to occur in nature (Sharma and Koul, 1995). The species reveals the polymorphic nature as has also been reported by Jain (1978b). Van Dijk *et al.* (1988) reported the presence of distinct ecotypes in the species. Presently, cytomixis has been recorded in the species *P. major* which results in aneuploidy as reported by Sharma and Koul (1995) in Kashmir Himalayan populations. However, there is no previous report on chromosomal associations found in this species. Geographical and ecotypic variations have a significant effect on morphology of the plant. This is basically due to genetic changes influenced by change in environmental conditions (Bradshaw, 1984). Altitude variations differentially influence the morphological and biochemical responses of *P. major* (Prakash *et al.*, 2011).

All accessions of *P. lanceolata* with n = 6 found to exist at diploid level and is in conformity with the previous reports (Brullo *et al.*, 1985; Sharma *et al.*, 1992). *P. lanceolata* is also a polymorphic species (Sharma *et al.*, 1984) as morphological variable individuals have been reported from the Kashmir Himalayas (Jain, 1978a). Our present attempt to study cytomorphological diversity has been made on lower as well as higher altitude populations of *P. lanceolata*. The phenomenon of gene flow and translocation heterozygosity and high level of gene flow in *P. lanceolata* (Bos and van Der Haring, 1988) led to the evolution in the species. It is characterized by a high degree of phenotypic plasticity and heterozygosity (Hammad, 2002). Munshi *et al.* (1994) reported the presence of aneuploid cytotypes (2n = 13, 14), besides the diploid cytotype in *P. lanceolata*. As the amount and the qualities of the economical part is known to vary with the climate and the chromosome constitutes, there is need to access these economic factors in the different taxa to mark out the elite germplasm for commercial cultivation and exploitation.

Amount of DNA/genome has a significant affect which showed that x = 6, most common base number and has more amount of DNA content as compared to x = 4, a rare number and has less amount of DNA content. The base number x = 6 is an ancestral number and its reduction to x = 4 through progressive reduction in DNA content made it to be a derivative of x = 6, a primary base number (Badr *et al.*, 1987). Phenomenon of transfer of chromatin material as a result of which aneuploidy (hypo and hyperploidy) besides normal ploidy were reported in *P. major* and *P. lanceolata*. These reports showed the presence of polyploid and aneuploid cytotypes raised from base number, x = 6 (Dhar and Sharma, 1999).

In the evolution of these two complex polymorphic species *P. lanceolata* and *P. major*, there has been evolutionary progress either through addition or a diminuation of DNA sequences. If we see the genetic distance between *Plantago* species, there is a marked difference of 5.3 and 3.54 picograms of DNA value in *P. lanceolata* and *P. major,* respectively (Sen and Sharma, 1990). The results showed a wide range of differences in these two species with *P. major* as an advanced one and an example of regressive evolution.

Exhaustive studies were made on this complex group *Plantago* to know its phylogeny with respect to its morphology, embryology and chemical analysis (Rahn, 1996) and physical mapping of rRNA (Ronsted *et al*., 2002). As a result of this, one genus, *Plantago* has been recongnized with approximately 200 species, in family Plantaginaceae and raised six subgenera. The iridoids distribution in *Plantago* sp. supports phylogenetic studies of DNA sequences (Ronsted *et al*., 2003) which is in accordance with the previous phylogenetic studies made by Rahn (1996).

CONCLUSION

The presence of intraspecific polyploid cytotypes, coupled with variations in morphology and meiotic behaviour within same cytotypes, clearly points out the fact that the different species of the genus are in active process of evolution, covering both chromosome number and mutations. The clear cut qualitative differences within the same species point out to the fact that these might be species complexes having distinct sub-species groups. Further, due to the variation in economically important constituents in different cyto-morphotypes, there is need to further explore the variability from different areas on accession basis to mark out the better genotype/ chemotypes for further exploitation and to understand the evolutionary processes operative in the species within the genus. In this regard, molecular and morphological markers are useful for the evaluation of diversity within populations of a species, *Plantago ovata* (Vahabi *et al*., 2008).

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