IRREGULAR MEIOTIC BEHAVIOUR RESULTED INTO ABNORMAL SPORAD FORMATION AND LOW POLLEN FERTILITY IN TWO ACCESSIONS OF *CALTHA PALUSTRIS*

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Abstract

In the present study, we report the phenomenon of cytomixis in some individuals of *Caltha palustris* 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, 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 spindle abnormalities, chromatin stickiness, asynchronous disjunction of bivalents and laggards. In the present case, the cytomixis and associated meiotic irregularities cause a significant reduction in pollen fertility, and resulted into the formation of heterogeneous-sized pollen grains. The role of such variably sized fertile pollen grains in the origin of polyploids and aneuploids can not be ruled out.

Keywords: *Caltha palustris*, cytomixis, meiotic irregularities, chromatin stickiness, pollen grains

INTRODUCTION

Caltha palustris L. also referred as ‘Marsh marigold’ is a perennial herb, commonly found growing along streams and is characterized by kidney-shaped, finely toothed leaves and clusters of yellow cup-shaped flowers (fig. 1a). The species is distributed in the Himalayas from Pakistan to Bhutan between altitudinal ranges of 2200-4000m. Flowering and fruiting appear during June-September.

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 Solang Nullah (2700m), PUN 59336 and Dhundhi (3050m), PUN 59337 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

Presently, two accessions analyzed meiotically depicted the same tetraploid chromosome count of n=16 (based on x=8) as confirmed from the presence of 16 bivalents at diakinesis (fig. 1b) and 16:16 chromosomal distribution at M-II (fig. 1c). Detailed meiotic analysis revealed that meiocytes in both the accessions showed the phenomenon of cytomixis involving chromatin transfer at different stages of meiosis (figs. 1d, 1e). Some of the meiocytes involved in chromatin transfer also showed extra chromatin material along the periphery (fig. 1f), pycnotic chromatin (fig. 1g), out of plate bivalent (fig. 1h), chromatin stickiness (fig. 1i), chromatin bridge and a micronucleus at T-II (fig. 1j) and consequently abnormal sporads

(fig. 1k) and sterile pollen grains and micropollen (11-24% figs. 1l, 1m). The data on cytomixis and cytomictically induced meiotic irregularities in the two accessions have been provided in Table 1.

Analysis of data revealed that %age of PMCs involved in chromatin transfer and meiocytes with meiotic abnormalities was found to be relatively higher in plants scored from Dhundhi (3050m) as compared to those collected from Solang Nullah (2700m). Analysis of data as provided in Table 1 revealed that reduction in pollen fertility as a consequence of cytomixis is found to be directly correlated with frequency of meiocytes involved in cytomixis and meiotic abnormalities which are significantly higher in the plants of Dhundhi (3050m).

Table 1: Data on cytomixis, meiotic irregularities and pollen fertility (%age) in the individuals of *Caltha palustris* (n=16) analyzed from Solang Nullah (2700m) and Dhundhi (3050m).

Meiotic irregularities	**Accessions**	
	Solang Nullah (2700m) PUN 59336	**Dhundhi (3050m) PUN 59337**
PMCs involved in cytomixis (%age)	16.34	23.69
Number of PMCs involved in cytomixis	2-3	2-3
PMCs showing pycnotic chromatin (%age)	5.91	18.76
PMCs showing fragmented chromosomes (%age)	9.34	15.62
PMCs showing chromatin stickiness (%age)	12.67	25.91
PMCs showing chromatin bridges and laggards at T-II (%age)	8.98	12.19
Triads (%age)	4.67	10.71
Tetrads with micronuclei (%age)	3.32	17.14
Pollen fertility (%age)	89	76

Discussion

The presently detected chromosome count of 2n=32 in the studied species is in agreement with the earlier chromosome reports by researchers from India as well as outside of India (Kumar and Singhal,

2008). However, perusal of chromosomal literature reveals that the species exhibits an array of chromosomes numbers, 2n=16, 28, 32, 34, 38, 40, 44, 48, 50, 52, 54, 55, 56, 57, 58, 60, 64, 68 which might have been facilitated in the species owing to its dual mode of propagation (sexual and vegetative) coupled with wide range of adaptation in Himalayas.

The existence of cytomixis in the meiocytes along with meiotic abnormalities and reduced pollen fertility have also been reported earlier in the plants growing around Rohtang Pass and Lahaul-Spiti Valley (Kumar and Singhal, 2008). Kumar, 2015 while exploring the cytomorphological diversity in the plants of Pangi Valley has also reported the phenomenon of cytomixis and associated meiotic irregularities in the species. It thus indicates that majority of the individuals growing in high altitudinal regions of North–West Himalayas existed at 4x level (2n=32) and showed the phenomenon of cytomixis seem to be associated with the genetic make-up of the species and is under the control of some genetic factors.

Till now, the phenomenon 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.

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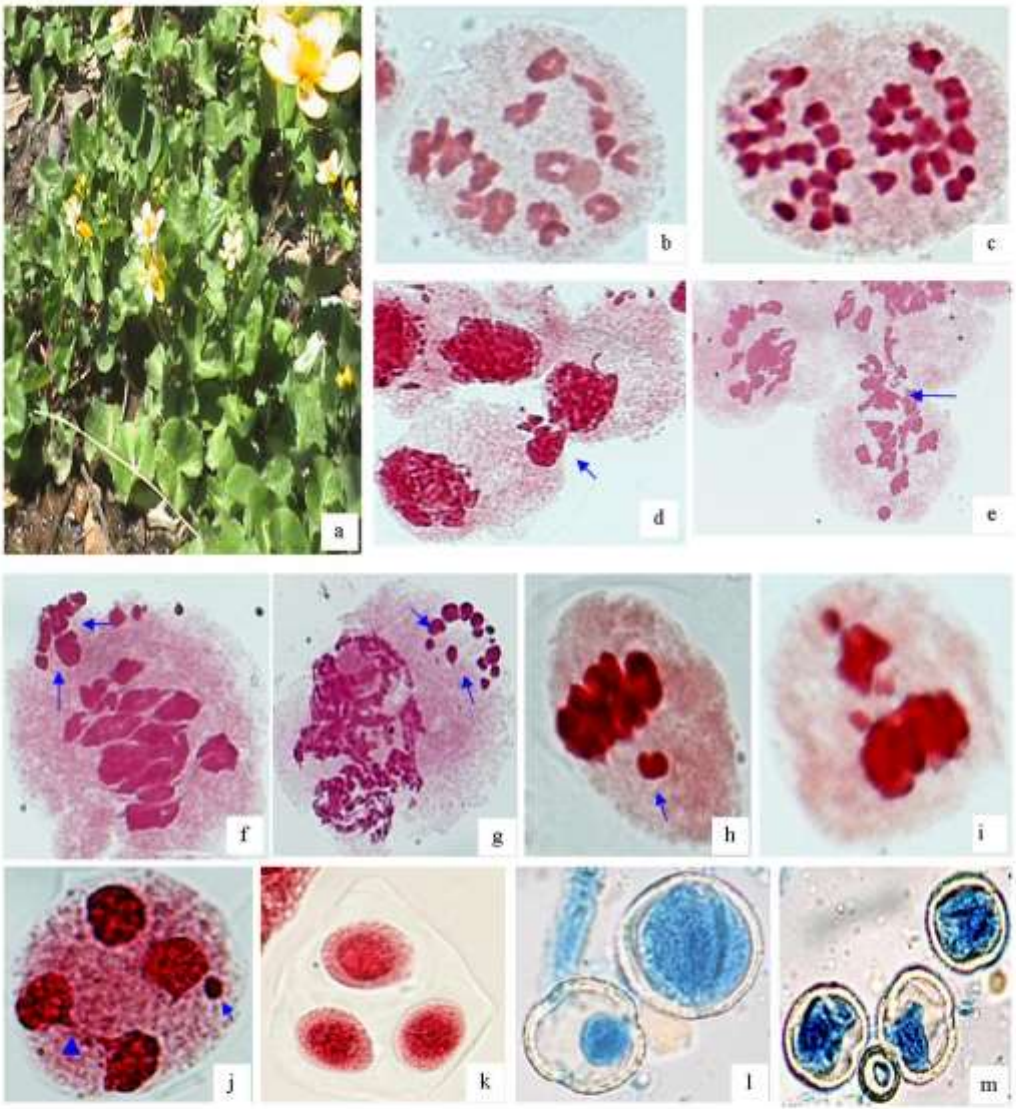

Figure: 1 (a-m). *Caltha palustris*, (a) A perennial herb forming loose clumps of kidney-shaped and finely toothed leaves with clusters of yellow cup-shaped flowers (inset). (b) A PMC showing 16 bivalents at diakinesis. (c) A PMC depicting 16:16 equal distribution of chromosomes at M-II. (d, e) PMCs showing cytomixis involving chromatin transfer at different stages of meiosis (arrowed). (f) A PMC showing extra chromatin material of different sizes along the periphery (arrowed). (g) A PMC showing pycnotic chromatin material at early Prophase-I (arrowed). (h) A PMC showing out of plate bivalent at M-I (arrowed). (i) A PMC showing chromatin stickiness. (j) A PMC showing a micronucleus at T-II (arrowed) and a chromatin bridge (arrow-head). (k) A triad. (l) Apparently fertile and sterile pollen grains. (m) Apparently fertile and sterile pollen grains along with a micropollen (arrowed).