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Cytological studies in two populations *Lychnis coronaria* Lamak. (Caryophyllaceae) from high altitude areas of Kashmir Himalaya

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ABSTRACT

At present, meiotic studies were performed in two populations of Lychnis coronaria collected from different areas of Kashmir Himalayas. The species depicted diploid chromosome count of 2n=24 at different stages of meiosis. The predominance of cytomixis is very high from early prophase-I upto tetrad stage of meiosis. A large number of PMCs were seen to be involved in chromatin transfer in groups of PMCs. PMCs with hypo- and hyperploid chromosome numbers were observed. Many other meiotic abnormalities including chromosomal laggards and bridges as well as chromatin stickiness were also present. As a result of the abnormalities, microsporogenesis was abnormal with the formation of micronuclei and production of heterogeneous sized fertile pollen grains with reduced pollen fertility.

Key words: Cytomixis, Lychnis coronaria, Meiotic abnormalities, Kashmir Himalaya

I. INTRODUCTION

The genus *Lychnis* L., of the family Caryophyllaceae comprises of 25 species, distributed in temperate Africa, Asia and Europe [1]. In India, it is represented by *L. coronaria* and *L. ischnopetala*. The first species is distributed in Jammu and Kashmir, and Karnataka while second one in temperate alpine regions of Eastern Himalayas and Sikkim. The genus is characterized by having flowers pentamerous in lax dichasia; calyx teeth 5; petals fringed; stamens 10; styles 5, opposite the calyx-teeth, carpophore reduced; capsule teeth entire, as many as styles.

L. coronaria Lamak., is commonly called as "Rose Campion", and grows on grassy slopes in exposed forests between 1,700-3,100m in Kashmir. The plants are tomentose with long straight silky hairs, petals obcordate and seeds biconvex (Fig. a). It flowers and fruits from May-August. Presently, meiotic studies were carried out in two populations from different areas of Kashmir Himalaya to examine the effect of cytomixis and other meiotic abnormalities on pollen fertility.

II. MATERIALS AND METHODS

To perform meiotic studies, flower buds were collected from different localities of selected areas of Kashmir Himalayas (Table 1). Smears of appropriate sized flower buds were made after fixing these in the Carnoy's fixative, using standard acetocarmine technique. Pollen fertility was estimated by mounting mature pollen grains

in glycero-acetocarmine (1:1) mixture. Well-filled pollen grains with stained nuclei were taken as apparently fertile, while shrivelled and unstained pollen grains were counted as sterile. Photomicrographs of pollen mother cells and pollen grains were made from freshly prepared slides using Nikon 80i eclipse Digital Imaging System.

III. RESULTS

In the present study, two populations belonging to *Lychnis coronaria* were cytologically worked out. The information about locality, altitude, present meiotic chromosome number, figure number and ploidy level are listed in Table 1. It is seen that chromosome number n=12 is invariably counted in all the populations at different stages of meiosis (Figs. b, c).

The meiotic course in both these populations was observed to be abnormal with the presence of cytomixis, chromosome stickiness, unoriented bivalents, chromatin bridges, laggards at anaphases and telophases, formation of micronuclei and abnormal microsporogenesis leading to variable pollen sterility (Tables 2). The cytomixis remained to be the chief phenomenon of the meiotic system in both the populations. The cytomixis in the form of cytoplasmic connections as well as transfer of chromatin between PMCs is seen right from the prophase-I to pollen formation and may involve many PMCs (Figs. d, e). Cytomixis in these populations results into the production of PMCs with different chromosome numbers and even empty PMCs. Chromatin stickiness involving few bivalents or whole complement is seen from prophase-I to metaphase-I (Fig. f). It was seen that mostly late or non-disjuncting bivalent bridges as well as chromosomal laggards were more common (Figs. g, h). The cytomixis inducing abnormalities ultimately end up in multipolarity with the formation of highly variable number of nuclei per PMC (Fig.i). This resulted into abnormal microsporogenesis leading to the formation of monads, diads, triads and polyads (Figs. j-l). Further, micronuclei were also been observed in both of these populations (Fig. l; Table 2). All these meiotic abnormalities lead to the formation of heterogeneous sized fertile pollen grains in both the populations (Figs.m,n).

IV. DISCUSSION

All the 25 taxonomical species of the genus have been cytologically worked out showing 2n=24, 32, 33, 36, 48, 72, but only limited number of species (28.00%) show polyploid nature with highest level attained as 6x. The genus is monobasic (x=12) with intraspecific euploidy exists in one species and intraspecific aneuploidy in one species each. From India, both the species have been cytologically worked out with one species at 4x and other with both 2x and 4x levels.

The phenomenon of cytomixis along with other meiotic abnormalities provide an evidence that cytomixis definitely affects the disturbance in the formation and functioning of the spindle during meiosis, resulting into irregular distribution of the chromatin within the PMCs and also between the PMCs showing the transfer of chromatin. The occurrence of PMCs with high and low chromosome numbers ultimately lead to the induction of abnormal polarity of spindle and formation of nuclei of variable numbers and sizes during microsporogenesis. This finally results in the production of heterogenous sized fertile pollen grains. These

findings are in conformity with the earlier observations in different plants [2, 3, 4]. Cytomixis leading to the production of aneuploids [5] and also of higher ploidy levels [6] seems to be equally operative in the presently investigated species. It is reported that cytomixis and meiotic abnormalities make integral appearance in both the populations but in varying frequencies (Table 2). The micronuclei observed in many PMCs may be due to the extra chromatin mass transferred by cytomixis as has also been suggested by Bhat *et al*. [7]. This phenomenon of cytomixis has been seen in many of the Angiosperms plant species with different possible causes being advocated as pathological/ physiological conditions, the effect of chemicals, temperature, stress factors or purely under genetic control. As both the populations have been meiotically worked out following the same standardized protocol of fixation, therefore in the presently investigated populations, the cytomixis seems to be under genetic control as pointed out earlier by some researchers [8, 9, 10]

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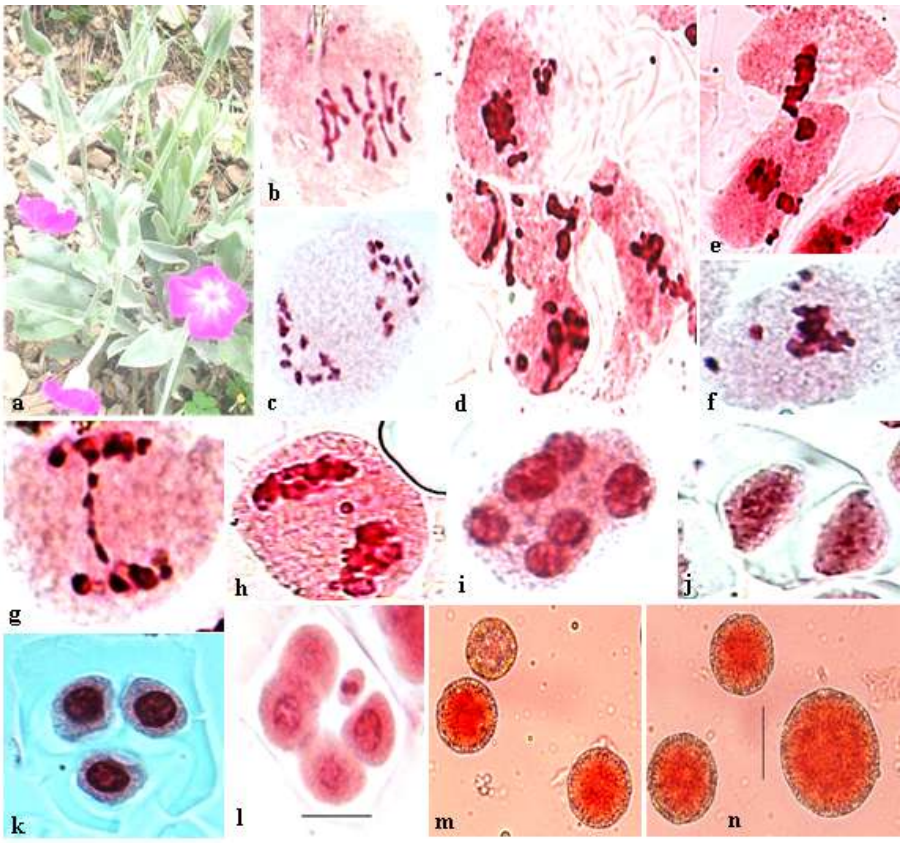

Figs. a-n: Meiosis in *Lychnis coronaria*. a) Morphology of plant. b) Metaphase-I showing 12II. c) Anaphase-I with 12:12 distribution of chromosomes. d-e) Group of PMCs showing cytomixis. f) Metaphase-I showing chromatin stickiness. g) Bridge at Anaphase-I. h) Laggard at Telophase-I. i) Multipolarity at Telophase-II (6 poles). j) A dyad. k) A triad. l) A tetrad with micronucleus. m) Fertile and sterile pollen grains. n) Heterogeneous sized fertile pollen grains. Scale 10 µm.

Table 1. Information about locality, altitude, accession number, chromosome numbers, ploidy level, pollen grain fertility and size of presently worked out populations of *Lychnis coronaria*.

S. No.	Accession number	Locality & altitude	Present chromosome number (2n)	Ploidy level/ Meiotic course*/ Pollen fertility (%)	Pollen grain size (µm)
1.	54395	Mahadev 3,000	24	2*x*/A/67.86	25.58×25.40- 21.45×21.20
2.	54396	Gulmarg 2,600	24	2*x*/A/62.87	

*A=Abnormal

Table 2. Data on abnormal meiotic course in populations *Lychnis coronaria*.

S. No.	Meiotic abnormalities	Accession Number	
		54395	**54396**
1.	PMCs involved in cytomixis at Meiosis-I /Meiosis-II (%)	1.33 (14/105)/ 5.71 (6/105)	8.18 (9/110)/ 5.45 (6/110)
2.	No. of PMCs involved in cytomixis at Meiosis-I /Meiosis-II (%)	2-5	2-3
3.	Chromosomal stickiness at M-I (%)	7.69 (5/65)	1.00 (7/70)
4.	Unoriented bivalents at M-I (%)	11.29 (7/62)	—
5.	Bridges at Meiosis-I/Meiosis-II (%)	9.18 (9/98)/ 8.16 (8/98)	11.76 (12/102)/ 8.82 (9/102)
6.	Laggards at Meiosis-I/Meiosis-II (%)	18.36 (18/98)/ 11.22 (11/98)	10.47(11/105)/ 10.47 (11/105)
7.	Multipolarity at T-II (%)	5.00 (2/40)	1.88 (1/53)
8.	Monads- WMN / WM (%)	3.12 (2/64)/ —	1.26 (1/79)/ 6.57(5/76)
9.	Dyads - WMN / WM (%)	1.56 (1/64)/ 3.12 (2/64)	5.06 (4/79)/ 3.79 (3/79)
10.	Triads - WMN / WM (%)	3.12 (2/64)/ 6.25 (4/64)	5.06 (4/79)/ 5.06 (4/79)
11.	Tetrads - WMN / WM (%)	67.18 (43/64)/ 15.62 (10.64)	59.96 (45/79)/ 18.98 (15/79)

Figures in parenthesis denote observed number of abnormal PMCs in the numerator and total number of PMCs observed in denominator.