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Cytomixis and associated meiotic abnormalities in Delphinium roylei Munz

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ABSTRACT

Meiotic studies have been carried out on four accessions of Delphinium roylei collected from different areas of Kashmir Himalayas. The cytomixis is observed from early prophase-I upto tetrad stage of meiosis. The percentage of PMCs involved in chromatin transfer is quite high and may involve group of PMCs. PMCs with hypo- and hyperploid chromosome numbers are also observed. The other meiotic abnormalities include chromosomal laggards and bridges as well as chromatin stickiness. Microsporogensis is abnormal with the formation of micronuclei and production of heterogeneous sized fertile pollen grains with reduced pollen fertility.

Key words: Cytomixis, Delphinium roylei, Meiotic abnormalities

I. INTRODUCTION

Delphinium roylei Munz. belongs to the family Ranunculaceae is commonly present near streams, on humus in forests and on open hill slopes with altitudinal range of 2,300-3,500m in the Kashmir valley. It is characterized by stem with a few branches; lower leaves petiolate and upper leaves shortly stalked to subsessile; inflorescence in dense central raceme with flowers deep blue in colour and spur straight. The flowering and fruiting is seen during the months of July-August. Presently, meiotic studies have been carried out on population basis from different areas of Kashmir Himalayas to analyze the effect of cytomixis on the meiotic behaviour and pollen fertility.

II. MATERIALS AND METHODS

For 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

During present study, four accessions belonging to *D. roylei* have been 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=16 is invariably counted in all the four accessions (Fig. 1a).

Meiotic abnormalities

The meiotic course in all these accessions has been 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, 3).The cytomixis remained to be the chief phenomenon of the meiotic system in all the accessions. 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 (Fig. 1b). Cytomixis in these accessions results into the production of PMCs with different chromosome numbers and even empty PMCs. It is seen that mostly late or non-disjuncting bivalent bridges as well as chromosomal laggards are more common (Figs. 1c,d). Chromatin stickiness involving few bivalents or whole complement is seen from prophase-I to metaphase-I (Fig. 1c). The cytomixis inducing abnormalities ultimately end up in multipolarity with the formation of highly variable number of nuclei per PMC. This results into abnormal microsporogenesis leading to the formation of monads, diads, triads and polyads (Figs. 1e-h). Further, micronuclei have also been observed in most of these accessions (Figs. 1e-g; Table 3). All these meiotic abnormalities lead to the formation of heterogeneous sized fertile pollen grains in all the accessions (Fig.1i).

IV. DISCUSSION

Cytomixis, co-existing with meiotic abnormalities in all these materials further give a clue 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. Although in low frequency, the occurrence of PMCs with high and low chromosome numbers is observed, leading ultimately to the induction of abnormal polarity of spindle and formation of nuclei of variable numbers and sizes during microsporogenesis and finally resulting in the production of heterogenous sized fertile pollen grains (Table 2). All these findings are in conformity with the earlier observations in different plants [1, 2]. Cytomixis leading to the production of aneuploids [3] and also of higher ploidy levels [4] seems to be equally operative in the presently investigated species. It is noted that cytomixis and meiotic abnormalities make integral appearance in all the accessions but in varying frequencies (Table 2). Hence, in general, the pollen fertility is not drastically affected in species with low frequency of cytomixis. Similar observations concerning the low frequency of cytomixis coupled with limited appearance of meiotic abnormalities and negligible low pollen sterility has also been reported in *Dactylis* [5] and *Hippohae rhamnoides* [6]. 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 angiospermic species with different possible causes being advocated as pathological/ physiological conditions, the effect of chemicals, temperature, stress factors or purely under genetic control. Since all the accessions 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]

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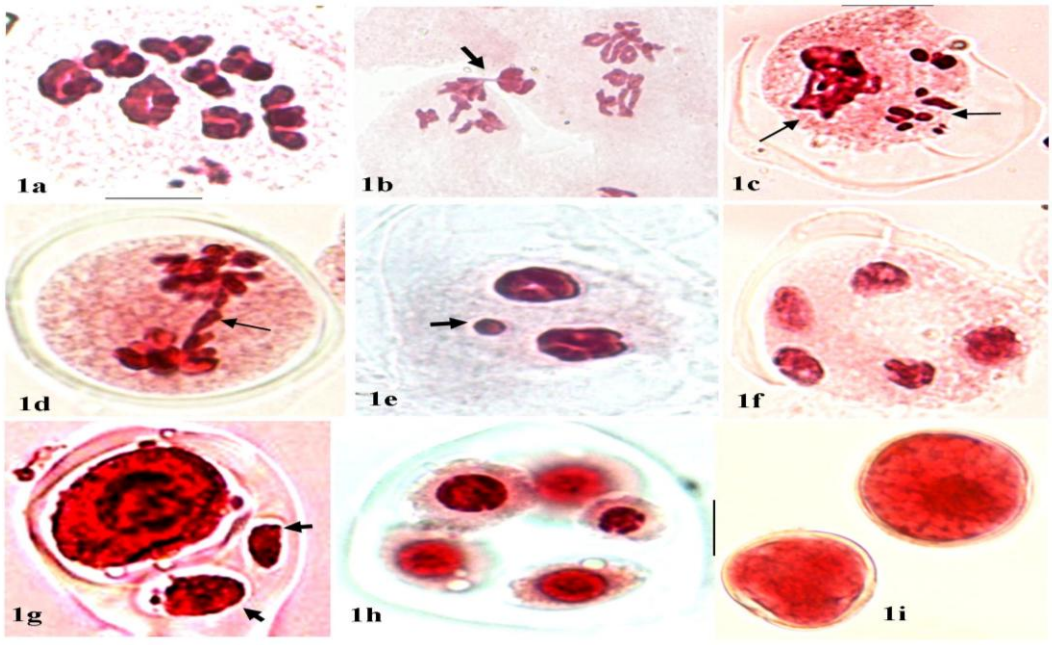

Figure 1: Meiosis in *D. roylei*: (a) Metaphase-I showing 8II. (b) Cytomixis at Metaphase-I involving two PMCs. (c) Fragmented chromatin material. (d) Bridge at Anaphase-I. (e) Laggard at Telophase-I. (f) Multipolarity at Telophase-II (5 poles). (g) A monad with micronuclei. (h) A polyad. (i) Heterogenous sized pollen grains. Scale 10 µm.

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Table 1. Information about locality, altitude, accession number, chromosome numbers, ploidy level, pollen grain fertility and size of presently worked out accessions of D. roylei.

S. No.	Accession number	Locality & altitude	Present chromosome number	Meiotic course*/ Pollen fertility (%)	Pollen grain size (µm)
1.	54266	Aharbal 2,500 m	2n=2x=16	A/ 70.55	23.35×24.92- 23.35×23.98
2.	54267	Mahadev 2,800 m	2n=2x=16	A/ 67.90	23.46×24.80- 23.37×23.82
3.	54465	Batnoor- Tral 2,300 m	2n=2x=16	A/ 76.29	23.16×24.65- 23.19×23.63
4.	54480	Chumnai 3,100 m	2n=2x=16	A/ 72.34	23.56×24.98- 23.36×23.76

*A=Abnormal

Table 2. Data on cytomixis, meiotic course, pollen fertility and pollen grain size in different accessions of *D. roylei*.

	Cytomixis at Meiosis-I/ Meiosis II		Meiotic course showing PMCs with		
Accession number	% of PMCs Laggards at Meiosis-I/ involved Meiosis-II (%)	Number Multipolar of PMCs PMCs at involved T-II (%)	Chromosomal stickiness at M-I (%)	Unoriented bivalents at M-I (%)	Bridges at Meiosis-I/ Meiosis-II (%)
54266	4.61 (6/130)/ 7.50 (9/120) 57 (4/112) 5.83 (7/120)	2-3 2.85 (3/105)	1.53 (2/130)	3.70 (5/135)	3.34 (4/120)/ 5.83 (7/120)
54267	12.85 (8/140)/ 3.12 (4/128)/	2-4 —	14.38 (20/139) 3.03 (4/132)	— 2.98 (4/134)	4.34 (6/138)/
54465	4.39(4/91)/ 2.86(2/70)/ 6.59(6/91)	2-3 6.45(4/62)	5.13(4/78)	2.63(2/76)	8.57(6/70)/ 7.14(5/70) —

54480 (6/105)/	11.66 (14/120)/ 3.06 (3/98)	2-5	5.60 (7/125)	2.85 (3/105)	6.03 (7/116)/	5.71

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

Table 3. Data on abnormal microsporogenesis in different accessions of *D. roylei*.

Accession number	Monads			Dyads		Triads		
	Tetrads		Polyads					
	WMN	WM	WMN WM	WMN	WM	WMN	WM	
54266	3.30 (3/91) 52.74 (48/91)	2.10 (2/91) 17.58 (16/91)	4.39 (4/91)	5.49 (5/91)		7.69 (7/91)	2.19 (2/91)	
54267 (70/115)	0.86 (1/115) 23.47 (27/115)	— 4.34	2.60 (3/115) (5/115)	0.86 (1/115)		4.34 (5/115)	2.60 (3/115)	60.86
54465 (65/85)	1.17 (1/85) 14.11 (12/85)	— 1.17	2.35 (2/85) (1/85)	1.17 (1/85)		1.17 (1/85)	2.35 (2/85)	76.47
54480 (60/94)	5.31 (5/94) 15.95 (15/94)	— 2.13	2.13 (2/94) (2/94)	3.19 (3/94)		—	7.44 (7/94)	63.82
54484	— 81.96 (50/61)	— 11.47 (7/61)	1.63 (1/61) —	—		3.27 (2/61)	1.63 (1/61)	

Figures in parenthesis denote observed number of abnormal PMCs in the numerator and total PMCs observed in denominator. WMN = without micronuclei; WM = with micronuclei.