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BRIEF COMMUNICATION

Cytomixis and associated meiotic abnormalities affecting pollen fertility in *Clematis orientalis*

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Abstract

Present cytological investigations from the cold desert regions of Lahaul-Spiti and Kinnaur (India) record the first ever tetraploid (2n=32) chromosome count and cytomixis in *Clematis orientalis* L. var. *acutifolia* Hook. f. *et* Thoms. The phenomenon of cytomixis (9.33 - 29.80 %) involving chromatin transfer among 2 - 3 proximate pollen mother cells (PMCs) during male meiosis occurs through narrow and broad cytoplasmic channels from early prophase to tetrad stage. However, frequency of its occurrence during the later meiotic stages is rather low. Chromatin transfer results into the formation of hypo-, hyperploid and enucleated PMCs. Various meiotic abnormalities associated with cytomixis such as chromatin stickiness, pycnotic chromatin, interbivalent connections, out of plate bivalents, late disjunction of bivalents, and laggards and bridges resulted into some pollen sterility (16.33 - 49.30 %) and heterogeneous pollen grains size.

Additional key words: chromatin stickiness, coenocytes, heterogeneous size of pollen grains, laggards and bridges, micronuclei, morphovariants.

Cytomixis involving transfer of chromatin material is reported in a large number of plants (Shnaider 1975, Singhal and Gill 1985, Bedi 1990, Lattoo *et al.* 2006, Singhal and Kumar 2008a,b). The phenomenon is better known to exist in genetically imbalanced plants like, hybrids, mutants and aneuploids, and polyploids (Premachandran *et al.* 1988, Peng *et al.* 2003, Zhou 2003, Sheidai and Attaei 2005). Recently, it has also been reported in transgenic plants of *Nicotiana tabacum* (Sidorchuk *et al.* 2007). Cytomixis is more commonly observed in meiotic cells but it has also been reported to occur in the somatic cells (Kostritsyna and Soldatov 1991, Wang *et al.* 2004, Guzicka and Wozny 2005).

Clematis orientalis, a widely distributed species of cold desert regions of Himalayas is quite variable morphologically and three varieties *acutifolia*, *obtusifolia* and *latifolia* based on the shape of leaflet and flower size are known in India (Hooker 1872). Intravarietal variation involving leaflet/lobe size and margin dentation is recorded presently in the individuals of var. *acutifolia* from Lahaul-Spiti and Kinnaur region. The species is also known to exhibit intraspecific diploid, 2n=16 (Sobti and Singh 1961, Shambulingappa 1966, Podlech and Dieterle 1969, Beskaravaynaja *et al.* 1979, Serov 1986, 1989, Khatoon and Ali 1993) and tetraploid, 2n=32 (Beskaravaynaja *et al.* 1979) cytotypes. With this idea in mind that the species is highly variable morphogenetically, an attempt has been made to study it cytomorphologically on population basis from the cold desert regions of Himalayas.

The materials for cytological studies were collected from the wild plants growing in the cold desert regions of Lahaul-Spiti and Kinnaur districts of Himachal Pradesh, India, during August in 2008. Presently, three morphovariants based on leaflet/lobe size and margin dentations have been identified in the variety *acutifolia* of *Clematis orientalis*. The morphovariant-1 (MV1), collected from Kibber at 4 270 m (Lahaul-Spiti), is with small

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Abbreviations: MV - morphovariant; PMC - pollen mother cell.

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leaflets/lobes (2.19 - 3.10 × 0.59 - 0.64 cm) and less toothed margins, morphovariant-2 (MV2), from Nako at 3 260 m (Kinnaur) is with large sized leaflets/lobes (7.27 - 9.10 × 3.75 - 4.25 cm) and more toothed margins, and morphovariant-3 (MV3), also from Nako, is with medium sized leaflets/lobes (3.20 - 4.6 × 0.80 - 2.10 cm) and more toothed margins. Voucher specimens of these cytologically worked out morphovariants are deposited in the Herbarium, Department of Botany, Punjabi University, Patiala (PUN: MV1 49995, MV2 49993, and MV3 49994). Young and unopened floral buds of suitable sizes (0.5 - 0.75 × 0.3 - 0.5 cm) randomly collected from five individuals in each morphovariant were fixed in Carnoy's fixative, transferred to 70 % alcohol and stored in refrigerator. Pollen mother cells (PMCs) were prepared by the squash technique and stained with 1 % acetocarmine. For somatic cells, no separate collection or fixation of seeds/shoot apex was made from the wild accessions. These somatic cells were observed in the same preparation for pollen mother cells. For all the three morphovariants (1234 pollen mother cells, 193 tetrads MV1; 965 PMCs, 247 tetrads MV2; 1860 PMCs, 628 tetrads MV3) freshly prepared slides were carefully examined to determine the chromosome number and meiotic abnormalities. Pollen fertility was estimated by squashing the anthers from mature and opened flowers in glyceroacetocarmine (1:1) mixture. For pollen fertility and pollen grain sizes (5140 pollen grains of MV1, 1436 pollen grains of MV2 and 3278 pollen grains of MV3) were observed. Well filled pollen grains with stained nuclei were taken as apparently fertile while shrivelled and unstained ones were counted as sterile. Pollen grain size was measured using occulomicrometre. Photomicrographs of pollen mother cells and pollen grains were made by *Nikon Eclipse 80i* microscope.

Meiotic investigations in three morphovariants revealed the occurrence of 16 large sized bivalents at diakinesis (Fig. 1*A*) and metaphase-I. The sporophytic chromosome count of 2n=32 was also confirmed from the presence of 32 chromosomes in somatic cells observed in the preparations for meiotic analysis. Besides, a polyploid somatic cell with 64 chromosomes and five nucleoli of different sizes was observed (Fig. 1*B*).

Cytomixis involving inter PMC transfer of chromatin material was observed in all the three morphovariants. Besides chromatin transfer, the species also showed various meiotic abnormalities and pollen grains of variable sizes and pollen sterility. The frequency of cytomixis in these morphovariants ranged between 9.33 - 29.80 % involving 2 - 3 PMCs (Fig. 1*C*). Chromatin transfer was maximum in MV2 (29.80 %) and minimum in MV3 (9.33 %). Transfer of chromatin occurred from early prophase to tetrad stage. However, frequency of its occurrence during the late meiotic stages was rather low. The transfer of chromatin occurred through narrow and broad cytoplasmic channels by the formation of one to many chromatin bridges (Fig. 1*D*). The chromatin transfer was either partial or complete resulting into the formation hypoploid, hyperploid and enucleated PMCs (Fig. 1*E*,*F*). Coenocytes resulting from the direct fusion of two PMCs were also observed (Fig. 1*G*).

Besides cytomixis meiotic abnormalities such as chromatin stickiness, pycnotic chromatin, interbivalent connections, out of plate bivalents, late disjunction of bivalents, and laggards and bridges were also recorded in this species. Chromatin stickiness was the most frequent meiotic anomaly from early prophase to telophase II. The chromatin stickiness was either partial or complete. PMCs with partial chromatin stickiness depicted 2 - 3 groups of chromatin masses (Fig. 1*H*). In complete chromatin stickiness whole of the complement was present in the form of a single chromatin mass (Fig. 1*H*). The MV2 individuals showed the highest frequency of PMCs (99.10 %) with chromatin stickiness. All the PMCs in anther showed chromatin stickiness at metaphase I, anaphases or telophases. Pycnotic chromatin was the other meiotic abnormality, which was quite common in PMCs during early stages. The number of pycnotic masses in PMCs varied between one to many (Fig. 1*I*). With the progress of meiosis these pycnotic masses were either eliminated or resulted into the formation of micronuclei in tetrads. Interbivalent chromatin connections among 2 - 4 bivalents were also observed at diakinesis in MV3 (Fig. 1*A*). In MV3, 7.62 % PMCs showed 1 - 3 bivalents which remain out of the metaphase plate (Fig. 1*J*). Late disjunction of 1 - 2 bivalents was also observed at anaphase I in MV1 and MV2. Lagging of 1 - 4 bivalents/chromosomes at anaphases/telophases were noticed in all the three morphovariants (Fig. 1*K*). Chromatin bridges were also observed at anaphases and telophases in MV1 and MV3. In MV1 both single and double bridges were present and their number varies from 1 to 5 (Fig. 1*L*). The microsporogenesis in 6.20 % (MV1), 4.40 % (MV2) and 4.12 % (MV3) cases was abnormal due to the presence of triads with unequal microspore units (Fig. 1*M*), polyads (Fig. 1*N*) and tetrads with 1 - 2 micronuclei (Fig. 1*O*). In some tetrads chromatin transfer occurred from micronucleus to microspore (Fig. 1*O*).

Consequent to cytomixis and associated meiotic abnormalities, individuals show 16.33 - 49.30 % pollen sterility (Fig. 1*P*) and heterogeneous sized pollen grains (Fig. 1*Q*). The frequency of pollen grains of size 25.48 - 28.50 × 25.48 - 27.90 μm (62.38 %) in MV1, 32.04 - 35.81 × 32.04 - 35.81 μm (91.55 %) in MV2 and 32.76 - 36.40 × 31.93 - 33.08 μm (91.40 %) in MV3 was higher as compared to pollen grain of other sizes.

The present chromosome count of n=16 and 2n=32 is the first ever record for var. *acutifolia*. All the three leaf morphovariants depicted the phenomenon of cytomixis and associated abnormalities during male meiosis. The occurrence of cytomixis in the species is reported for the first time. Although cytomixis involving chromatin

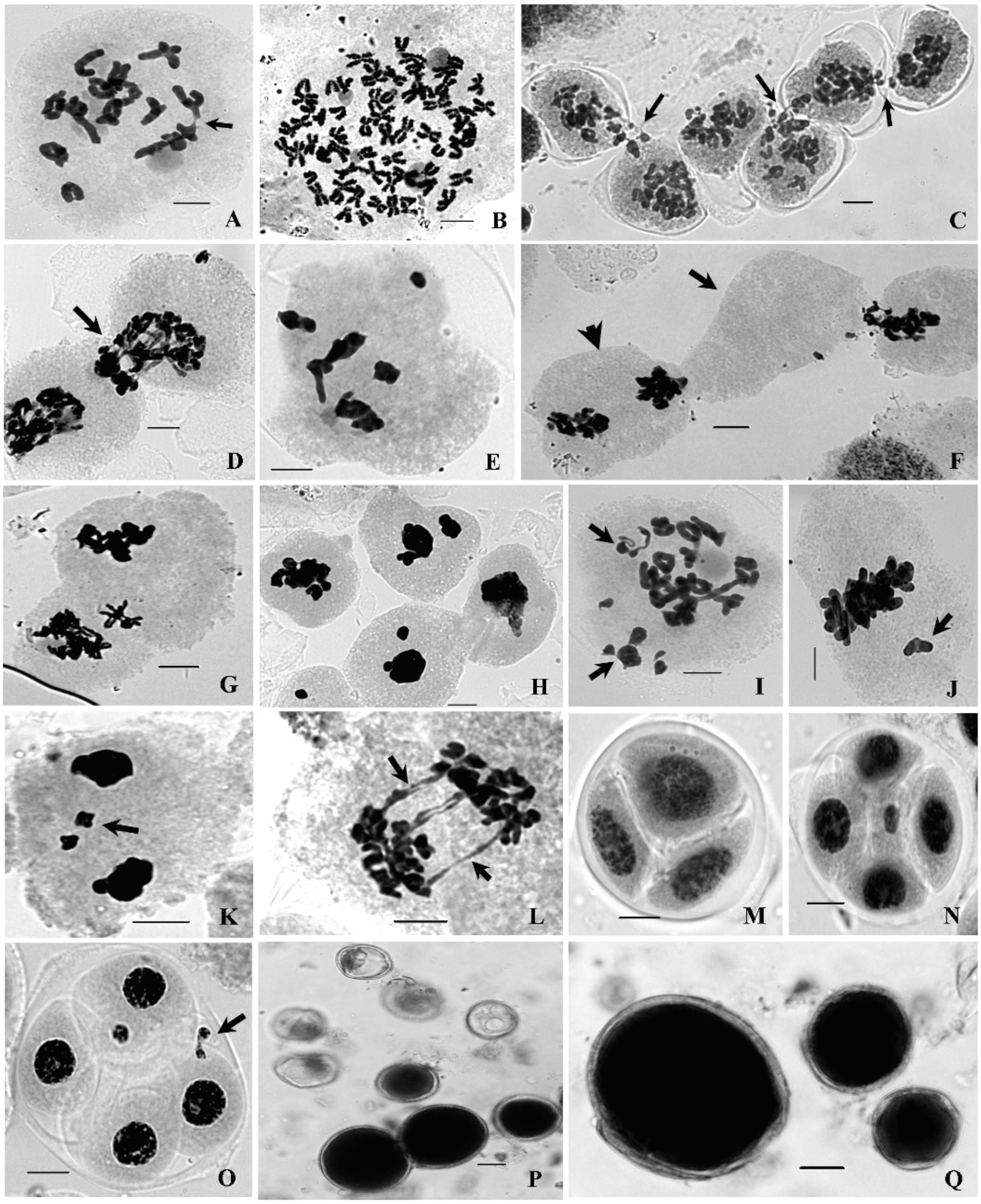

Fig. 1. Cytology of *Clematis orientalis* L. var. *acutifolia*: *A* - PMC at diakinesis showing 16 large sized bivalents and interbivalent connection (*arrow*); *B* - polyploid somatic cell with 64 chromosomes and five different sized nucleoli; *C* - group of 2 - 3 PMCs involved in chromatin transfer (*arrow*); *D* - transfer of chromatin through multiple chromatin bridges (*arrow*); *E* - hypoploid PMC; *F* - enucleated PMC (*arrow*) and another proximate PMC with double chromosome complement (*arrow head*); *G* - Coenocytes with double chromosome complement at the same meiotic stage; *H* - group of PMCs showing partial and complete chromatin stickiness; *I* - PMC showing pycnotic chromatin masses (*arrow*); *J* - PMC showing out of plate one bivalent at metaphase-I (*arrow*); *K* - PMC at anaphase-I showing 2 bivalents as laggards (*arrow*); *L* - PMC showing two double and a single chromatin bridge at anaphase-I (*arrow*); *M* - triad with unequal microspores; *N* - polyad; *O* - transfer of chromatin material from micronucleus to microspore (*arrow*) in a tetrad; *P* - transparent sterile and stained apparently fertile pollen grains; *Q* - stained apparently fertile heterogeneous sized pollen grains. *Scale bar* =10 µm.

transfer is prevalent throughout the meiotic course but its occurrence during meiosis-I is more frequent as has been noticed by other workers (Consolaro and Pagliarini 1995, Pierozzi and Benatti 1998). The cytomixis at tetrad stage has been reported in many cases but transfer of chromatin from micronucleus to microspore within a tetrad is not common. So far we have not come across any reference regarding this phenomenon in any species. Further, the nature of chromatin transfer is either partial or complete. Complete transfer of chromatin or fusion of PMCs resulted into enucleated and hyperploid PMCs. Direct fusions of PMCs are also observed that resulted into coenocytes with synchronized and unsynchronized chromatin material.

The phenomenon of cytomixis and associated meiotic abnormalities like chromatin stickiness, pycnotic chromatin, interbivalent connections, out of plate bivalents, late disjunction of bivalents, and laggards and bridges in the species resulted into partial pollen sterility and pollen grains of variable sizes. Similar effects of cytomixis and associated meiotic abnormalities on pollen viability and pollen size has also been reported in *Papaver rhoeas* (Chauhan 1981), *Vicia faba* (Haroun *et al*. 2004), *Caltha palustris* (Kumar and Singhal 2008), *Meconopsis aculeata* (Singhal and Kumar 2008a) and *Hippophae rhamnoides* (Singhal *et al*. 2008).

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