Cytomixis and associated meiotic abnormalities resulted into abnormal sporads and low pollen fertility in *Ranunculus laetus*.

MANINDER*[1], VIJAY KUMAR SINGHAL[1]

1 DEPARTMENT OF BOTANY, PUNJABI UNIVERSITY, PATIALA, PUNJAB, INDIA

Abstract

In the present study, we report the phenomenon of cytomixis in some individuals of *Ranunculus laetus* showing chromatin transfer at the different stages of meiosis I. In most of the cases, two to six PMCs are involved in chromatin transfer. The migration of chromatin is either complete or partial, resulting in denucleated, double nucleolated, hypoploid and hyperploid PMCs. Single or multiple cytoplasmic channels between two or more proximate PMCs have been observed at prophase I. PMCs involved in cytomixis showed various other meiotic irregularities such as chromatin stickiness, asynchronous disjunction of bivalents and laggards. Present study depicts cytomixis and associated meiotic irregularities causing a significant reduction in pollen fertility which further resulted into formation of heterogeneous-sized fertile and sterile pollen grains.

Keywords: *Ranunculus laetus*, cytomixis, meiotic irregularities, chromatin stickiness, pollen grains

INTRODUCTION

Ranunculus laetus, also known as 'Cheerful buttercup', is a diffuse perennial herb with woody rootstock, usually found in moist shady places, dry waste grounds and along roadsides. The yellow-coloured flowers which appear during the months of June to October are present on tall, slender, upright leafless stems (fig. 1a). The species is widespread throughout the Himalayas in India between altitudes of 1500-3000m. It is highly variable and polymorphic species in its habitat, hairiness of stem, and leaf and flower size (Aswal and Mehrotra, 1994).

The phenomenon of cytomixis is defined as the migration of chromatin material among proximate cells through cytoplasmic connections or intercellular bridges and cytomictic channels as well as through cell wall dissolution (Falistocco *et al.*, 1995). It was first observed by Körnicke (1901) in pollen mother cells (PMCs) of *Crocus sativus*. Subsequently, Gates (1908) observed delicate threads of cytoplasm connecting adjacent PMCs in *Oenothera* species. Gates (1911) suggested that these connections constitute an important pathway for the exchange of genetic material and cytoplasm between proximate PMCs, and described the transfer of nuclear material through them from one meiocyte to another, coined the term 'cytomixis'. Cytoplasmic connections between meiocytes originate from pre-existing system of plasmodesmata which develop in anther tissues and then, in general, becomes obstructed by the progressive deposition of callose (Heslop-Harrison, 1966). However, in some cases, these may exist till the later stages of meiosis and their size may increase to form conspicuous inter-PMC cytomictic channels through which transfer of chromatin or chromosomes may takes place (Mursalimov and Deineko, 2011).

Materials and methods

Collection of materials for cytomorphogical investigations, wild accessions were collected from the high altitudinal regions of Palchan (2400m) and Rohtang Tunnel (3100m) of Himachal Pradesh. The accessions were identified by consulting floras and were compared with the specimens lying in the Herbaria (PUN), Punjabi University, Patiala and Northern Regional Centre, Botanical Survey of India, Dehra Dun (BSD). The duly identified specimens were deposited in the Herbarium, Department of Botany, Punjabi University, Patiala. For meiotic and pollen grain studies, floral buds of appropriate sizes were fixed in a freshly prepared Carnoy's fixative (mixture of alcohol–chloroform– acetic acid in a volume ratio 6 : 3 : 1) and preserved in 70% ethanol in a refrigerator. Photomicrographs of well spread chromosomes, tetrads, pollen grains were taken with a digital imaging system of Leica Q Win Digital system.

Male Meiosis, Pollen Grain and Morphometric Analyses

Meiocytes were prepared by squashing the young and developing anthers in 1% acetocarmine. These were observed at various stages of prophase-1, metaphase-1 (M-I), anaphase-1/II (A-I/-II), telophases-1/II and sporads. Pollen fertility was assessed through stainability tests by squashing mature anthers from various blossoms in glycerol–acetocarmine (1:1) mixture. Well filled pollen grains with stained nuclei were scored as apparently fertile, while shriveled and flaccid pollen grains with unstained or poorly stained cytoplasm as sterile.

RESULTS

The present study based on four accessions uniformly shared the same gametic chromosome count of n=16 as confirmed from the presence of 16 large-sized bivalents at M-I (fig. 1b) and 16:16 distribution of chromosomes at A-I (fig. 1c). Based on x=8, the presently studied accessions existed at 4x level. While the three accessions scored from Palchan (2400m) depicted perfectly regular meiotic course and normal sporad formation, resulting into high pollen fertility (92-98%), the accession collected around Rohtang Tunnel (3100m) showed the phenomenon of cytomixis involving chromatin transfer among 2-4 PMCs at M-I (fig. 1d, Table 1). As a consequence of cytomixis, meiocytes involved in cytomixis also showed some meiotic irregularities as, chromatin stickiness, unequal (15:17) distribution of chromosomes at A-I, laggards and chromatin bridges (fig. 1e) and multi-polar distribution of chromosomes along with multiple nucleoli at T-II (fig. 1f). Consequent to these meiotic abnormalities, irregular microsporogenesis in the form of sporads with micronuclei (figs. 1g, 1h) were resulted. Pollen fertility was reduced to 28% and heterogenous sized fertile pollen grains as, large (25.39 x 23.43μm), medium (22.34 x 20.58 μm) and small-sized (19.72 x 18.54μm) were resulted (fig. 1i).

Table 1: Data on cytomixis and associated meiotic irregularities in *Ranunculus laetus* accession scored from Rohtang Tunnel (3100m).

Meiocytes involved in cytomixis and associated meiotic irregularities	Accession Rohtang Tunnel (3100m) PUN 59346
PMCs involved in cytomixis (%age)	15.21
Number of PMCs involved in cytomixis	2-3
PMCs showing chromatin stickiness (%age)	11.59
PMCs showing unequal distribution of chromosomes (%age)	9.4
PMCs with laggards and bridges at anaphases/telophases (%age)	10.86
PMCs with multipolar chromosomal distribution (%age)	14.97
Sporads with micronuclei (%age)	8.21
Pollen fertility (%age)	72

Discussion

The tetraploid chromosome count of 2n=32 (based on x=8) reported presently from the different localities of Solang Valley agrees with the earlier chromosome reports by workers from India and outside of India. The species is also known to exist at diploid level with 2n=16 (Khatoon and Ali, 1993; Vaidya and Joshi, 2003). Intraspecific polyploid cytotypes such as tetraploid, 2n=28 (Bir and Thakur, 1984; Himshikha, 2014; Kumar, 2015), hexaploid, 2n=42 (Roy and Sharma, 1971) and octoploid, 2n=56 (Kabu *et al*., 1988) (based on x=7) are also known to exist in the Himalayas, respectively. Kaur, S. *et al*. (2010) reported the presence of 3 B-chromosomes in the plants from Dalhousie hills. It thus indicates that the species which is highly variable morphogenetically depict considerable amount of chromosomal diversity involving intraspecific polyploidy at two different basic numbers, x=7 and x=8.

Till now, the phenomenon of cytomixis is known to be reported in a wide range of angiosperms both dicots and monocots (Kaur, M. and Singhal, 2014; Kumar, R. *et al*., 2015; Kumar, G. and Chaudhary, 2016). Cytomixis has been suggested to be more prevalent in genetically, physiologically and biochemically imbalanced plants such as triploids, haploids, hybrids, mutants, apomicts, trisomics and aneuploids (Li *et al*., 2009) where it causes irregularities during the meiotic process and its end-products.

CONCLUSION

Although opinions about the significance of cytomixis are varied and conflicting, most researchers agreed that it must have an evolutionary significance (Boldrini and Pagliarini, 2006). It was also considered as a possible cause of aneuploidy and polyploidy (Lattoo *et al.*, 2006), or produce unreduced pollen grains as reported in several grass species including *Dactylis* (Falistocco *et al.*, 1995), *Aegilops* (Sheidai *et al.*, 1999), and other flowering plants including *Anemone rivularis* (Kumar, R. *et al*., 2015), and *Lippia alba* (Reis *et al*., 2016). Present studies indicates that the occurrence and frequency of meiocytes involved in cytomixis has no correlation with ploidy level, rather it is the genetic makeup and prevailing environmental conditions which are responsible for the presence or absence of cytomixis.

Referances

Bir, S.S. and Thakur, H.K. 1984. In: SOCGI Plant chromosome number reports-II. J. Cytol. Genet. 19: 114-115.

Boldrini KR, Pagliarini, MS 2006. Cell fusion and cytomixis during microsporogenesis in *Brachiaria humidicola* (Poaceae). S African J Bot 72: 478-481.

Falistocco E, Tosti N, Falcinelli M. 1995. Cytomixis in pollen mother cells of diploid *Dactylis*, one of the origins of '2n' gametes. J Hered 86: 448-453.

Gates RR. 1908. A study of reduction in Oenothera rubrinervis. Bot Gaz 46: 1-34.

Gates RR. 1911. Pollen formation in Oenothera gigas. Ann Bot 25: 909-940.

Heslop-Harrison J 1966. Cytoplasmic connections between angiosperm meiocytes. Ann Bot 30: 221-234.

Himshikha. 2014. Cytomorphological Explorations of Dicots from Parvati Valley in Kullu District (Himachal Pradesh). Ph.D. Thesis, Pbi. Univ., Patiala.

Kabu, R., Wafai, B.A. and Kachroo, P. 1988. Studies on the genus Ranunculus L. I. Natural diploidy in R. laetus Wall. ex. Hook. et Thoms. and impact of intraspecific chromosome variability on the phenotype of the species. Phytomorphology 38: 321-325.

Kaur M and Singhal VK. 2014. First report of cytomixis and meiotic abnormalities in Nepeta govaniana from Solang Valley, Kullu district, Himachal Pradesh. Cytologia 79: 227-233.

Kaur, S., Singhal, V.K. and Kumar, P. 2010. Male meiotic studies in some plants of Polypetalae from Dalhousie hills (Himachal Pradesh). Cytologia 75: 289-297.

Khatoon, S. and Ali, S.I. 1993. Chromosome Atlas of the Angiosperms of Pakistan. Department of Botany, Univ. of Karachi, Karachi.

Körnicke M. 1901. Uber ortsveranderung von Zellkarnern S B Niederhein Ges Natur-U Heilkunde Bonn A. pp. 14-25.

Kumar G, Chaudhary, N 2016. Induced cytomictic variations and syncyte formation during microsporogenesis in Phaseolus vulgaris L. Cytol Genet 50: 121–127.

Kumar P, Singhal, V.K. 2008. Cytology of Caltha palustris L. (Ranunculaceae) from cold regions of Western Himalayas. Cytologia 73: 137-143.

Kumar P. 2015. Cytomorphological Studies in the Dicotyledonous Plants from Pangi Valley and its Adjoining Areas of District Chamba (H.P.). Ph.D. Thesis, Pbi. Univ., Patiala.

Kumar R, Rana PK, Himshikha, Kaur D, Kaur M, Singhal VK, Gupta RC, Kumar P. 2015. Structural heterozygosity and cytomixis driven pollen sterility in *Anemone rivularis* Buch.-Ham. ex DC. from Western Himalaya (India). Caryologia 68: 246–253.

Kumar, Pawan 2015. Cytomorphological Studies in the Dicotyledonous Plants From Pangi Valley and its Adjoining Areas of District Chamba (H.P.). Ph.D. Thesis, Pbi. Univ., Patiala.

Lattoo SK, Khan S, Bamotra S, Dhar AK. 2006. Cytomixis impairs meiosis and influences reproductive success in Chlorophytum comosum (Thunb.) Jacq. - an additional strategy and possible implications. J Biosci 31: 629-637.

Li XF, Song ZQ, Feng DS, Wang, HG. 2009. Cytomixis in *Thinopyrum intermedium*, *Thinopyrum ponticum* and its hybrids with Wheat. Cereal Res Commun 37: 353-361.

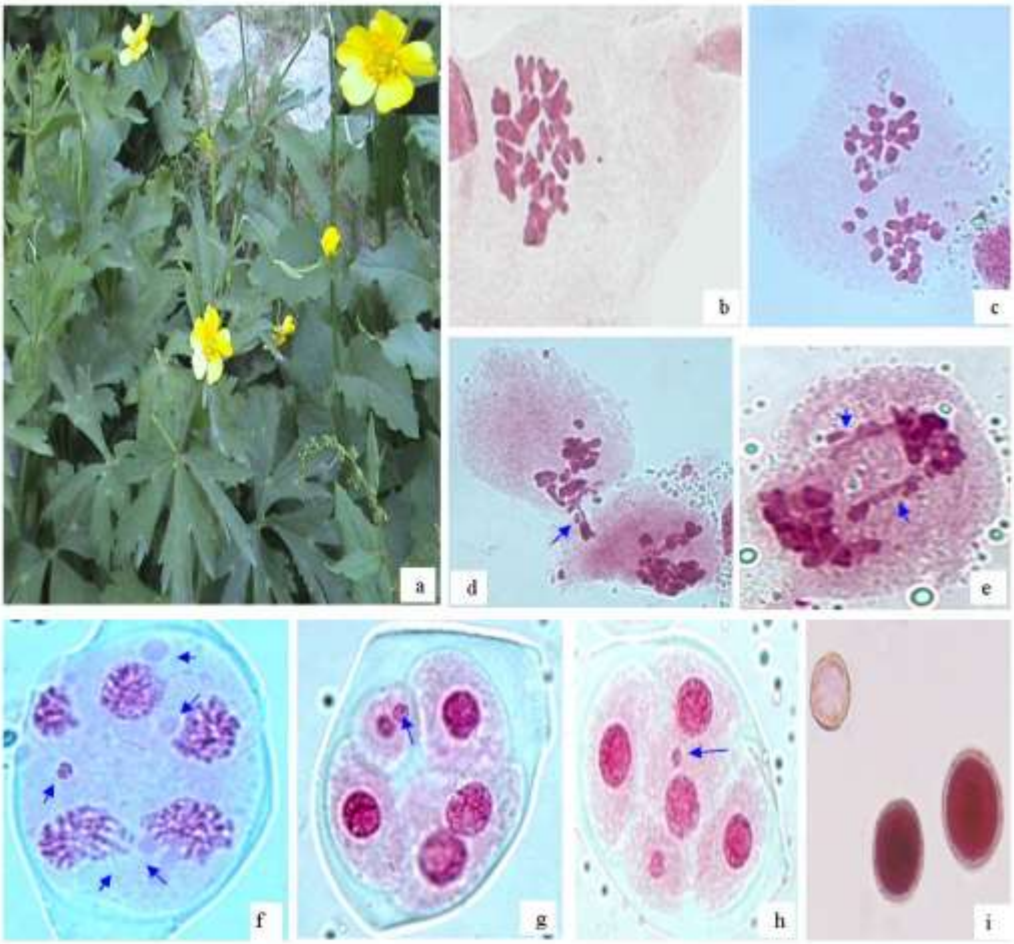
Mursalimov SR, Deineko EV. 2011. An ultrastructural study of cytomixis in tobacco pollen mother cells. Protoplasma 248: 717-724.

Reis AC, Sousa SM, Viccini LF. 2016. High frequency of cytomixis observed at zygotene in tetraploid *Lippia alba*. Pl Syst Evol 302: 121-127.

Roy SC, Sharma AK. 1971. Cytotaxonomic studies in Indian Ranunculaceae. Nucleus 14: 132-143.

Sheidai M, Attaei S. 2005. Meiotic studies of some Stipa (Poaceae) species and population in Iran. Cytologia 70: 23-31.

Vaidya BL, Joshi, KK. 2003. Cytogenetic studies of some species of Himalayan *Anemone* and *Ranunculus* (Ranunculaceae). Cytologia 68: 61-66.



***Ranunculus laetus*, (a-i),** a) An erect perennial herb with hairy, much branched stem with deeply dissected leaves and yellow flowers (inset). (b) A PMC showing 16 large-sized bivalents at M-I. (c) A PMC showing 16:16 equal distribution of chromosomes at A-I. (d) Two proximate PMCs depicting cytomixis (arrowed). (e) A PMC showing chromatin bridges and laggards (arrowed) at A-I. (f) A multipolar PMC at T-II with multiple nucleoli (arrowed). (g) A tetrad with two micronuclei (arrowed) (h) A tetrad with micronucleus (arrowed) (i) Heterogenous-sized fertile and sterile pollen grains.