First ever record of meiotic chromosome count, cytomixis and associated meiotic irregularities in Silene gallica L

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Abstract:- Presently, we report the first ever record of meiotic chromosome count in Silene gallica from India. In addition, the phenomenon of cytomixis showing chromatin transfer and related meiotic irregularities at the different stages of meiosis I in some individuals of the species has also been recorded in the species for the first time on the world wide basis. In most of the cases, two to six PMCs are involved in chromatin transfer. The migration of chromatin resulted into 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: Silene gallica, cytomixis, meiotic irregularities, chromatin stickiness, pollen grains

INTRODUCTION

Silene gallica is an erect annual herb, 10-45 cm high, is a native to Eurasia and North Africa, but can be found throughout the temperate world as a common roadside weed. In India, it can be seen growing wild in the sub-Himalayan region. The species possesses linear-oblong or spathulate leaves with bright pink coloured flowers on a long, unilateral raceme (fig. 1a). Flowering and fruiting occur in the months of July-October.

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 Bahang (2450m), and Vashisht Village (2475m) of Solang Valley, 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. 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 two accessions scored from Bahang (2450m), and Vashisht Village (2475m) shared the same gametic chromosome count of n=12 (fig. 1b). However, the accession scored from Vashisht Village (2475m) also showed the phenomenon of cytomixis involving chromatin transfer in 17.31% PMCs (figs. 1c, 1d). The PMCs involved in cytomixis also showed extra-chromatin material, laggards at anaphases/telophases (11.25%) (figs. 1e, 1f, 1g) and scattered chromosomes due to abnormal spindle activity (9.33%) (fig. 1h). Consequent to these, abnormal sporads such as dyads (4.59%), triads (6.12%), and tetrads with micronuclei (figs. 1i-1l) were observed. The products of such sporads yielded sterile pollen (8%) and heterogenous-sized pollen grains (fig. 1m). The pollen grains were of two sizes as, large 35.62 x 16.86 µm (58.46%) and typical sized 23.18 x 21.51 µm (41.54%). The data on cytomixis and associated meiotic irregularities in the accession scored from Vashisht Village (2475m) is presented in Table 1.

Table 7: Data on cytomixis and associated meiotic irregularities in S. gallica scored from Vashisht Village (2475m).

Meiotic irregularities	Accession Vashisht Village (2475 m) PUN 59214
PMCs involved in cytomixis (%age)	17.31
Number of PMCs involved in cytomixis	2-3
PMCs showing pycnotic chromatin, laggards at anaphases/telophases (%age)	11.25
PMCs showing scattered distribution of chromosomes (%age)	9.33
Dyads (%age)	4.59
Triads (%age)	6.12
Tetrads with micronuclei (%age)	2.53
Pollen sterility (%age)	8

DISCUSSION

Present chromosome count of 2n=24 is the first ever chromosomal record for the species from India which is in line with the earlier reports for the species from outside of India. Earlier the species has been counted chromosomally from other regions of the world (Yildiz & Cirpici, 1996; Valdés *et al.*, 1997).

The phenomenon of cytomixis and associated meiotic irregularities has been reported for the first time in the species on the worldwide basis.

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.

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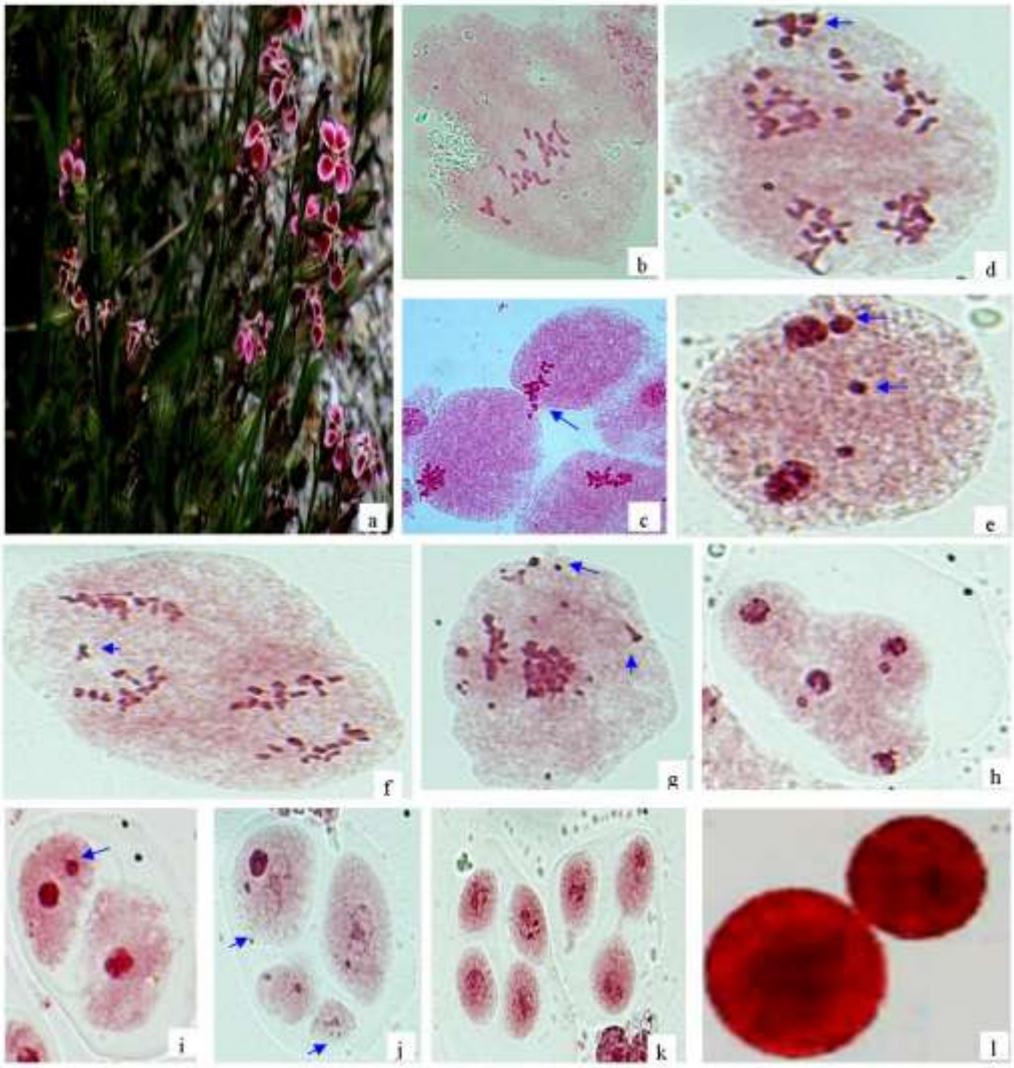

Figure 1. (a-m) *Silene gallica*, (a-l), a) An annual herb with linear-oblong leaves and bright pink flowers on a long, unilateral raceme. (b) A PMC at M-I with 12 bivalents. (c) Two adjacent PMCs depicting cytomixis involving chromatin transfer at M-I (arrowed). (d) Three proximate PMCs showing cytomixis at T-II (arrowed). (e) A PMC showing extra chromatin towards periphery (arrowed). (f) A PMC showing laggards at T-I (arrowed). (g) A PMC showing laggard at A-II (arrowed). (h) A PMC showing scattered chromatin material due to abnormal spindle activity. (i) A tetrad with two micronuclei. (j) A dyad with included micronuclei (arrowed). (k) A tetrad with unequal-sized microspore units (arrowed). (l) A triad and a tetrad (m) Heterogenous sized fertile/ stained pollen grains.