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CYTOGENETIC EXPLORATION OF *PLANTAGO LANCEOLATA* LINN.: THE SOLDIER'S HERB, IN JAMMU & KASHMIR (INDIA)

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Abstract: The present investigation deals with the detailed meiotic, karyotypic and cyto-morphological analysis of diploid and trisomic populations with chromosome numbers ranging from 2n=12 to 2n=13 in Plantago *lanceolata* belonging to family plantaginaceae from the region of Jammu and Kashmir. The mitotic and morphological irregularities were recorded in different polyploids.

Key words: Plantaginaceae; *Plantago lanceolata;* Karyotype; Trisomic; Meiosis; Chromosome number

INTRODUCTION

The botanical name *"Plantago lanceolata"* is derived from the word "Planta", a foot, and "ago" a wort (meaning plant) in allusion to the shape of the broad leaves. Tradition maintains that English plantain springs up wherever English people set foot, no matter what the climate is. *Plantago lanceolata* is a species of genus *Plantago* and is known by different common names given in table 1. It is a common weed of cultivated land. The plant likes sandy, medium (loamy) and heavy (clay) soils but prefers well-drained soil. It can grow in nutritionally poor soil with neutral, basic and/or alkaline nature and cannot grow in the shade and tolerates maritime exposure. The plant succeeds in any moderately fertile soil in a sunny position (Huxley A. 1992) and also in very poor land (Grieve 1984).

Table 1: Common names of *Plantago lanceolata*

1.	English Plantain
2.	Plantain
3.	Snake Plantain
4.	Black Plantain
5.	Buckhorn Plantain
6.	Lance-leaf Plantain
7.	Long Plantain
8.	Narrow leaved Plantain
9.	Soldier's Herb
10.	Rib leaf
11.	Ribwort
12.	Ribgrass
13.	Black Jack
14.	Jackstraw
15.	Hen Plant
16.	Wendles
17.	Kemps
18.	Cocks
19.	Quinquenervia
20.	Costa Canina
21.	Blackjack
22.	Lamb's Tongue
23.	Ribble Grass

The leaves are gray-green to dark green, dense basal rosette, linear-elliptical shaped, spreading or erect, smooth or scarcely toothed around edges, 3 to 5 well-defined parallel veins along the length of each leaf. The roots are shallow, crown of coarse fibrous roots early which become thick rhizome and fibrous roots later. The stems are wiry, erect, leafless, silky, slender, 4 to 16 inches tall, bearing one or more leafless, green, unbranching, oblong flower spike at tip, often with a slight groove going up the stem or angular, scattered hairs toward the base of the plant (Figure 1, a-c). The plant is in flowering from April to August and the flowers are hermaphrodite, tiny, white in color, hairy, scentless, densely clustered together, facing in all directions along a capsule-shaped spike. The inflorescence is 1/2 to 3 or 4 inches long and 1/3 to 1/2 inch thick, surrounded by a halo of anthers at the ends of long thin stamens protruding from the flowers. During bud stage flower spike is gray-green and is cone shaped at the top, but it becomes light brown and capsule-like as the flowers bloom from bottom to top. The sepals and bracts which are brownish give the spike its predominantly dark color. The petals are white, 4 lobed that are bent back with brown midribs. The stamens are 4 in number; long in size with white slender filaments which bear off-white anthers on tips (Figure 1, d-f).

Ribwort plantain is a safe and effective treatment for bleeding; it quickly staunches blood flow and encourages the repair of damaged tissue (Chevallier A., 1996). The leaves contain mucilage, tannin and silica acid (Phillips & Foy, 1990). An extract of these has antibacterial properties (Chopra *et al.*, 1986), have a bitter flavor and are astringent, demulcent, mildly expectorant, haemostatic and ophthalmic (Launert E., 1981; Triska 1975; Lust J., 1983; Singh *et al,* 1976; Mills 1988; Foster *et al,* 1990; Chevallier A., 1996). Internally, they are used in the treatment of a wide range of complaints including

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diarrhea, gastritis, peptic ulcers, irritable bowel syndrome, hemorrhage, hemorrhoids, cystitis, bronchitis, catarrh, sinusitis, asthma and hay fever (Bown D., 1995; Chevallier A., 1996). They are used externally in treating skin inflammations, malignant ulcers, cuts, stings etc (Grieve 1984). The heated leaves are used as a wet dressing for wounds, swellings etc (Weiner M. A., 1980; Foster & Duke, 1990). The root is a remedy for the bite of rattlesnakes; it is used in equal portions with *Marrubium vulgare* (Coffey T., 1993). The seeds are used in the treatment of parasitic worms (Weiner M. A., 1980). Plantain seeds contain up to 30% mucilage which swells up in the gut, acting as a bulk laxative and soothing irritated membranes (Bown D., 1995). Sometimes the seed husks are used without the seeds (Bown D., 1995). Distilled water made from the plant makes an excellent eye lotion (Chiej R., 1984). A good fibre obtained from the leaves (Grieve 1984), is suitable for textiles. Mucilage from the seed coats is used as a fabric stiffener (Grieve 1984). It is obtained by macerating the seed in hot water (Polunin 1969). Grae I. (1974) reported that the gold and brown dyes are obtained from the whole plant and it is an important food plant for the caterpillars of many species of butterflies (Carter D., 1982). The current paper deals with the varied cytology and cyto-morphological analysis of diploid and trisomic populations of *Plantago lanceolata* of Jammu and Kashmir, India.

Materials and Methods

The plants of *P. lanceolata* were collected from different zones of Jammu & Kashmir and grown in the earthen pots in School of Biotechnology, University of Jammu, Jammu (Figure 1).

Figure 1 (a-c): Vegetative Morphology of *Plantago lanceolata*

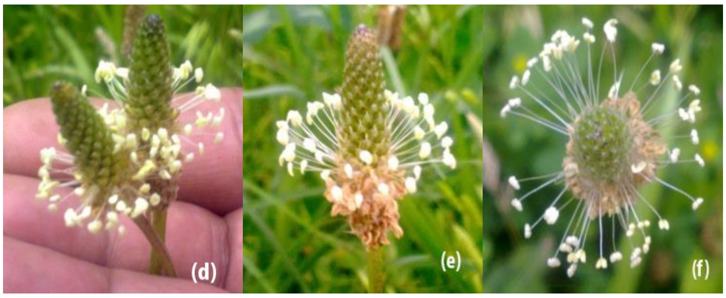

Figure 1 (d-f): Inflorescence of *Plantago lanceolata*

The protocols of Pandita *et al,* (2013) and Pandita (2013) were followed for cytogenetic exploration in these populations of *P. lanceolata* wherein the PDB (Para-Dichlorobenzene) treated root tips were initially fixed in Carnoy's fluid, hydrolyzed in 1NHCL, stained in feulgen stain and finally squashed in a drop of 1% acetocarmine. All the cytological observations were done from temporary mounts. The observations and photography was done under the light microscope. The karyotypic parameters of both the cytotypes were studied from photo micrographs. The relative chromosome length and T.C.L. % were calculated by applying the following formulae:

$$\text{Relative Chromosome Length} = \frac{\text{Absolute Length of a chromosome}}{\text{Length of Longest chromosome}} \times 100$$

$$\text{T.C.L. \%} = \frac{\text{Absolute Length of a chromosome}}{\text{Total Length of chromosome Complement}} \times 100$$

Further, the Mean Chromosome Length (MCL), ratio of the size of the Longest to Smallest Chromosome (LC/SC) of the somatic complements was also studied. For meiotic studies, the young plant spikes containing anthers were fixed in a mixture of 4 parts chloroform, 3 parts ethyl alcohol and 1 part of acetic acid and a pinch of ferric chloride and then preserved in 70% ethyl alcohol at 4°C. The anthers were squashed in 1% acetocarmine and different meiotic stages were observed in pollen mother cells. The chiasmata frequency and recombination index was calculated at Diakinesis and Metaphase-I. The terminalization coefficient was also calculated. These were calculated by applying the following formulae:

Recombination index = n + Chiasmata frequency/cell

Where n is the number of bivalents

$$\text{Terminalization coefficient} = \frac{\text{Average number of terminalized chiasmata / PMC}}{\text{Average number of total chiasmata / PMC}}$$

Results and Discussion

A large number of species of the genus *Plantago* have been worked out cytologically by Heitz (1927); Turesson (1922a), (1922b), (1928); Hyde (1945, 1953); Basset (1965-1967) etc. The information about *P. lanceolata* is, however, scanty though Nakajima (1930); McCullagh (1934); Boocher (1943) and Basset (1967) have tackled this species.

Meiosis in all the four forms proceeds regularly. The different stages of meiotic chromosomes viz; Diakinesis, Metaphase and Anaphase were observed (Figure 2). Meiotic base number for *P. lanceolata* is 06 (Darlington and Wylie, 1958). The Nucleolus was prominent at Prophase-I stage of meiosis. Chromosomes at metaphase stage were present on Metaphase plate. During Anaphase-I regular chromosome segregation (06< >06) was observed. Six bivalents are formed which show perfect segregation at anaphase 1. Rod bivalents, in general, are larger in number than rings. The number of Rod Bivalents was 04 and Ring Bivalent was 02 & nucleolar chromosomes were 02 in *P. lanceolata.* The Anaphase-I was regular in all the PMCs. The number of late separating bivalents was 01 (Figure 2). The Chiasmata Frequency was calculated at Metaphase-I as 04/PMC & 01-02/Bivalent and at Diakinesis was 11/PMC & 02/Bivalent. The other parameters studied included Recombination Index and Terminalization Coefficient. The Recombination Index at Diakinesis of *P. lanceolata* was 17 and at Metaphase was 07-08. The Terminalization Coefficient was 0.25 in *P. lanceolata.* Comparative data on bivalent form, Chiasmata frequency (at Diplotene and Metaphase) and terminalization coefficient are summed up in (Table 2).

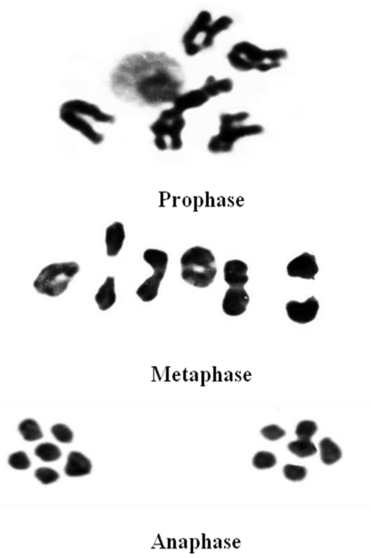

Figure 2: Meiotic Chromosomal stages of the Diploid *Plantago lanceolata*

Table 2: Details of PMC Meiosis in Diploid Cytotype of *Plantago lanceolata*

Number of Nucleolar Chromosomes			02
Number of Ring Bivalents			02
Number of Rod Bivalents			04
Diakinesis	Number of Chiasmata	Per PMC	11
		Per Bivalent	02
		Recombination Index	17
Metaphase- I	Number of Chiasmata	Per PMC	04
		Per Bivalent	01-02
		Recombination Index	07-08
		Terminalization Coefficient	0.25
Anaphase -I	Regular/Irregular		Regular
	Number of Late Separating IIs		01

The chromosome complements of *P. lanceolata* reported earlier are 2n = 12, 18, 24. Our count of 2n=12 agrees with that given by McCullagh (1934), Briggs (1973), Brullo (1985), Matsuo & Noguchi (1989) and Sharma *et al.*, (1992). Boocher *et al.*, (1943) reported 2ns, 12 + 1 in some French forms in accordance with our studies. McCullagh (1934) has reported tetraploids and hexaploids of this species. The diploid chromosome complement of *Plantago lanceolata* has 12 chromosomes (Figure 3a), which can be categorized into two groups. The first group comprises of 8 Sub-Median chromosomes and the second group consists of 4 Sub-Terminal chromosomes, each bearing a satellite at the distal end of short arm (Figure 4). All metaphase spreads of the trisomic have 13 chromosomes (Figure 3b). The habitat, geography and enviormental variations instigate genetic changes which have a significant upshot on plant morphology (Bradshaw 1984; Prakash *et al*, 2011). The diploid plants were evaluated for different morphological traits (Table 3; Figure 1, 3). The trisomic plants are broad and stronger in contrast to the diploids; which bear thicker and broader leaves. The plant height, petiole length and peduncle length exhibit slight increase in size. The most striking modification is in the leaf number and spike number per plant. The effect of polyploidy on the phenotype of individual cells has been estimated by comparing the size, frequency and index of stomata.

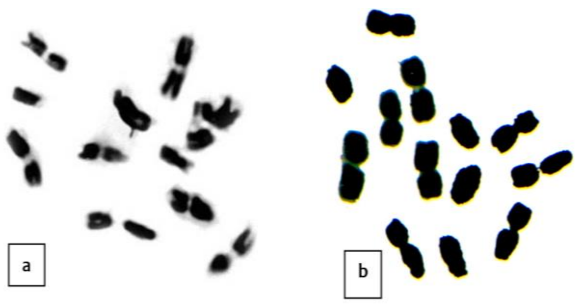

Figure 3: Somatic Chromosome Complements of the (a) Diploid *P. lanceolata* & (b) Trisomic *P. lanceolata*

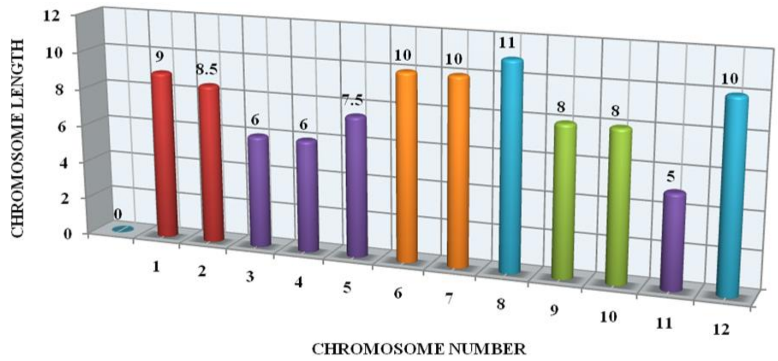

Figure 4: Histogram of the Chromosome Number versus Chromosome Length

Table 3: Morphometry of Somatic Chromosome Complement of Diploid *Plantago lanceolata*

Chromosome Number	Short Arm (mm)	Long Arm (mm)	Absolute Length (mm)	Arm Ratio (mm)	Index Number (mm)	Relative	T.C.L. %
1	4	5	9	1.25	0.8	81.81	9.09
2	3.5	5	8.5	1.42	0.7	77.27	8.58
3	2	4	6	2	0.5	54.54	6.06
4	3	3	6	1	1	54.54	6.06
5	2.5	5	7.5	2	0.5	68.18	7.57
6	5	5	10	1	1	90.90	10.10
7	2	8	10	4	0.25	90.90	10.10
8	4	7	11	1.75	0.57	100	11.11
9	4	4	8	1	1	72.72	8.08
10	4	4	8	1	1	72.72	8.08
11	1	4	5	4	0.25	45.45	5.05
12	4	6	10	1.5	0.66	90.90	10.10
T.C.L.				99mm			
M.C.L.				8.25mm			
LC/SC				1.83 mm			

Conclusion

To conclude, the intraspecific polyploid cytotypes, exhibiting chromosome number and morphological variations in plants conclude that this species is in vigorous evolutionary process. So, the need of the hour is to explore this species from different geographical and ecological niches to understand the evolutionary processes involved in this species which can be accomplished with further application of morphological and molecular markers.

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Conflict Of Interest

We confirm that there are no known conflicts of interest associated with this manuscript. The manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed and the order of authors listed in the manuscript has been approved by all of us.

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