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Cytological study in some species of *Lactuca* L. (Asteraceae) from high altitudinal regions of Kinnaur district, Himachal Pradesh, India

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ABSTRACT: The Present study includes cytomorphological analysis on five species of *Lactuca* L. (Asteraceae) from the hilly district of Kinnaur, Himachal Pradesh (India). *Lactuca orientalis* (Boiss.) Boiss. (2*n*=18) which has been counted chromosomally for the first time from India also adds a new intraspecific 2*x* cytotype to the earlier tetraploid chromosomal report of (2*n*=36) from outside of India. The chromosome counts for *L. dissecta* D. Don (*n*=8), *L. dolichophylla* Kitam. (*n*=8), *L. macrorhiza* (Royle) Hook. f. (*n*=8) and *L. serriola* L. (*n*=9) confirm the earlier reports from India and other parts of the world. The wild accessions of *L. serriola* L. from the region exhibit considerable morphological variability in leaf characters but shared the same diploid chromosome number (2*n*=18). Such variation in morphological characters where morphovariants grow under similar ecological condition could be attributed to genetic reasons. Meiotic analysis reveals that chromosomes are small-sized in *L. dissecta*, and *L. dolicophylla* compared to *L. macrorhiza*, *L. orientalis* and *L. serriola* which possessed larger sized chromosomes. Besides, *L. orientalis* and *L. serriola* depict relative differences in the size of bivalents in a genome which could be attributed to unequal reciprocal translocations.

KEY WORDS: Asteraceae, Chromosome number, Chromosome size, *Lactuca*, Meiosis, Morphovariants, Pollen fertility.

INTRODUCTION

The genus *Lactuca* L. belonging to tribe Cichorieae of the family Asteraceae, is represented by 147 species of annuals, biennials or perennials worldwide (Lebeda *et al*. 2007; Bhellum and Singh, 2015). *Lactuca* has been analyzed for its wide geographical distribution (Lebeda *et al*. 2004), genetic diversity (Lebeda *et al*. 2014), and chromosomal studies (Matoba *et al*. 2007; Kaur and Singhal 2015; Singhal *et al*. 2018). Majority of the species of genus *Lactuca* L. are xerophytes, well adapted to dry climatic conditions except some endemic species of Central African mountains and Eastern African which are confined to subtropical to tropical rain forests. The species are characterized by abundant latex, rhizomatous and fusiform/tuberous roots. Leaves are very variable, radical or alternate, entire or dentate or variously toothed, often lobed, pinnate, pinnatifid or runcinate-pinnatifid and sessile or petioled. Perusal of chromosome literature reveals that in spite of attempts by several cytolgists (Gupta and Gill, 1983, 1989; Mathew and Mathew, 1988; Malik, 2012; Bala and Gupta, 2013; Kaur and Singhal, 2015; Rana *et al*. 2015; Gupta *et al*. 2017), only 13 species of the genus are known chromosomally from the hilly region and plains of India. Still some regions of Indian Himalayan are yet to be explored chromosomally and one such region is Kinnaur district of Himachal Pradesh. The present study includes the cytomorphological analysis of five species of *Lactuca* L. (Asteraceae) from the cold desert and high altitude region of this tribal district.

MATERIALS AND METHODS

Collection and submission of samples

For exploration of cytomorphological diversity, field surveys were conducted in the different localities of Kinnaur district of Himachal Pradesh (India) during the months of April to September for five years (2007–2011). In each species 1–2 individuals were analyzed for chromosome counts, meiotic analysis and pollen fertility. Voucher specimens of the cytologically worked out individuals were deposited in the Herbarium, Department of Botany, Punjabi University, Patiala (PUN).

Cytological Analysis

Young capitula were fixed in a freshly prepared Carnoy's fixative (6 ethanol: 3 chloroform: 1 acetic acid v: v: v) for 24h and preserved in 70% alcohol at 4°C. Meiocyte preparations were made by squashing the young and developing anthers in 1% acetocarmine following standard method of Belling (1926). In each case, 400 meiocytes were observed at different meiotic stages of prophase-I, metaphase-I/II, anaphase-I/II, anaphase-I/II and sporads. Pollen viability was estimated through stainability tests for which anthers from mature flowers were squashed in glycerol-acetocarmine (1:1) mixture and 1% aniline blue dye (Marks, 1954) Well filled pollen grains with fully stained nuclei and cytoplasm were scored as fertile while shrivelled with partially or unstained cytoplasm were counted as sterile.

Table 1: Information on locality with altitude, accession number/s (PUN), meiotic chromosome number, ploidy level, pollen fertility % age, and previous chromosome reports on the cytomorphologically investigated ***Lactuca*** species.

Taxon	Locality with altitude (m)	Accession number/s (PUN)	Meiotic chromosome number (*n*)	Ploidy level	Pollen fertility (%)	Previous chromosome reports
Lactuca dissecta D. Don	Sunnum, 2680	55757	8	2*x*	100	**2n=16** Mehra & Sidhu (1960), Mehra *et al.* (1965), Shetty (1967), Kaul & Singh (1972), Kaul (1974), Remanandan & Mehra (1974), Koul *et al.* (1976), Gupta & Gill (1983, 1989), Khatoon & Ali (1988), Razaq *et al.* (1994).
	Ropa, 3000	55755	8	2*x*	100	
	Ropa, 3000	55756	8	2*x*	100	
	Karchham, 1900	53943	8	2*x*	100	
L. dolichophylla Kitam. (= ***L. longifolia*** DC.)	Reckong Peo, 2670	53942	8	2*x*	100	**2n=16** Stebbins *et al.* (1953), Mehra et al. (1965), Shetty (1967), Kaul & Singh (1972), Remanandan & Mehra, (1974), Gill *et al.* (1980).
L. macrorhiza (Royle) Hook. f. (= ***Cicerbita macrorhiza*** (Royle) Beauv.)	Chango, 3050	53945	8	2*x*	100	**2n=16** Mehra *et al.* (1965), Shetty, (1967), Remanandan & Mehra (1974), Gupta *et al.* (1989)
	Nako, 3660	53946	8	2*x*	100	
	Nako Lake, 3660	53947	8	2*x*	100	
	Kalpa, 2760	53944	8	2*x*	100	
L. orientalis (Boiss.) Boiss.	Nako, 3660	53948	9	2*x*	54	**2n=36** Babcock *et al.* (1937).
	Ropa, 3000	53949	9	2*x*	62	
L. serriola L.						**2n=18** Gates & Rees (1921), Ishkawa (1921), Tischler (1934), Cooper & Mahony (1935), Babcock *et al.* (1937), Whitaker & Jagger (1939), Mulligan (1957), Mehra & Sidhu (1960), Mehra *et al.* (1965), Kaul & Singh (1972), Remanandan & Mehra (1974), Koul *et al.* (1976), Tomkins & Grant (1978), Kuzmanov & Georgieva (1980), Gupta & Gill (1989), Ghaffari (1999). **2n=18+0-1F** Gupta & Gill (1983).
Leaf morphovariants						
MVI – oblong pinnatifid leaves	Spello, 2800	53951	9	2*x*	100	
	Pooh, 2840	53950	9	2*x*	100	
	Akpa, 2700	53987	9	2*x*	100	
MVII – **oblong**-lanceolate pinnatifid leaves	Khab, 2800	53952	9	2*x*	100	
	Pooh, 2840	53954	9	2*x*	100	
MVIII – **oblong**-lanceolate leaves	Pooh, 2840	53953	9	2*x*	100	
MVI – **linear** leaves						

Morphometric analysis

Morphological analysis was made on the basis of various leaf characters in morphovariants of *L. serriola.* For stomatal studies, abaxial epidermal peels were obtained from the middle portion of the mature leaves through KOH treatment. Thoroughly washed peels were mounted in glycerol. Stomatal index was calculated by using the expression: SI = S/E+S × 100 Where, SI = stomatal index, S = number of stomata per field, E = number of epidermal cells per field.

Photomicrographs

Photomicrographs of meiocytes were made from the freshly prepared slides using Leica Qwin Digital Imaging System and Nikon Eclipse 80*i* microscope.

RESULTS

The study includes the gametic chromosome number, detailed meiotic course, and pollen fertility in five species of *Lactuca*, *L. dissecta*, *L. dolichophylla*, *L. macrorhiza*, *L. orientalis* and *L. serriola* collected at the varying altitudes in Kinnaur district (Himachal Pradesh) (Table 1).

Chromosome number and meiotic course

L. dissecta D. Don

It is an annual, sub-erect, glabrous and dichotomously branched herb commonly and gregariously distributed in orchards, forests and waste lands between 1200m to 3000m. All the meiotically analyzed accessions (PUN 55757, 55755, 55756, 53943) shared the same gametic chromosome number, n=8 as confirmed from the presence of 8 equal sized bivalents at M-I (Fig. 1A), 8:8 chromosomes at A–I (Fig. 1B) and 8:8:8:8 daughter chromosomes at A–II (Fig. 1C). Microsporogenesis was perfectly normal resulting into 100% fertile pollen grains.

L. dolichophylla Kitam. (= ***L. longifolia*** DC.).

The species is an annual or biennial herb, stems erect. Leaves sessile, lanceolate or linear-lanceolate and more or less sagittate at the base, heads small, peduncled and light blue. The accession collected from Reckong Peo, 2670m (PUN 53942) revealed the presence of 8 bivalents at M-I (Fig. 1D). Further meiotic course was regular resulting into normal microsporogenesis and 100% pollen fertility.

L. macrorhiza (Royle) Hook. f. (= ***Cicerbita macrorhiza*** (Royle) Beauv.)

A perennial herb with elongated rootstock and ascending stems. Leaves are variable, lower leaves petiolated with toothed auricle, upper ones sessile, auricled at the base. All the four accessions scored from Chango (PUN 53945), Nako (PUN 53946), Nako Lake (PUN 53947), and Kalpa (PUN 53944) shared the same gametic chromosome number of *n*=8 as confirmed from

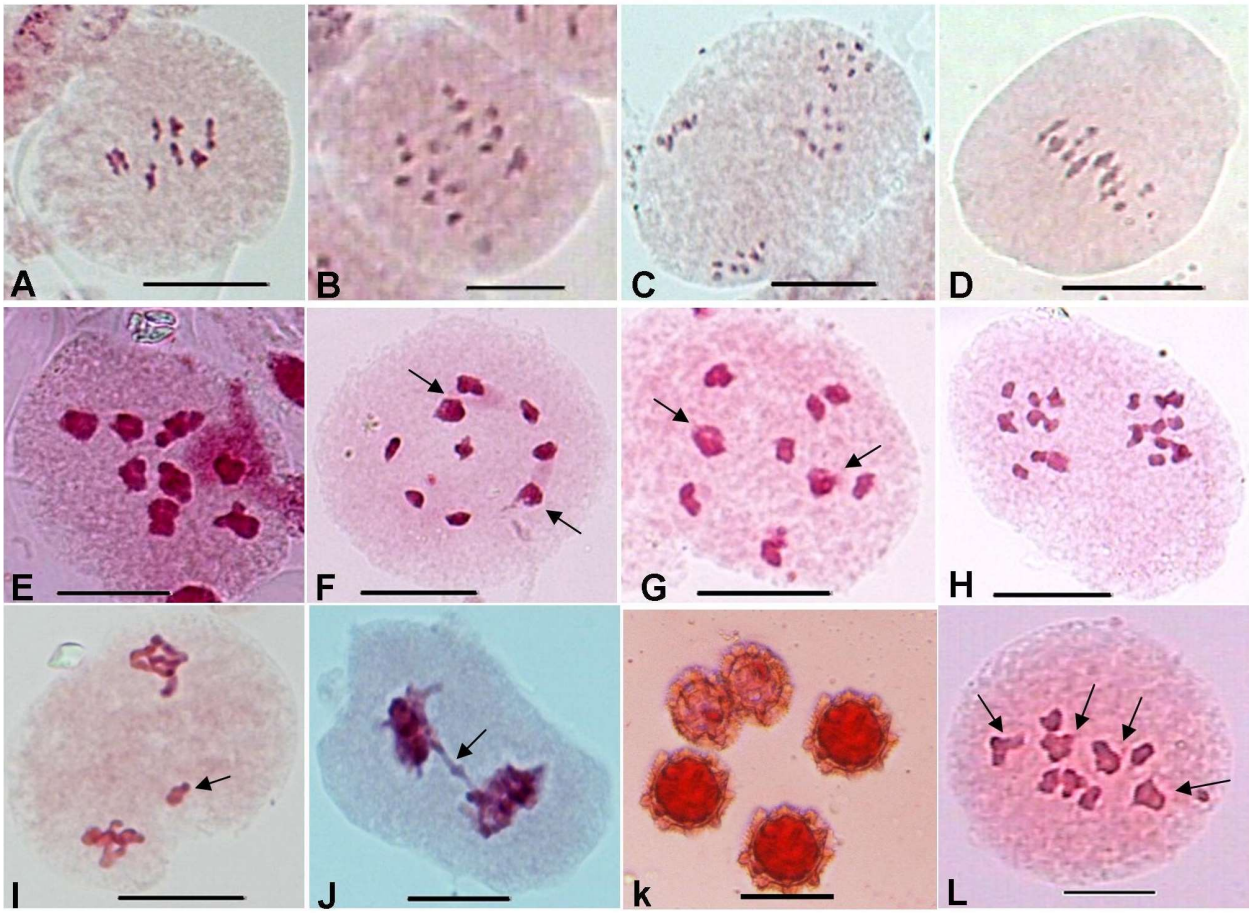

Fig. 1. Male meiosis in genus ***Lactuca*. A**. ***L. dissecta*;** PMC with 8 equal sized bivalents at M-I. **B**. A PMC showing 8:8 chromosomes at A-I. **C**. A PMC showing 8:8:8:8 daughter chromosomes at A-II. **D**. ***L. dolichophylla*; A** PMC showing 8 bivalents at M-I. **E**. ***L. macrorhiza*;** A PMC showing 8 at M-I. **F** & **G**. ***L. orientalis*;** PMCs showing 9 unequal sized bivalents (2 larger sized; arrowed) at diakinesis and M-I. **H**. A PMC showing 9:9 chromosomes distribution at A-I. **I**. A PMC showing a laggard at A-I (arrowed). **J**. A PMC showing a chromatin bridge at A-I (arrowed). **K**. A large number of unstained/sterile pollen grains. **L**. ***L. serriola* ;** PMC at M-I with 9 bivalents (4 larger sized; arrowed). Scale bar 10µm

the presence of 8 large sized bivalents at M-I (Fig. 1E). Microsporogenesis was normal resulting into cent per cent pollen fertility.

L. orientalis (Boiss.) Boiss.

A perennial herb with woody rootstock, glabrous intricately branched stem, pinnatifid leaves and yellow florets is distributed between 2000–4600 m in India, Afghanistan, Pakistan, Central Asia, Iraq and Syria. Meiotically analyzed accessions of the species were collected from dry slopes around Nako (PUN 53948) and Ropa (PUN 53949) between 3000-3660m shared the chromosome count of n=9 as confirmed from the presence of 9 unequal sized bivalents (2 larger sized) at diakinesis (Fig. 1F) and M–I (Fig. 1G) and 9:9 chromosomes distribution at A–I (Fig. 1H). These large sized bivalents in majority of the PMCs showed late disjunction and left as laggards during anaphases (Fig. 1I). In a few PMCs late disjunction of bivalents due to interlocking of chiasmata resulted into chromatin bridges (Fig. 1J). Both the accessions showed considerable amount of sterile pollen grains (54–62 %) (Fig. 1K).

L. serriola L.

Prickly lettuce, native to Europe, commonly grows as a weed of orchards along roadsides, on moist shady slopes and in field crops. The species in the region exhibits considerable morphological variability in leaf characters (Fig. 2 A–D). Four morphovariants (MVI, MVII, MVIII, MVIV) differing in leaf shape and size are recognized presently. These morphovariants also differ significantly in stomatal characters (Fig. 2 E-H, Table 2). All the morphovariants shared the same gametic chromosome number of n=9 as confirmed from the presence of nine bivalents at M-I (Fig. 1L). The bivalents were noticed to be of unequal sized which include 4 large and 5 small sized. However, all the bivalents showed regular segregation resulting to normal sporad formation and 100% fertile pollen grains.

Table 2. Data on leaf characters of the four morphovariants of ***Lactuca serriola*** L.

Leaf Characters & Location	Morphovariants			
	MVI (*n*=9)	MVII (*n*=9)	MVIII (*n*=9)	MVIV (*n*=9)
Distribution	Less common	More common	More common	Less common
Leaf size	2.00-5.00 × 1.28-4.00	2.5-8.90 × 0.22-3.24	2.00-14.00 × 0.30-3.80	2.50-8.00 × 0.42-1.44
Leaf shape	Oblong-pinnatifid	Oblong-lanceolate pinnatifid	Oblong-lanceolate	Linear
Leaf lobes	Broad lobes	Thin lobes	May or may not be lobed	No lobes
Leaf margin	Finely spinulose toothed	Finely spinulose toothed	Spinulose toothed	Entire
Stomatal size (*μ*m)	18.85-19.22 × 15.08-18.85	22.62-23.75 × 15.08-15.83	22.62-24.50 × 15.08-16.96	22.62 × 15.08
Site of collection	Spello, 2800 Pooh, 2840	Akpa, 2700 Khab, 2800	Pooh, 2840	Pooh, 2840

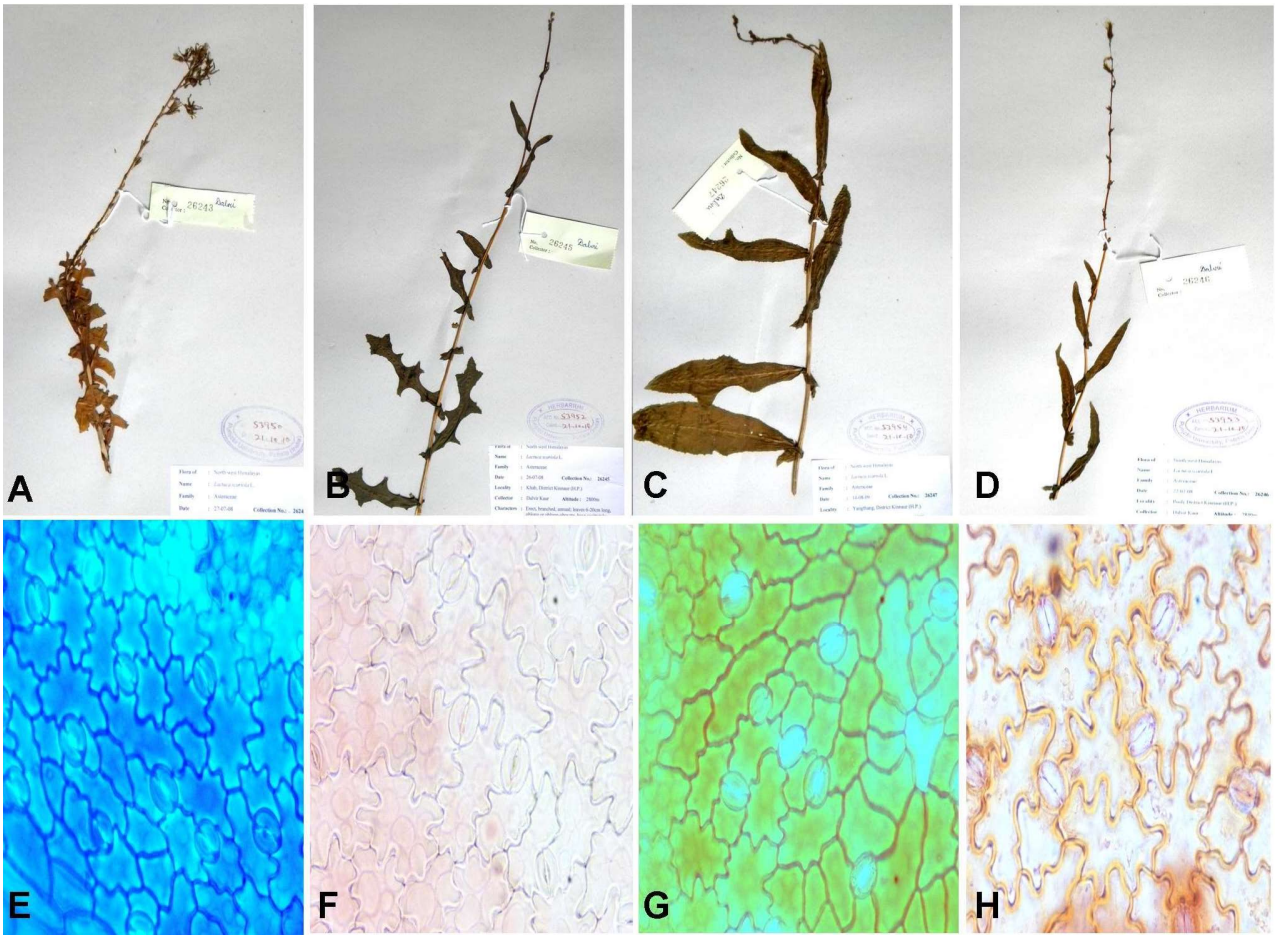

Fig. 2. Leaf morphovariants of ***L. serriola*.** **A**. MVI individual with oblong-pinnatifid leaves. **B**. MVII individual with oblong-lanceolate pinnatifid leaves. **C**. MVIII individual with oblong-lanceolate leaves. **D**. A MVIV individual with linear leaves. **E**. Stomata of MVI individual. **F**. Stomata of MVII individual. **G**. Stomata of MVIII individual. **H**. Stomata of MVIV individual. Scale bar 10μm.

DISCUSSION

Chromosomal counts

All the species of *Lactuca* have been worked out cytomorphologically for the first time from the Kinnaur district (Himachal Pradesh) and exist at diploid level based on gametic chromosome number *x*=8, and 9. The present study confirms the earlier chromosome reports for *Lactuca dissecta* (2*n*=16), *L. dolichophylla* (2*n*=16), and *L. macrorhiza* (2*n*=16) (see: Table 1). *L. orientalis*, which has been counted chromosomaly for the first time from India, adds a new 2*x* cytotype (2*n*=18) to the earlier tetraploid chromosomal report of 2*n*=36 from North America (Babcock *et al*., 1937). In *L. serriola*, diploid chromosome counts of 2*n*=18 is in agreement with the chromosomal reports from Indian Himalayas and from other regions of the world (see: Table 1). However, Gupta and Gill (1983) have reported the presence of a fragment (2*n*=18+0-1F) in plants from Punjab state of India.

Chromosome size and meiotic course

The analyzed species of *Lactuca* exhibit chromosome size differences. The chromosomes/bivalents are small sized in *L. dissecta*, *L. dolichophylla*, compared to *L. macrorhiza*, *L. orientalis* and *L. serriola* which possessed larger sized chromosomes. *Lactuca orientalis* and *L. serriola* depict relative chromosome size differences where some bivalents are larger sized compared to the remaining ones. Many evolutionary processes are known to affect such relative chromosome size differences which include reciprocal translocations, deletions and inversions, unequal crossover, dispersion of repetitive sequences, genome duplication, and chromosome fusion and fission and mis-segregation (Schubert, 2007). Among these, reciprocal translocations (simple or unequal) have often been considered as one of the major forces to shape chromosome size variation as suggested by a number of cytologists (Stebbins, 1950, 1971; Bickmore and Teague, 2002; Schubert, 2007; Li *et al*., 2011). The meiotic course has been noticed to be perfectly regular in *L. dissecta, L. dolichophylla*, *L. macrorhiza* and *L. serriola* resulting into normal sporad formation and high pollen fertility. However in *L. orientalis* pollen fertility was significantly reduced due to abnormal chromosomal segregation during anaphases. The larger size of bivalents in the species seem to be responsible for delay in their segregation during anaphases as has also been reported in *Cyathocline purpurea* and *Blumea* spp. (Gupta, 1981).

Morphological variation

Intraspecific morphological variation involving leaf characters detected presently in the wild accessions of *L. serriola* L. does not appear to be due to any apparent cytological reasons as all the morphovariants shared the same chromosome number (x=9) and ploidy level ($2x$). It thus indicates that morphovariation in the species might have a genetic basis as suggested by Kumar *et al*. (2018) in a number of such wild species.

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