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A MALE STERILE MUTANT WITH DESYNAPTIC BEHAVIOUR OF CHROMOSOMES IN *NIGELLA SATIVA* L.

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A male sterile mutant of *Nigella sativa* L. (black cummin) was isolated from the fertile mutant progenies of *broad elongated lax pinnae* (6 hours 0.25%, EMS-induced) at M_3 generation, which showed chlorophyll deficiency in the shoot apex of the primary axis at the onset of floral bud initiation and produced small yellowish white anthers and those were non-dehiscent and pollenless at anthesis. Male sterility has been found to be associated with desynapsis and defective female gametophytic tissues. Desynaptic behaviour of chromosomes studied in the male sterile plant was of "strong" type. Meiosis was found to progress upto to tetrad stage followed by complete degeneration of microspores leading to pollenless condition in anthers.

Keywords : *Broad elongated lax pinnae* mutant; Desynapsis; Male sterility; Meiotic continuation; Microspore degeneration.

Introduction

A male sterile plant was screened from the fertile mutant progenies at M_3 generation of *Nigella sativa* L. (black cummin), a spice yielding member of the family Ranunculaceae. Morphological and cytogenetical nature of the male sterile plant has been described in the present text.

Materials and Methods

The Male sterile plant was isolated from the M_3 segregating progeny raised from 109 selfed seeds of the mutant *broad elongated lax pinnae* (6 hours 0.25%, EMS-induced), which produced 74 normal and 35 mutant plant types. Morphological nature of the aberrant plant was studied which possessed the mutant trait. Studies on the pollen and pollen mother cells were made following single anther squashes in 1% propionocarmine solution. For meiotic analysis, anther squashes were performed from 8 flower buds of the male sterile plant fixed (1:3 propiono-alcohol) at different blooming periods (first formed flower bud, mid-flowering stage and drying up stage of the plant). Cytological data was estimated from the pooled frequency of all squash preparations.

Morphological and cytological observations were also made in normal and male fertile mutant plants under similar conditions for comparison. Photomicrographs were taken from temporary slide preparations.

Observations

Chlorophyll deficiency (yellowish green in clour) in the shoot apex of the primary axis at the onset of floral bud initiation and non-dehiscent anthers at anthesis allowed phenotypic differentiation of the male sterile plant from other male fertile mutant plants in the population. The male sterile plant exhibited *broad elongated lax pinnae* nature and attained a height of 26.3 cm at maturity as compared to rather erect (range : 31.3-71.5cm; mean : 55.95 cm ± 1.5) and compact habit of the normal plants (Figs. 1-2). Number of primary branches produced by the marked plant (6.0) was more or less same as control (range : 4-10, mean : 6.26±0.3). All floral buds initiated in the main flowering season and out of 24 flower buds produced by the male sterile plant, 11 bloomed (buds collected for cytological studies and used for crossing experiments were not considered) after 89 to 110 days from sowing instead of 80-95 days in control plants. Each flower (small yellowish white) of the male sterile plant had

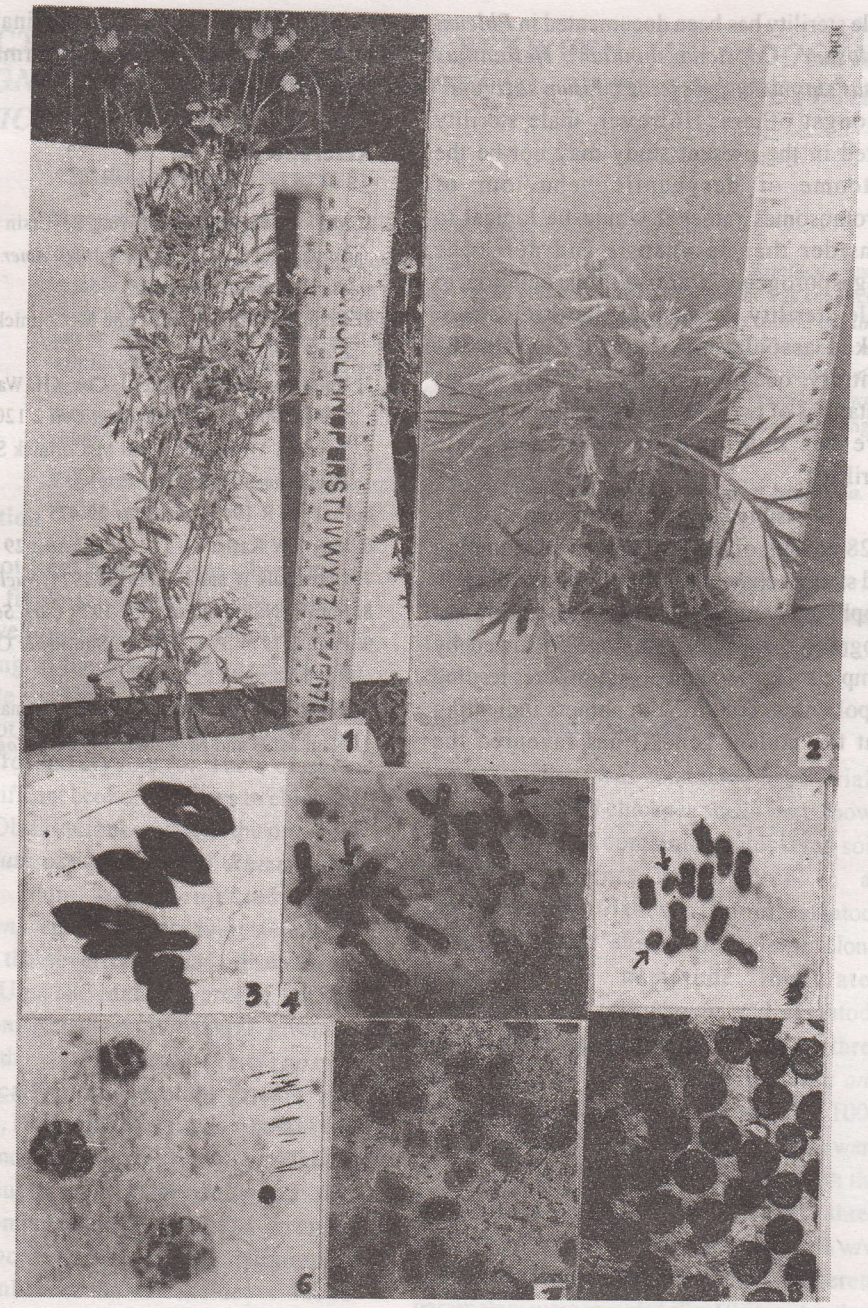
10-12 stamens and the microsporophylls were with short style (2.6mm ± 0.2) and small (4.13mm ± 0.06) indehiscent yellowish white anthers which were completely pollenless at maturity. The flowers of the male sterile plant dried up on selfing and on cross pollination with pollens from normal plants produced rudimentary fruits with abortive seeds and the plant died after 121 days from sowing. On the contrary, control plants produced 27.6 ± 1.74 flowers per plant (range : 11-44) and each flower demonstrated 3 to 4 whorls of stamens (range : 28-45; mean : 33.5 ± 0.8) with medium sized (6.24 mm ± 0.6) dehiscent anthers yielding 90.5 to 97.8 (92.648 mean value) percent stainable (Fig. 8) pollen grains (size: 38.6 μ ± 1.4). Plant height (range : 15.3 to 35.0 cm; mean : 25.63 cm ±1.5), number of primary branches per plant (range : 3-10, mean : 5.0 ±0.5), number of flowers per plant (range : 11-47; mean : 20.57 ± 2.7), number of stamens per flower (31.7 ± 0.3), size of anthers (6.17 mm ± 0.8) and frequency of stainable pollen grains (77.48% mean value) were also assessed in male fertile mutant plants. Seed setting was normal in the male fertile mutants.

All anther squashes performed in the male sterile plant have shown similar meiotic events. Clumping and sticky behaviour of chromosomes was observed at early prophase and has been recorded in 21 to 28 percent PMCs per anther. The male sterile plant showed desynaptic behaviour of chromosomes (Figs. 4-5) and the chromosomal associations studied at diplotene, diakinesis and metaphase I (168 PMCs estimated) were 6II (4.76%), 5II+2I, (9.52%), 4II+4I (4.76%) and 12I (80.95%). Mean frequency of univalents and bivalents per cell was estimated to be 10.1 and 0.9 respectively. Chiasma frequency per nucleus observed in the male sterile plant was 0.31 ± 1.2 as compared to 9.9±0.74 in normal plants. Unequal separations of chromosomes were studied in AI(71.43%) and AII (42.62%) from 84 and 122 cells respectively. The male sterile plant produced tetrads mostly with unequal spory (Fig. 6) followed by complete degeneration of microspores (Fig. 7) leading to pollenless condition. On the contrary, normal meiosis (2n = 12) was studied both in control and male fertile mutant plants (Fig. 3); however in a few PMCs, 2-4 univalents occurred possibly due to precocious separation of chromosomes.

Discussion

The male sterile plant possessing the mutant trait appeared at M_3 generation of *broad elongated lax pinnae* mutant and was phenotypically aberrant to normal and male fertile mutant plants. Male sterility was confirmed in the phenotypic marker plant by their non-dehiscent anthers which were pollenless at maturity. The male sterile genes possibily have arisen as a consequence of gene mutation and it has been suggested that such genes act on the genetically programmed steps of meiosis and results in non-development of functional pollen and/or in the non-dehiscence of anthers[1-4]. Male sterility in the present case was also found to be associated with defective female gametophytic tissues as the male sterile plant yielded only abortive seeds on crossing with fertile pollens from normal plants.

Similar meiotic events were studied from all the flower buds fixed at different blooming periods of the male sterile plant. Cytological studies performed in the male sterile plant have shown desynaptic behaviour of chromosomes and the desynapsis was of "strong" type[5] as the mutant demonstrated high frquency of univalents per cell with cocomitant decrease in bivalent frequency per cell and chiasmata per nucleus. The male specific gene action leading to a synapsis or desynapsis has demonstrated a close linkage between anther specific and desynapsis genes in higher plants[6-8]. Desynapsis as a cause of



Figs. 1-2 : Normal (Fig. 1) and male sterile (Fig. 2) plants of *Nigella sativa* L.

Figs. 3-8 : 3. 6 II at MI. of controls. 4-5. 12I at diplotene and MI respectively of male sterile plant (➜ telocentric chromosomes).

6. Male sterile plant showing unequal spores at early tetrad stage.

7. Degenerating microspores in the male sterile plant. 8. Stainable pollens in controls.

male sterility has been documented in *Phleum nodosum*[6], *Capsicum annuum*[8], *Helianthus annus*[9], *Jasminum pubescens*[10], *Pisum sativum*[11] amongst others. However, male sterility noted in the present study may not be the outcome of desynaptic behaviour of chromosomes rather it would be logical to consider that desynapsis and defective megasporogenesis are with association to male sterility suggesting pleiotropism or linked association of the traits. On the contrary, occurrence of two simaltaneous mutations of two closely placed genes though rare can exhibit desynapsis and male sterility[10].

Possibly due to meiotic arrestation, 21 to 28 percent of the PMCs showed clumping and sticky behaviour of chromosomes at early prophase but meiosis in the male sterile plant progressed upto tetrad stage followed by complete degeneration of microspores leading to pollenless condition in anthers indicating that the mutant gene(s) has favoured the continuation of meiosis and the final meiotic arrest was between microspore formation and their maturation.

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