Cytology of *Ranunculus laetus* Wall. ex Royle from cold desert regions and adjoining hills of North-west Himalayas (India)

Kumar Puneet, Singhal Vijay Kumar*, Rana Pawan Kumar, Kaur Shubhpreet and Kaur Dalvir

Department of Botany, Punjabi University, Patiala-147002, Punjab, India.

Abstract — Present work pertains to the cytological investigations performed in *Ranunculus laetus* for the first time from Indian cold deserts and adjoining high hills of North-west Himalayas which covers Chamba, Kinnaur, Kullu and Lahaul-Spiti districts of Himachal Pradesh. We here also report for the first time the presence of 1 B-chromosome in two accessions studied from Dalhousie hills. Twelve accessions scored presently from these regions uniformly shared the same meiotic chromosome number, n=14 and existed at tetraploid level (based on x=7). Of these, eight accessions showed abnormalities during male meiosis, such as pollen mother cells (PMCs) involved in chromatin transfer at different stages of meiosis, chromosome stickiness, pycnotic chromatin material, out of plate bivalents at metaphase-I, nonsynchronous disjunction of some bivalents, and laggards at anaphases/telophases. Consequent to these meiotic irregularities, microsporogenesis in meiocytes is abnormal characterized by the presence of dyads, polyads, and micronuclei and included micronuclei in sporads. These irregularities during meiotic course resulted into varying percentage of pollen sterility (9-31%) and pollen grains of heterogeneous sizes. The remaining four accessions showed regular meiotic course, normal microsporogenesis and nearly cent percent pollen fertility (97.90-100%).

Key words: B-chromosome, Chamba, cytomixis, Kinnaur, Kullu, laggards and bridges, Lahaul-Spiti.

INTRODUCTION

***Ranunculus laetus* Wall. ex Royle** (= *R. distans* Royle ex D. Don, Family Ranunculaceae) grows as a diffuse perennial herb with woody rootstocks, stems strigose often covered with yellowish-grey deflexed hairs in the lower part. The yellow coloured flowers present on slender, tall, upright leafless stems appeared during the months of June-September. Referred as 'Lalbutty' in the cold deserts of Himalayas in India used to reduce the harmful effects of smoking, purifies the blood, removal of intestinal worms and for cuts, wounds, stomachache, sinusitis (Manandhar 2002). The species is widespread throughout the Himalayas in India and Chitral, the Pamir Alai and Tian Shan between at altitudes of 1500-3000 m (Grey-Wilson 1974; Suyal *et al*. 2010). It is a very variable and polymorphic species in its habitat, hairyness of stem, and leaf and flower size (Aswal and Mehrotra 1994). The species has been worked out quite extensively from the various region of Himalayas in India viz. Eastern Himalayas (Sharma and Sarkar 1970; Roy and Sharma 1971), Western Himalayas (Mehra and Kaur 1963; Sobti and Singh 1961), Kashmir hills (Bhat *et al*. 1972; Mehra and Remanandan 1972; Kabu *et al.* 1988) and Garhwal hills (Bir and Thakur 1984; Bir *et al*. 1986) and from outside India from the hills of Nepal (Vaidya and Joshi 2003), Russia (Goepfert 1974; Agapova and Zemskova 1985) and Pakistan (Baquar and Abid Askari 1970a; b; Khatoon 1991; Khatoon and Ali 1993). On the basis of chromosome information gathered by these workers through meiotic and karyotypic studies the species depicted considerable variation in chromosome number among different populations as depicted from the presence of intraspecific polyploidy at two different

*Corresponding author: e-mail: vksinghal53@gmail.com

basic numbers (4x, 6x, 8x on x=7, 2x, 4x on x=8). Keeping in view the presence of heterogeneity in chromosome number and polymorphic nature of the species present studies were undertaken on male meiosis, microsporogenesis, pollen fertility, and pollen grain size in different accessions collected from the cold deserts region and high hills falling in the districts of Himachal Pradesh. Effects of cytomixis and chromatin transfer among meiocytes and spindle abnormalities on meiotic course, microsporogenesis, pollen fertility and pollen size have also been discussed.

MATERIAL AND METHODS

Materials for male meiotic studies were sampled from the wild plants collected from Chamba, Kinnaur, Kullu and Lahaul-Spiti districts of Himachal Pradesh (Table 1) in June-September during three years (2007-2009). The voucher specimens of the cytologically worked out plants were deposited in the Herbarium, Department of Botany, Punjabi University, Patiala (PUN). For meiotic chromosome counts, young and unopened floral buds were fixed in freshly prepared Carnoy's fixative for 24 h and then preserved in 70% alcohol at 4°C in the refrigerator. Smears of pollen mother cells were made in 1% acetocarmine. A number of temporary slides were examined to determine the chromosome number at different stages and meiotic abnormalities. Pollen fertility was estimated through stainability tests for which anthers from mature flowers were smeared in glycerol-acetocarmine mixture (1:1) and aniline blue dye (1%). Well filled pollen grains with stained nuclei were taken to be apparently fertile while those with shriveled up nuclei and unstained/faintly cytoplasm as sterile. Photomicrographs of chromosome counts, sporads and pollen grains were made from the freshly prepared slides using *Leica Qwin Digital Imaging System* and *Nikon 80i Eclipse* microscope. Information on meiotic chromosome numbers, localities with altitude, plant accession numbers, ploidy level, % age pollen fertility and previous chromosome reports of the presently studied accessions are provided in the Table 1. For previous chromosome reports, various Indexes to Plant Chromosome Numbers were consulted which include, Darlington and Wylie (1955), Fedorov (1969), Moore (1973; 1974; 1977), Goldblatt (1981; 1984; 1985; 1988), Goldblatt and Johnson (1990; 1991; 1994; 1996; 1998; 2000; 2003; 2006), Kumar and Subramanian (1986), and Khatoon and Ali (1993).

RESULTS

Detailed cytological observations have been recorded on male meiosis, microsporogenesis, pollen fertility, and pollen grain size in *R. laetus* from twelve accessions collected from different localities falling in the districts of Chamba, Kinnaur, Kullu and Lahaul-Spiti of Himachal Pradesh (Table 1). All the accessions shared the same meiotic chromosome number (n=14) and ploidy level (4x) as confirmed from the presence of 14 large sized bivalents at diakinesis (Fig. 1) and metaphase-I (Fig. 2), and 14:14 chromosomes distribution at metaphase-II (Fig. 3). The accessions scored from Kukumsheri (2800 m), Gulaba (3200 m), Sangla (2630 m), Nichar (2150 m), Bharmour (2300 m), Muhani (2300 m), Hillour Dhar (3300 m), and Khajjiyar (1950 m) showed some abnormalities during male meiosis, such as pollen mother cells (PMCs) involved in chromatin transfer at different stages of meiosis, chromosome stickiness, pycnotic chromatin material, nonsynchronous disjunction of some bivalents and spindle irregularities (out of plate bivalents at metaphase-I, laggards and bridges at anaphases/telophases) and presence of 1 B-chromosome (Figs. 4-13). The laggards in such PMCs are resulted into the formation of micronuclei and included micronuclei in sporads (Figs.14, 15). Besides, the presence of dyads and polyads are also observed in some cases (Fig.16). Consequently, these accessions depict varying percentage of pollen sterility (9-31%) and pollen grains of heterogeneous sizes (Figs.17, 18) However, four accessions studied from Gondhla (3160 m), Banikhet (1800 m), Bathri (1900 m) and Dalhousie Cantonment (2100 m) showed regular meiotic course, normal microsporogenesis and nearly cent per cent pollen fertility (97.90-100%). Brief results covering meiotic course, microsporogenesis, pollen fertility and pollen size in each accession are given as under.

A. Lahaul-Spiti

(i) *Kukumsheri (2800 m), Lahaul Valley* - The accession which is growing in the moist shady places showed the inter PMCs transfer of chromatin material and associated meiotic abnormalities at various stages of meiosis from early prophase-I to T-II. Transfer of chromatin material involving 2-4 PMCs is existent in 23.13% of the observed PMCs (Figs.4 -6). Chromatin transfer among meiocytes occurred through narrow as well as broad cytoplasmic channels forming 1-2 chromatin strands. In few PMCs only cytoplasmic connections were observed without showing any transfer of chromatin material. PMCs were also noticed to be directly fused in some occasions to facilitate the chromatin transfer (Fig. 6). Chromatin migration was observed to be both uni- and bidirectional (Fig.4). Simultaneous transfer of chromatin material from one PMC to 2 adjacent PMCs was also noticed in some cases (Fig. 4). Transfer of chromatin material was either partial or complete which resulted into the formation of hypo-, hyperploid and enucleated PMCs (Figs. 5, 6). Extra chromatin masses other than the normal complement are also noticed in some PMCs. Other meiotic anomalies associated with cytomixis included chromatin stickiness, pycnotic chromatin, and laggards and bridges at anaphases/telophases (Figs. 8, 11, 12). Lagging of 1-3 bivalents/chromosomes during anaphases and telophases were observed in 41.43% PMCs. The lagging chromatin material which failed to get included into the telophases nuclei resulted into the formation of micronuclei (Fig. 14) or included micronuclei (Fig. 15). Consequent to this, microsporogenesis was also abnormal characterized by the presence of polyads (6.45%), tetrads with 1-3 micronuclei (58.06%) and tetrads with included micronuclei (9.68%) (Fig. 15). Pollen fertility was low (69%). Also the pollen grains of two different sizes as large (33.90 μm ×31.90 μm, 25.74%) and normal sized (26.47-28.76 μm ×26.30-25.58 μm, 74.26%) were resulted.

(ii) *Gondhla (3160 m), Lahaul Valley* - Meiotic behaviour in this accession which was found growing on moist sun facing slopes was perfectly regular, and resulted into normal microsporogenesis and nearly cent per cent pollen fertility (99%).

B. Kullu

(i) *Gulaba (3200 m), Manali hills* - The accession is collected from the moist hilly slopes, showed normal meiotic course in majority of the PMCs, characterized by the presence of 14 bivalents at metaphase-I and their regular segregation during anaphase-I. Only few PMCs depicted 1-2 bivalents which remained out of metaphasic plate during meiosis-I (Fig. 9) and showed late disjunction at anaphase-I. Products of such meiocytes resulted into some pollen malformation (2%).

C. Kinnaur

(i) *Sangla (2630 m)* - This accession which grows in moist places along cultivated fields in the Sangla Valley of Kinnaur district depicts the phenomenon of cytomixis involving transfer of chromatin material in 24.05% PMCs during meiosis-I. In a group, 2-4 PMCs were observed to be involved in chromatin transfer. Chromosome stickiness was recorded in 72.38% PMCs during metaphase-I and anaphase-I. Chromatin material in the form of laggards and bridges during anaphases/telophases was noticed in 28.21% and 22.46% PMCs, respectively. Micronuclei were observed in the meiocytes at telophase-II (16.32% PMCs) and sporad stage (22.22%). Few sporads with more than four units were also observed in this accession (1.58%). The accession showed some pollen sterility (10.03%). Pollen grains of two different sizes as, normal (21.87-22.62 μm ×23.37- 23.75 μm, 79.5%) and small sized (18.09-18.85 μm ×19.60-19.98 μm, 20.5%) were resulted.

(ii) *Nichar (2150 m)* - Another accession which also grows in moist places along roadsides in the Nichar village in Kinnaur district showed the phenomenon of cytomixis involving chromatin transfer among 2-3 proximate PMCs (22.51%) during the early stages of prophase-I. PMC with pycnotic chromatin material consequent to cytomixis is recorded in 2.5% of the observed meiocytes. Abnormal sporads such as tetrads with micronuclei (5.7%) and polyads (4.90%) were also observed. Cytomixis and associated meiotic abnormalities resulted into some pollen sterility (9.37%) and two different sized pollen grains, as normal (24.50 μm ×26.39 μm, 84%) and small sized (22.62-22.99 μm ×20.73-21.87 μm, 16%).

D. Chamba

(i) *Bharmour (2300 m), Manimahesh hills* - The accession which grows on moist slopes showed highly abnormal meiosis due to cytomixis and other associated meiotic irregularities like stickiness in chromosomes, laggards and bridges during anaphases/telophases, and spindle irregularities. The phenomenon of cytomixis involving transfer of chromatin material among 2-4 PMCs was observed in 7.85% cases. The phenomenon of chromatin transfer which occurred through cytoplasmic channels was noticed from early stages of prophase-I to T-I. In some cases only cytomictic channels are present among proximate PMCs. Chromatin stickiness was observed in 54.48% PMCs at metaphase-I and anaphases/telophases (Fig. 8). Narrow as well as broad chromatin bridges due to stickiness in chromatin material are recorded in 24.73% PMCs at telophases. Lagging of 1-4 bivalents/chromosomes during anaphases/telophases is noticed in 62.69% PMCs (Fig. 11). These meiotic irregularities in the meiocytes resulted into the formation of abnormal sporads, as dyads and tetrads with micronuclei (Fig. 16) and consequently pollen sterility (91.16%) and heterogeneous sized pollen grains (Fig. 17). On

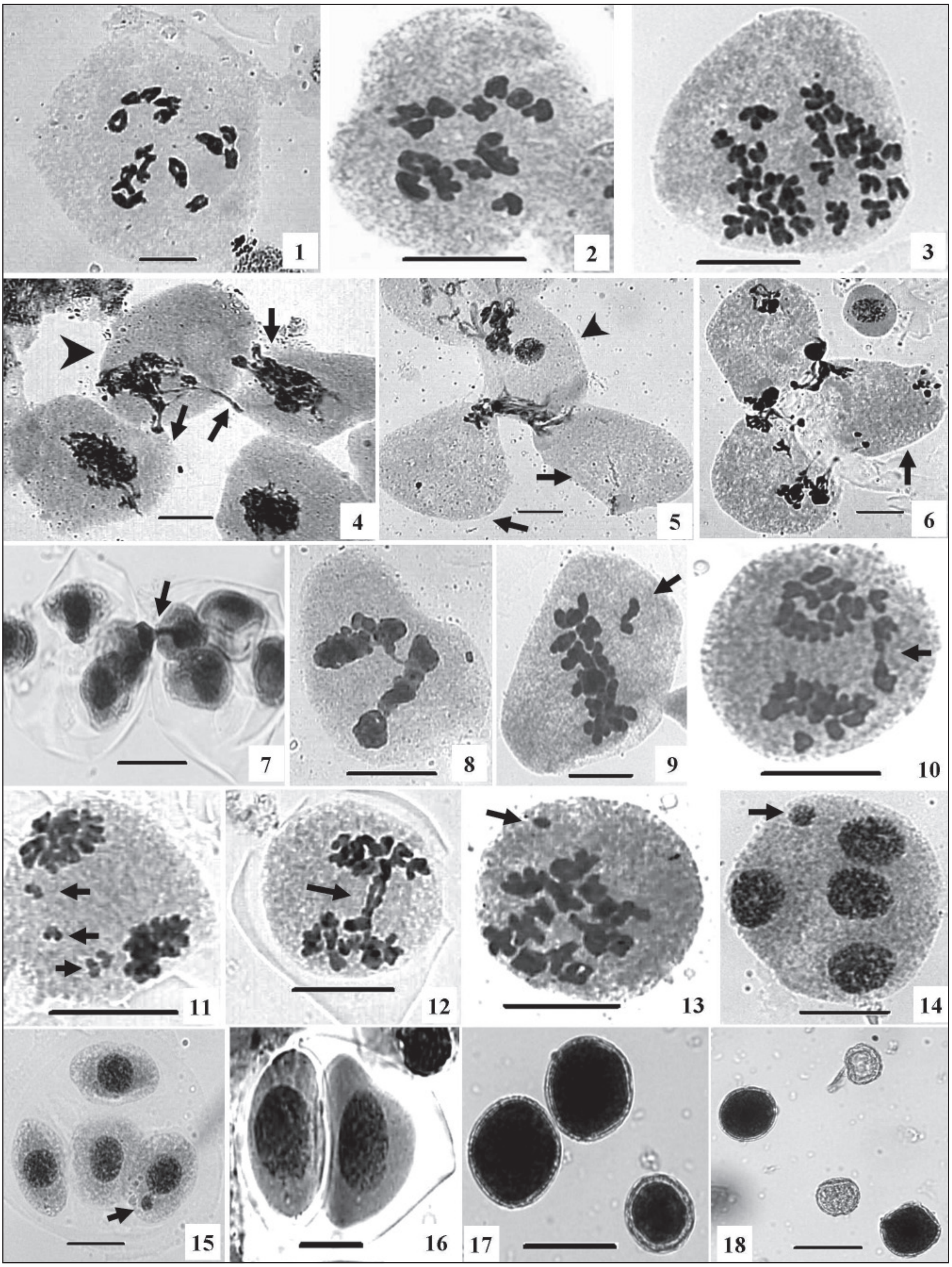

Fig. 1-18 — (1) A PMC showing 14 bivalents at diakinesis. (2) A PMC showing 14 bivalents at metaphase-I. (3) A PMC showing 14:14 chromosomes distribution at metaphase-II. (4) Two PMCs involved in bidirectional chromatin transfer (arrows) of which one PMC (arrow head) also simultaneously transfer chromatin to other adjacent PMCs. (5) Two enucleated PMCs (arrows) resulted after complete transfer of chromatin material. (6) A group of three PMCs involved in chromatin material transfer of which one is hypoploid (arrow). (7) Microspores of two different tetrads involved in chromatin material transfer (arrow). (8) A PMC showing chromosome stickiness at anaphase-I. (9) Out of plate bivalent at metaphase-I (arrow). (10) Nonsynchronous disjunction of one bivalent at anaphase-I (arrow). (11) Laggards at anaphase-I (arrows). (12) A chromatin bridge at anaphase-I (arrow). (13) A PMC at metaphase-I showing 1B-chromosome and 14 bivalents. (14) A micronucleus at telophase-II (arrow). (15) A tetrad showing included micronucleus in a microspore (arrow). (16) A dyad. (17) Apparently fertile heterogeneous sized pollen grains. (18) Stained apparently fertile and unstained sterile pollen grains. (bar =10µm).

the basis of size, the pollen grains in the accession are categorized as small (19.18-20.72 μm × 21.56-23.82 μm), normal (25.15-27.54 μm × 22.98-24.65 μm) and large sized (29.13-33.56 μm × 24.87-31.72 μm). The normal sized pollen grains are noticed to be relatively in high frequency (50.45%) compared to the small (9.30%) and large (40.25%) ones.

(ii) *Muhani (2300 m), Pangi Valley* - The accession from Pangi Valley in the district Chamba, which grows around agricultural fields at Muhani village along the sides of running water streams, depicted some meiotic abnormalities, as out of plate bivalents (7.14%) and presence of lagging chromatin material during anaphases (2.5%). The lagging chromatin at sporad stage constitutes micronuclei (2.00%) and included micronuclei (0.66%). Although the pollen grains are of uniform sized but there is some malformation in the form of sterile/unstained pollen grains (2.84%).

(iii) *Hillour Dhar (3300 m), Pangi Valley* - Another accession collected from Hillour Dhar in Pangi Valley of district Chamba, grows on moist hilly open meadows along the sides of running water streams at an altitude of 3300 m. The meiotic course in majority of the PMCs in this accession was normal. Analysis revealed that (19.26% PMCs) during anaphases/telophases showed lagging chromatin material which failed to get included into telophase nuclei. Consequently (21.15%) sporads showed the presence of micronuclei and included micronuclei. Some sterile pollen grains were also resulted (6.63%).

(iv) *Banikhet (1800m), Dalhousie hills* - The accession which grows on moist places showed regular meiotic course resulting into normal tetrad formation and cent per cent pollen fertility.

(v) *Bathri (1900m), Dalhousie hills* - Meiotic course in this accession growing on moist shady places was also normal leading to high pollen fertility (97.90%).

(vi) *Dalhousie Cantonment (2100m), Dalhousie hills* - The accession scored from Dalhousie Cantonment area growing on moist slopes depicted normal meiotic course and high pollen fertility (95.80%). However, some PMCs show the presence of 1 B-chromosome at metaphase-I (Fig. 13).

(vii) *Khajjiyar (1950m), Dalhousie hills* - The accession which grows in moist places along the boundaries of *Cedrus deodara* forest depicted some meiotic abnormalities like, chromatin bridges during anaphases/telophases (0.91% PMCs) and presence of out of metaphasic plate few bivalents (13.98% PMCs). Consequently, the product of PMCs yielded some pollen sterility (8.67%). Besides, some PMCs in the accession also showed the occurrence of 1 B-chromosome at metaphase-I.

DISCUSSION

R. laetus a highly polymorphic species exhibited considerable diversity in habitat, hairyness of stem and in quantitative characters like plant height and leaf size. The species also exhibited chromosomal diversity and an array of chromosome numbers are reported by workers from different parts of India and outside India (2n= 16, 28, 32, 42, 56).The species has been worked out chromosomally for the first time from Indian cold deserts and adjoining high hills. All the accessions scored presently from the cold deserts regions of India uniformly shared the same meiotic chromosome number, n=14 and existed at tetraploid level (based on x=7). Incidentally majority of earlier chromosome reports from other regions of the Indian Himalayas, viz., Eastern Himalayas (SHARMA and SARKAR 1970; ROY and SHARMA 1971), Western Himalayas (MEHRA and KAUR 1963; SOBTI and SINGH 1961), Kashmir Himalayas (BHAT *et al*. 1972; MEHRA and REMANANDAN 1972; KABU *et al.* 1988) and Garhwal Himalayas (BIR and THAKUR 1984; BIR *et al*. 1986) and from the hills of Russia (GOEPFERT 1974; AGAPOVA and ZEMSKOVA 1985) also shared the same tetraploid chromosome count of 2n=28. We here reported for the first time the presence of 1 B-chromosome in the two accessions scored from Dalhousie hills.

Other interesting finding in the species on the basis of present studies is that some of the individuals scored from the cold deserts Himalayas in Lahaul-Spiti, Kinnaur and Manimahesh hills showed the phenomenon of cytomixis involving chromatin transfer among proximate PMCs and associated meiotic abnormalities like chromosome stickiness, pycnotic chromatin material, nonsynchronous disjunction of some bivalents and spindle irregularities (out of plate bivalents at metaphase-I, laggards and bridges at anaphases/telophases). These abnormalities are reported here for the first time in the species.

Cytomixis in PMCs - Transfer of chromatin material or chromosomes among the adjacent PMCs occurs through cytomictic channels as well as through cell wall dissolution (FALISTOCCO *et al*. 1995). These cytoplasmic channels originate from the pre-existing connections of plasmodesmata formed within the anther tissues. The plasmodesmata become completely ob-

TABLE 1 — Locality with altitude, accession number, meiotic chromosome number and ploidy level of wild plants of *Ranunculus laetus*.

S. No	Localities with altitude	Accession number (PUN[1])	Meiotic chromosome number (n)	Ploidy level	% age pollen fertility	Previous chromosome reports
	A. Lahaul-Spiti,					**2n=16**
	Lahual Valley:					VAIDYA and JOSHI, 2003;
1.	Kukumsheri, 2800 m	51147	14	4x	69.00	
2.	Gondhla, 3160 m	51352	14	4x	99.00	**2n =16, 28**
						KABU *et al.*1988;
	B. Kullu,					
	Manali Hills:					**2n =28**
3.	Gulaba, 3200 m	51348	14	4x	98.00	MEHRA and KAUR, 1963; SHARMA and
						SARKAR, 1967-1968; ROY and SHARMA,
	C. Kinnaur:					1971; BHAT *et al.* 1972; MEHRA and RE-
4.	Sangla, 2630 m	50946	14	4x	89.97	MANANDAN, 1972; GOEPFERT, 1974; BIR
5.	Nichar, 2150 m	52272	14	4x	90.63	and THAKUR, 1984; AGAPOVA and ZEM-
						SKOVA, 1985; BIR *et al.* 1986;
	D. Chamba					
	Manimahesh hills:					**2n =28, 32**
6.	Bharmour, 2300 m	51344	14	4x	91.16	MEHRA and REMANANDAN, 1972;
	Pangi Valley:					**2n =32**
7.	Muhani, 2300 m	52213	14	4x	97.16	SOBTI and SINGH, 1961; BAQUAR and
8.	Hillour Dhar, 3300 m	52216	14	4x	93.37	ABID ASKARI, 1970a, b; KHATOON, 1991;
	Dalhousie hills:					**2n =32, 56**
9.	Banikhet, 1800 m	51732	14	4x	100	KHATOON and ALI, 1993;
10.	Bathri, 1900 m	51730	14	4x	97.90	
11.	Dalhousie Cantonment, 2100 m	51726	14	4x	95.80	**2n=42**
12.	Khajjiyar, 1950 m	51728	14	4x	91.33	ROY and SHARMA, 1971.

[1] Herbarium code of Botany Department, Punjabi University, Patiala as per "Index Herbariorum" by HOLMGREN and HOLMGREN (1998).

structed with the progress of meiosis by the deposition of callose, but in some cases they persist during meiosis and increase in size forming conspicuous inter-pollen mother cell connections or cytomictic channels that permit the transfer of chromatin or chromosomes (FALISTOCCO *et al.* 1995; SHABRANGI *et al.* 2010). This phenomenon reported in a large number of plants was considered at one time to be of less evolutionary importance. But the analysis may resulted into the formation of aneuploid plants or produced unreduced pollen grains (gametes) as reported in several numerous plant species (FALISTOCCO *et al.* 1995; LATTOO *et al.* 2006; SINGHAL and KUMAR 2008a; b; 2010; SHABRANGI *et al.* 2010). Unreduced gamete formation is of evolutionary importance leading to the production of plants with higher ploidy level (SHEIDAI and BAGHERI-SHABESTAREI 2007).

Of the 12 accessions scored presently 8 showed meiotic abnormalities and among these four accessions of *R. laetus* depicted the phenomenon of cytomixis. The chromatin transfer occurred through narrow as well as broad cytoplasmic channels forming 1-2 chromatin strands involving 2-4 PMCs and the % age of PMCs with cytomixis ranged between 7.85-24.05%. The percentage of PMCs involved in the chromatin material transfer was high during the first meiotic division however it also extended up to sporad stage though with lesser frequency. Some of the PMCs were also directly fused to facilitate the chromatin transfer. In some cases, cytomixis may lead to the migration of the whole chromatin material among the neighbouring meiocytes and lead to the formation of unreduced gametes. In the present case partial or complete chromatin transfer resulted into the formation of PMCs which depicts hypo-, hyperploid and enucleated nature. And the products of such PMCs in these individuals yield variable sized fertile and sterile pollen grains.

Spindle abnormalities -The spindle apparatus is normally bipolar and acts as a single unit, playing a crucial role in chromosome alignment during metaphase (Shabrangi *et al*. 2010). Any kind of disturbance in the spindle apparatus may result in random unorientation of chromosomes in the PMCs and consequent sub-grouping of the chromosomes which function independently. Spindle abnormalities in the presently studied species resulted into out of metaphase plate bivalents that have led to the formation of laggards which subsequently failed to include into the telophase haploid nuclei and yielded micronuclei at late telophases and in the sporads. Spindle abnormalities mainly resulted due to the duality of nucleus in foreign cytoplasm, environmental influence, and disharmonious gene interaction as has been suggested by Nirmala and Rao (1996).

Other meiotic abnormalities - Among other meiotic abnormalities, chromosome stickiness was the most common. It resulted into the pycnosis of chromatin material and sticky bridges at anaphases/telophases. Nonsynchronous disjunction of some bivalents at anaphase was also observed which might have arisen due to late terminalization of chiasma.

The phenomenon of cytomixis and associated meiotic irregularities in *R.laetus* resulting into abnormal sporad formation, and some pollen malformation seem to be under some genetic factors as has been reported in several woody species (Singhal and Gill 1985; Singhal *et al*. 2007), *Vicia faba* (Haroun *et al.* 2004), *Medicago sativa* (Bellucci *et al.* 2003), *Polygonum tomentosum* (Haroun 1995), *Chlorophytum comosum* (Lattoo *et al*. 2006), *Caltha palustris* (Kumar and Singhal 2008), *Clematis flammula* (Kumar *et al*. 2008), *Hippophae rhamnoides* (Singhal *et al*. 2008), *Meconopsis aculeata* (Singhal and Kumar 2008 a), *Withania somnifera* (Singhal and Kumar 2008b), *Lychnis indica* (Singhal *et al*. 2009 a), *Anemone rivularis* (Singhal *et al*. 2009b), *Clematis orientalis* (Kumar *et al*. 2010) and *Clematis montana* (Singhal *et al*. 2010).

Acknowledgements — The authors are grateful to the University Grants Commission, New Delhi for providing financial assistance under the DRS SAP I and II and ASIST programme and CSIR for providing Senior Research Fellowship to Mr. Puneet Kumar. Thanks are also due to the Head, Department of Botany for necessary laboratory and library facilities.

REFERENCES

Agapova N.D. and Zemskova E.A., 1985 — *Chromosome numbers in some species of the genus Ranunculus (Ranunculaceae)*. Botaniceskjij Žurnal SSSR, 70: 855-856.

Aswal B.S. and Mehrotra B.N., 1994 — *Flora of Lahaul-Spiti* (*a Cold desert in North West Himalaya*). Bishen Singh Mahendra Pal Singh, Dehra Dun, India.

Baquar S.R. and Abid Askari S.H., 1970a — *Chromosome numbers in some flowering plants of West Pakistan*. Génet. Ibér., 22: 1-11.

Baquar S.R. and Abid Askari S.H., 1970b — *Chromosome studies in some flowering plants of West Pakistan II*. Génet. Ibér., 22: 41-51.

Bellucci M., Roscini C. and Mariani A., 2003 — *Cytomixis in pollen mother cells of Medicago sativa L.* Journal of Heredity, 94: 512-516.

Bhat B.K., Bakshi S.K. and Kaul M.K., 1972 — *In IOPB Chromosome Number Reports XXXVIII*. Taxon, 21: 679-684.

Bir S.S. and Thakur H., 1984 — SOCGI plant chromosome number reports-II. Journal of Cytology and Genetics, 19: 114-115.

Bir S.S., Thakur H. and Chatha G.S., 1986 — *Chromosomal studies in certain members of Ranunculaceae and Menispermaceae*. Nucleus, 29: 183-186.

Darlington C.D. and Wylie A.P., 1955 — *Chromosome atlas of flowering plants*. George Allen & Unwin Ltd., London.

Falistocco E., Tosti T. and Falcinelli M., 1995 — *Cytomixis in pollen mother cells of diploid Dactylis, one of the origins of 2n gametes.* Journal of Heredity, 86: 448-453.

Fedorov A.N.A., 1969 — *Chromosome Numbers of Flowering Plants*. Academy of Science of the USSR Komarov Botanical Institute, Leningard (Reprint 1974).

Goepfert D., 1974 — *Karyotypes and DNA content in species of* Ranunculus *L. and related genera.* Botaniska Notiser, 127: 464-489.

Goldblatt P., 1981; 1984; 1985; 1988 — *Index to Plant Chromosome Numbers 1975-1978, 1979-1981, 1982-1983, 1984-1985*. Monographs in Systematic Botany. Missouri Botanical Garden, USA.Vols. 5, 8, 13, 23.

Goldblatt P. and Johnson D.E., 1990; 1991; 1994; 1996; 1998; 2000; 2003; 2006. *Index to Plant Chromosome Numbers 1986-1987, 1988-1989, 1990-1991, 1992-1993, 1994-1995, 1996-1997, 1998-2000, 2001-2003.* Monographs in Systematic Botany. Missouri Botanical Garden, USA.Vols. 30, 40, 51, 58, 69, 81, 94, 106.

Grey-Wilson C., 1974 — *Some Notes on the Flora of Iran and Afghanistan*. Kew Bulletin, 29: 19-81.

Haroun S.A., 1995 — *Cytomixis in pollen mother cells of Polygonum tomentosum Schrank.* Cytologia, 60: 257-260.

Haroun S.A., Al Shehri A.M. and Al Wadie H.M., 2004 — *Cytomixis in the microsporogenesis of Vicia faba L.* (Fabaceae). Cytologia, 69: 7-11.

Holmgren P.K. and Holmgren N.H. 1998 — *Index Herbariorum: A global directory of public herbaria and associated staff.* New York Botanical Garden's Virtual Herbarium, http://sweetgum.nybg.org/ih/.

KABU R., WAFAI B.A. and KACHROO P., 1988 — *Studies on the genus Ranunculus Linn. I. Natural diploidy in R. laetus Wall. ex H. & T. and impact of intraspecific chromosome variability on the phenotype of the species.* Phytomorphology, 38: 321-325.
KHATOON S., 1991— *Polyploidy in the flora of Pakistan - an analytical study*. Ph. D. Thesis, Department of Botany, University of Karachi, Karachi, Pakistan.
KHATOON S. and ALI S.I., 1993 — *Chromosome Atlas of the Angiosperms of Pakistan.* Department of Botany, BCC & T Press, University of Karachi, pp. 232.
KUMAR P. and SINGHAL V.K., 2008 — *Cytology of Caltha palustris L. (Ranunculaceae) from cold regions of Western Himalayas*. Cytologia, 73: 137-143.
KUMAR P., SINGHAL V.K. and KAUR J., 2008 — *Cytomixis induced meiotic abnormalities in pollen mother cells of Clematis flammula L. (Ranunculaceae).* Cytologia, 73: 381-385.
KUMAR P., SINGHAL V.K., KAUR D. and KAUR S., 2010 — *Cytomixis and associated meiotic abnormalities affecting pollen fertility in Clematis orientalis*. Biologia Plantarum, 54: 181-184.
KUMAR V. and SUBRAMANIAN B., 1986 — *Chromosome Atlas of Flowering Plants of the Indian Subcotinent Vol.I. Dicotyledon.* BSI Calcutta.
LATTOO S.K., KHAN S., BAMOTRA S. and DHAR A.K., 2006 — *Cytomixis impairs meiosis and influences reproductive success in Chlorophytum comosum (Thunb.) Jacq. - an additional strategy and possible implications.* Journal of Biosciences, 31: 629-637.
MANANDHAR N.P., 2002 — *Plants and people of Nepal*. Timber Press, Inc. Portland, Oregon, U.S.A.
MEHRA P.N. and KAUR B., 1963 — *Cytological study of some Himalayan Ranunculaceae.* Proc. 50th Indian Sci. Congr. Part 3: 453-454.
MEHRA P.N. and REMANANDAN P., 1972 — *Cytology of some W.Himalayan Ranunculaceae.* Cytologia, 37: 281-296.
MOORE R.J., 1973, 1974, 1977 — *Index to Plant Chromosome Numbers*. 1967-1971, 1972. 1973-74 Regnum Vegetabile, 90, 91, 96.
NIRMALA A. and RAO P.N., 1996 — *Genesis of chromosome numerical mosacism in higher plants*. Nucleus, 39: 151-75.
ROY S.C. and SHARMA A.K., 1971 — *Cytotaxonomic studies in Indian Ranunculaceae*. Nucleus, 14: 132-143.
SHABRANGI A., SHEIDAI M., MAJD A., NABIUNI M. and DORRANIAN D., 2010 — *Cytogenetic abnormalities caused by extremely low frequency electromagnetic fields in canola.* ScienceAsia, 36: 292-296.
SHARMA A.K. and SARKAR A.K., 1970 — *Chromosome number reports of plants*. In Annual Report 1967-1968, Cytogenetics Laboratory, Department of Botany, University of Calcutta**.** Research Bulletin, 2**:** 38-48.
SHEIDAI M. and BAGHERI-SHABESTAREI E.S., 2007 — *Cytomixis and unreduced pollen formation in some Festuca L. species of Iran*. Caryologia, 60: 364-371.
SINGHAL V.K. and GILL B., 1985 — *Cytomixis in some woody species*. Biologica, 1: 168–175.
SINGHAL V.K. and KUMAR P., 2008a — *Impact of cytomixis on meiosis, pollen viability and pollen size in wild populations of Himalayan poppy (Meconopsis aculeata Royle).* Journal of Biosciences, 33: 371-380.
SINGHAL V.K. and KUMAR P., 2008b — *Cytomixis during microsporogenesis in the diploid and tetraploid cytotypes of Withania somnifera (L.) Dunal, 1852 (Solanaceae).* Comparative Cytogenetics, 2: 85-92.
SINGHAL V.K. and KUMAR P., 2010 — *Variable sized pollen grains due to impaired male meiosis in the cold desert plants of North West Himalayas (India).* In Benjamin J.K. (ed), "Pollen: Structure, Types and Effects", p. 101-126. Nova Science Publishers, New York, USA.
SINGHAL V.K., GILL B.S. and DHALIWAL R.S., 2007 — *Status of chromosomal diversity in the hardwood tree species of Punjab state.* Journal of Cytology and Genetics, 8: 67-83.
SINGHAL V.K., KAUR S., KAUR D. and KUMAR P., 2009a — *New detection of haploid chromosomes, pollen size and sterility in Lychnis indica Benth. var. fimbriata Wall.* Chromosome Botany, 4: 53-56.
SINGHAL V.K., KAUR D. and KUMAR P., 2008 — *Effect of cytomixis on the pollen size in 'Seabuckthorn' (Hippophae rhamnoides L., Elaeagnaceae).* Cytologia, 73: 167-172.
SINGHAL V.K., KAUR S. and KUMAR P., 2010 — *Aberrant male meiosis, pollen sterility and variable sized pollen grains in Clematis montana Buch. Ham. ex DC. from Dalhousie hills, Himachal Pradesh.* Cytologia, 75: 31-36.
SINGHAL V.K., KUMAR P., KAUR D. and RANA P.K., 2009b — *Chromatin transfer during male meiosis resulted into heterogeneous sized pollen grains in Anemone rivularis Buch. - Ham. ex DC. from Indian cold deserts.* Cytologia, 74: 229-234.
SOBTI S.N. and SINGH S.D., 1961 **—** *Chromosome survey of Indian medicinal plants*. Proceedings of the Indian Academy of Sciences, A54: 138-144.
SUYAL S., SHARMA C.M., GAIROLA S., GHILDIYAL S.K., RANA C.S. and BUTOLA. D.S., 2010 — *Phytodiversity (Angiosperms and Gymnosperms) in Chaurangikhal forest of Garhwal Himalaya, Uttarakhand, India*. Indian Journal of Science and Technology, 3: 267-275.
VAIDYA B.L. and JOSHI K.K., 2003 — *Cytogenetical studies of some species of Himalayan Anemone and Ranunculus (Ranunculaceae).* Cytologia, 68: 61-66.

Received March 30th 2010; accepted November 19th 2010