First ever record of cytomixis, and associated meiotic irregularities resulting into reduced pollen fertility (%age) in Clematis *buchananiana* DC.

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Abstract: We report the phenomenon of cytomixis for the first time while studying some populations of *Clematis buchananiana* DC. which shows chromatin transfer at the different stages of meiosis-I. In most of the cases, 2-3 PMCs are involved in chromatin transfer. Transfer of chromatin material through cytomixis resulted into various other meiotic irregularities like hypoploid and hyperploid PMCs, out of plate bivalents, extra-chromatin masses lying away from the main chromosome complement, and chromatin stickiness. In the present case, as a result of cytomixis and other associated meiotic irregularities, a significant reduction in pollen fertility is observed.

Keywords: *Clematis buchananiana* DC., Cytomixis, Chromatin stickiness, Hyperploids, Hypoploids

Introduction

Clematis buchananiana DC. is a deciduous woody climber with pinnately compound leaves, commonly found on forest margins, generally climbing on small trees and bushes. Pale yellow-coloured flowers are borne in cymes in leaf axils. The species is widely distributed in Eastern Asia from Himalayas to Tibet, Burma and Western China, between altitudinal ranges of 1700-3000m. Flowering and fruiting appear during July-September.

Materials and Methods

The materials for cytological studies were collected from wild plants growing at various altitudes in Solang Valley of Himachal Pradesh, India, during the months of April-September, from two different localities. Voucher specimens of the cytologically worked out accessions were deposited in the Herbarium, Department of Botany, Punjabi University, Patiala. Young and unopened floral buds of suitable sizes randomly collected from five individuals in each population, were fixed in Carnoy's fixative, transferred to 70% alcohol and stored in a refrigerator. Pollen mother cells (PMCs) were prepared by the squash technique and stained with

1% acetocarmine. Freshly prepared slides were carefully examined to determine the chromosome number and meiotic abnormalities. Pollen fertility was estimated through stainability tests by squashing the anthers from mature and opened flowers in glyceroacetocarmine (1:1) mixture, or 1% aniline blue dye. Well-filled pollen grains with stained nuclei were taken as apparently fertile, while shriveled and unstained ones were counted as sterile. Photomicrographs of pollen mother cells, sporads, and pollen grains were taken by a Nikon Eclipse 80i microscope. Pollen size was measured by an oculo-micrometer.

Two accessions collected from Bahang (2450m) and Solang Nullah (2700m) showed the same gametic chromosome count of n=8 as confirmed from the presence of 8 bivalents at M-I (fig. 1a). While the accession collected from Bahang (2450m) showed normal meiotic behaviour with perfect microsporogenesis and nearly cent per cent pollen fertility (98%) (fig. 1b), the accession scored from a relatively high altitude site along Solang Nullah (2700m) depicted the phenomenon of cytomixis involving chromatin transfer among 2-3 PMCs (fig. 1c) and other associated meiotic irregularities like, chromatin masses lying away from the main chromosome complement (fig. 1d), out of plate bivalent (fig. 1e), and chromatin stickiness (fig. 1f) resulting into hypoploid (fig. 1g), and hyperploid PMCs (figs. 1h, 1i), tetrads with micronuclei (fig. 1j) resulting into reduced pollen fertility (79%) (fig. 1k). Data regarding cytomixis and associated meiotic irregularities and pollen fertility (%age) in the studied accession around Solang Nullah (2700m) are provided in Table 1.

Table 1: Data on cytomixis, meiotic irregularities and pollen fertility (%age) in the accession scored from Solang Nullah (2700m) in *C. buchananiana.*

Meiotic irregularities	**Accession**
	Solang Nullah (2700m) PUN 59339
PMCs involved in cytomixis (% age)	19.20
Number of PMCs involved in cytomixis	2-3
Meiotic stages at which cytomixis occur	Prophase-1, M-I
PMCs showing pycnotic chromatin and extra chromatin masses (% age)	6.67

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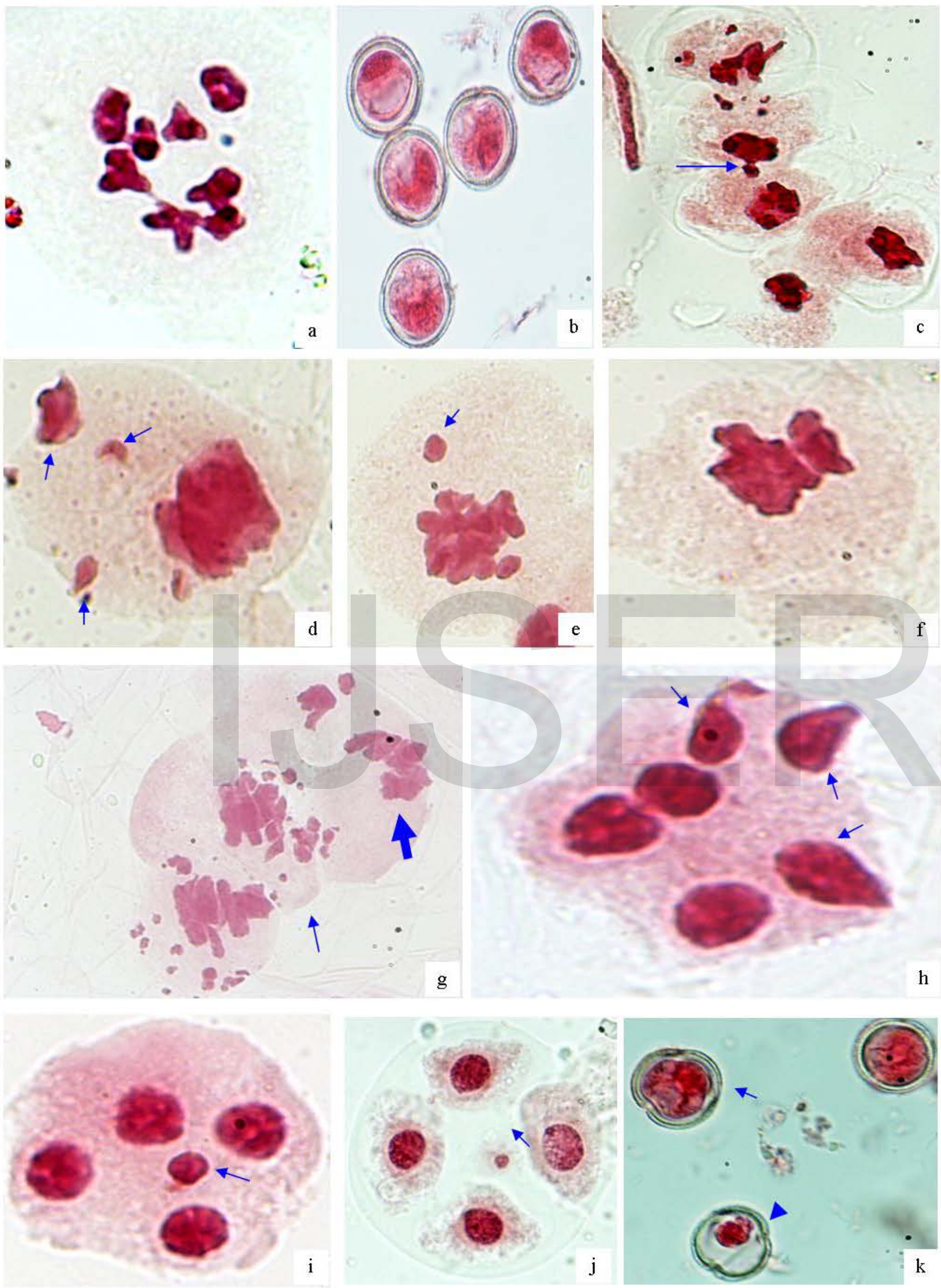
PMCs with spindle abnormalities in the form of out of plate bivalent (% age)	5.83
PMCs showing chromatin stickiness (% age)	12.03
Abnormal sporads (%age)	27.72
Pollen fertility (%age)	79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Discussion

The present study substantiates the earlier diploid chromosome count of 2n=16 reported by several workers from Indian Himalayas (Mehra and Kaur, 1963; Bhattacharjee, 1976; Sharma and Bhattacharya, 1976; Bhattacharjee and Sharma, 1980) and also from the Soviet Union (Volkova and Ulanova, 1986). Bir and Thakur (1984) and Bir *et al.* (1987) have reported the presence of 1 B-chromosome in the individual analyzed from Garhwal Himalayas. However, the phenomenon of cytomixis and associated meiotic irregularities observed during the present analysis has been reported for the first time in the species.

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 (Falistocco *et al.*, 1995; Haroun, 1995; Singhal and Kumar, 2008a, b, 2010; Shabrangi *et al.*, 2010; Mursalimov and Deineko, 2011).

Till now, the phenomenon is known to be reported in a wide range of angiosperms both dicots and monocots (Boldrini and Pagliarini, 2006; Lattoo *et al.*, 2006; Kumar *et al.*, 2010, 2012, 2013, 2016; Singhal *et al.*, 2008, 2009, 2010, 2011, 2014; Sheidai *et al.*, 2009a, b, 2010; Fadaie *et al.*, 2010; Mursalimov *et al.*, 2010; Mandal *et al.*, 2013; Rana *et al.*, 2013, 2014; Kaur, M. and Singhal, 2014; Kumar, R. *et al.*, 2015). 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 (Haroun *et al.*, 2004; Li *et al*., 2009) where it causes irregularities during the meiotic process and its end-products.

Although opinions about the significance of cytomixis are varied and conflicting, most researchers agreed that it must have an evolutionary significance (Ghanima and Talaat, 2003; 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), *Alopecurus* and *Catbrosa* (Sheidai *et al.*, 2009a), *Hordeum* (Sheidai *et al.*, 2010), *Sorghum bicolor* (Ghaffari, 2006) and other flowering plants such as *Meconopsis aculeata* (Singhal and Kumar, 2008a), *Clematis flammula* (Kumar *et al.*, 2008), *Houttuynia cordata* (Guan *et al.*, 2012), *Nepeta govaniana* (Kaur and Singhal, 2014), and *Anemone rivularis* (Kumar *et al*., 2015).

In the presently analyzed species, the phenomenon among meiocytes was noticed to be more common during the early stages of meiosis-I. The frequency of chromatin transfer was also reported to be much higher during the first meiotic division than the second division as has been shown by Bellucci *et al.* (2003), Song and Li (2009).

Phenomenon of cytomixis in the presently studied species seems to have induced various meiotic abnormalities in the meiocytes which included chromatin stickiness, pycnotic chromatin, out of plate bivalent, spindle irregularities, aneuploid (hypo-/hyperploid) meiocytes, and aberrant microspore tetrads. The products of such aberrant sporads resulted into the formation of sterile and fertile pollen grains. Similar findings regarding the effects of cytomixis and associated meiotic irregularities on meiotic behaviour have been reported in *Polygonum tomentosum* (Haroun, 1995), *Hordeum vulgare* (Haroun, 1996), *Brassica napus* var. *oleifera* and *B. campestris* var *oleifera* (Alice and Maria, 1997), *Vicia faba* (Haroun *et al.*, 2004), and *Meconopsis aculeata* (Singhal and Kumar, 2008).

Although cytomixis has been reported in several plant species, yet its origin is still unclear. While cytomixis was considered in the past to be an anomalous meiotic behaviour, either due to pathological reasons (Bobak and Herich, 1978; Morrisset, 1978), or may be induced by mechanical injury (Takats, 1959), or induced by fixation (Heslop-Harrison, 1966; Haroun, 1995). It is now considered to be a normal cytological phenomenon and not an artifact of cytological preparations. Some of the factors thought to have been responsible for cytomixis include, the action of chemical agents such as colchicine (Dwivedi *et al.*, 1988), use of herbicides (Bobak and Herich, 1978), partial or total inhibition of cytokinesis during microsporogenesis (Risueño *et al.*, 1969), physiological and environmental factors (Bellucci *et al.*, 2003; Lattoo *et al.*, 2006; Boldrini and Pagliarini, 2006), temperature (De and Sharma, 1983), pressure differences (Morrisset, 1978), stress factors and genetic control (Malallah and Attia, 2003). Although environmental factors especially freezing temperature stress certainly influence the meiotic process, cytomixis seems to be a natural phenomenon under the genetic control as proposed by many authors (Lattoo *et al.*, 2006; Sidorchuk *et al.*, 2007; Singhal *et al.*, 2008, 2009a, b, 2010, 2011; Singhal and Kumar, 2008a, b, 2010; Kaur and Singhal, 2012; Kravets, 2011, 2013; Rana *et al.*, 2013, 2014; Kaur and Singhal, 2014, Kumar *et al.*, 2015).

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FIGURE LEGENDS

FIGURE 1 (a-k). a) A PMC with 8 large-sized bivalents at M-I. (b) Apparently fertile equal-sized pollen grains. (c) Three proximate PMCs showing cytomixis involving chromatin transfer (arrowed). (d) A PMC showing extra chromatin masses at M-I (arrowed). (e) A PMC showing out of plate bivalent (arrowed). (f) A PMC showing chromatin stickiness. (g) A hyperploid (arrowed) and hypoploid (arrowhead) PMC. (h) A hyperploid PMC showing 6 masses of chromatin material (arrowed). (i) A PMC showing micronucleus at T-II (arrowed). (j) A tetrad with a micronucleus (arrowed). (k) Apparently fertile (arrowed) and sterile (arrow-head) pollen grains.