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ACCESSORY NUCLEOLI IN SOME MUTANT LINES OF *NIGELLA SATIVA* L. (BLACK CUMIN)

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Accessory nucleoli ranging from 1 to 5 per cell have been studied in different mutant lines (raised from selfed M_2 seeds of the mutants which were EMS-induced) of *Nigella sativa* L. (black cumin), while the control plants consistently showed one nucleolus per cell (size : 8.36 μ ±0.08). The *lax branching* and *viridis* mutants revealed 1-2 nucleoli per cell. The nucleoli were of different sizes in the mutants (8.30 μ to 1.67 μ) and were either found free or seen associated to different bivalents. Occurrence of multiple nucleoli in the variant plant types has been attributed to gene mutations which possibly have induced changes in the regulatory system of the cells.

Keywords : Accessory nucleoli; Black cumin; Mutant lines.

Introduction

Nucleoli producing the bulk of cellular RNA (80-90%) provides the basic patterns of ribosomal organization and is remarkably similar in fine structure[1] although they demonstrate variation in size and number. Sybenga[2] reported one nucleolus per genome in majority of the plant and animal species, while more than one nucleolus have been reported in only a few[3-6].

The present paper report on the occurrence of multiple nucleoli in some mutant lines of *Nigella sativa* L. (black cumin), a spice yielding member of the family Ranunculaceae. The nature of the nucleolus and the probable cause of their formation have also been dealt with.

Materials and Methods

PMCs with more than one nucleolus have been encountered in different mutants at M_2 generation (3 and 6 hours treatment with 0.25 and 0.5 per cent EMS respectively) but identification of nucleoli and chromosomes were made in the mutant lines (5 randomly selected plants were assessed in each mutant) at M_3 generation raised from selfed M_2 seeds using the staining scheduled described by Levin[3]. Identical observations have also been made in the control plants grown under similar environmental conditons. Photomicrographs were taken from suitable preparations.

Results and Discussion

The control plants revealed consistent presence of single nucleolus (size 8.36μ ± 0.08) per cell (Table 1). This is in agreement to the number of chromosome with secondary constriction in the comlement[7]; however, nucleolus number is not commensurable to the number of secondarily constricted chromosomes and it has been proved that those chromosomal regions which code for 18S and 24S RNA are nucleolar organizing in nature[8].

The nucleoli ranging in number from 1-5 were observed in most of the mutants, while *lax branching* and *viridis* mutants showed 1-2 nucleoli per cell (Table 1). In the mutant lines, 65.57 to 48.39 per cent PMCs showed one nucleolus (size : 8.30μ ± 0.18) per cell; however the rest demonstrated the occurrence of 2 to 5 nucleoli per cell manifestating size differences and they were in between 8.30 μ and 1.67 μ. Nucleoli was either free or found in association to different bivalents (Fig. 1-6) but occasionally two nucleoli of different or same sizes were seen attached to a single bivalent (Figs. 5-6).

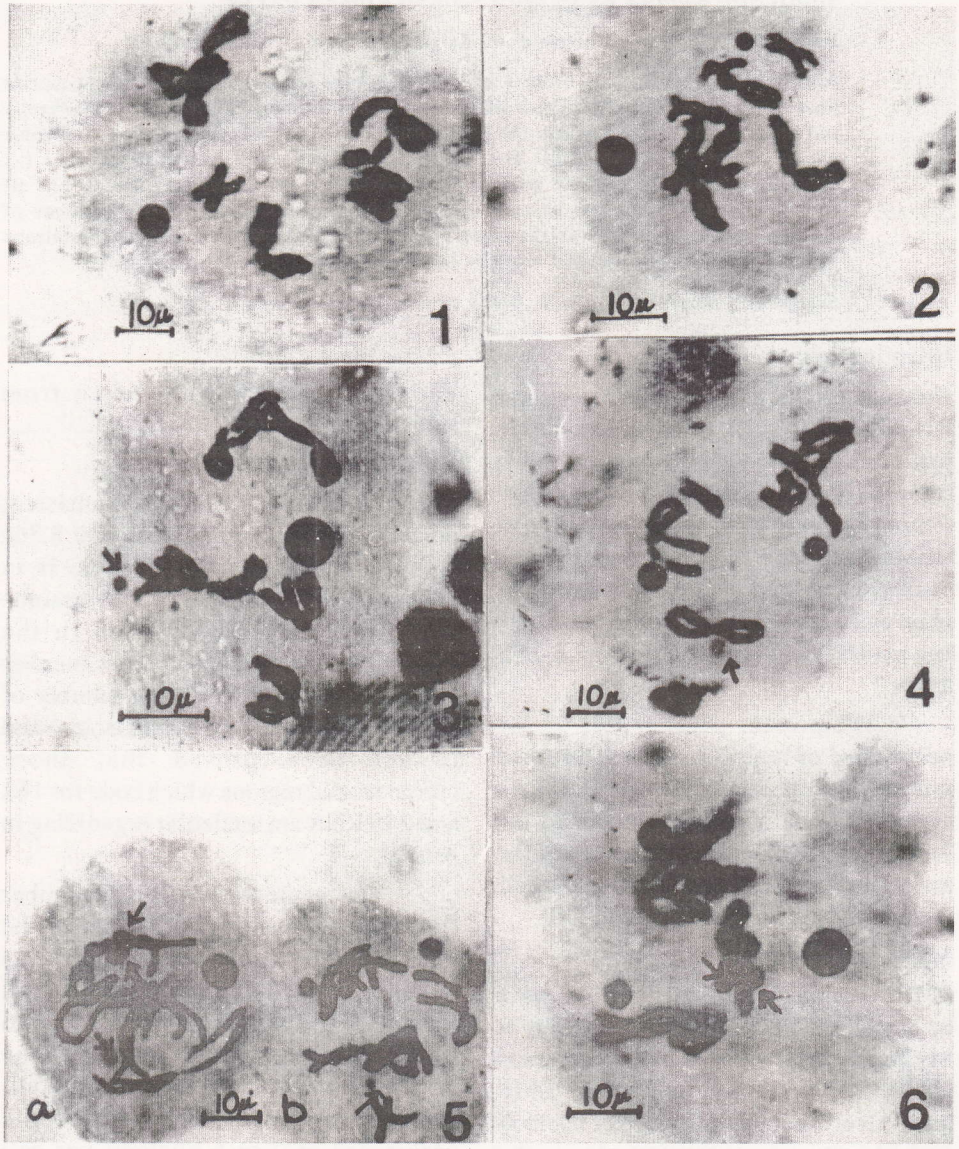

Fig. 1-6 : PMCs at prophase I showing variation in number and size of nucleoli in mutants of *N. sativa.* I, one nucleolus; 2-3, two unequal sized nucleoli, unattached to bivalents; 4, three nucleoli; 5, four nucleoli of which 2 are attached to a bivalent (a) and 3 unequal sized nucleoli (b); 6, 5 nucleoli.

Table 1. Nucleolus number at prophase I in PMCs of *N. sativa* plant types.

Plant types	PMCs scored	Number of Nucleolus per cell				
		1	2	3	4	5
Control	106	106	–	–	–	–
Bushy	172	100	52	16	2	2
Chloroxantha	153	81	30	27	9	6
Crinkle leaf	87	54	24	3	3	3
Feathery leaf	124	60	50	6	4	4
Lax branching	115	71	44	–	–	–
Narrow leaf	116	70	30	8	6	2
Viridis	122	80	42	–	–	–

Supernumerary nucleoli have also been reported in *Oxlis dispar* (1 to 10)[4], *Phlox pilosa* (1 to 5)[3], *Crotalaria* (1 to 6)[5], *Trigonella foenum-graecum* (2 to 10)[6] amongst others.

Various causes such as breaks across the secondarily constricted region[9], suppression of nucleolar organizing region and activation of several latent loci[2], hybridity[1] and change in environment[10] have been attributed for the formation of multiple nucleoli in different plant species. In the present case, occurrence of accessory nucleoli due to hybridity and change in environment can easily be ruled out as the mutant lines were raised from selfed M_2 mutant seeds and were grown with normal plants in the same environment. Persistent occurrence of nucleolus having the size of 8.36 $\mu \pm 0.08$ in control and 8.30 $\mu \pm 0.18$ in the mutant lines has indicated the presence of a distinct nucleolar organizer in the plant types and breaks across the secondarily constricted chromosomes to the formation of multiple nucleoli is rather difficult to consider in the context.

The plant types having the mutation background showed nucleolar variation in size and number. Multiple and variable sized nucleoli formation have been presumed as an outcome of disturbed genetic state of the plant types caused by gene mutation and the mutant genes possibly have induced changes in the regulatory system of the cell thereby activating various latent loci capale of synthesizing tiny nucleoli. Hiko-Lchi and Chen-Hui Kao[11] attributed size variation of nucleolus on the basis of difference in the intensity of nucleolar forming power.

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