Meiotic behaviour and pollen fertility in three species of *Zephyranthes* (*Amaryllidaceae*)

T. THOIBI DEVI and P.K. BORUA

Department of Life Sciences, Dibrugarh University, Dibrugarh - 786 004, Assam, India

Abstract

Studies on meiotic behaviour and pollen fertility have been carried out in *Zephyranthes candida*, *Z. grandiflora* and *Z. flava*. Maximum meiotic abnormalities in chromosome behaviour were observed in *Z. candida* and *Z. grandiflora*. There were variations in the number of bivalent formation, multivalents and anaphasic separation. All types of abnormalities were found to be associated with low percentage of pollen fertility. In *Z. flava*, chromosomal aberrations were low and pollen fertility was high.

Additional key words: chromatin bridges, laggard, tripolar spindle.

Introduction

The genus *Zephyranthes*, represented by about 60 species is one of the evolutionary dynamic genera of the family *Amaryllidaceae*. Three species important for horticulture and pharmaceutics are found in the north eastern region of India: *Z. candida* Haines, *Z. grandiflora* Lindl. and *Z. flava* Haines. *Z. candida* and *Z. grandiflora* are propagated vegetatively while *Z. flava* is propagated also by seeds.

The study of chromosomes of *Zephyranthes* shows that polyploidy and aneuploidy are widespread in the genus (Darlington and Wylie 1955, Ornduff 1968, 1969, Moore 1973, 1977, Naranjo 1974, Greizerstein *et al.* 1987, Davina and Fernandez 1989, Thoibi Devi and Borua 1996). However, reports on the chromosome behaviour of meiosis in the genus are scarce (Nagao and Thakusagawa 1932, Yokouchi 1965, Raina and Khoshoo 1972, Sharma and Ghosh 1954, Tandon and Mathur 1965). The reason behind this may be the difficulties in getting buds of appropriate size since they occur inside the bulbs before the development of inflorescence which is a common feature throughout the bulbous genera of the family. Chromosome numbers at metaphase I are important contributions to the knowledge of the basic chromosome number and the origin and nature of polyploidy in the genus (Davina and Fernandez 1989). The present study compares the meiotic behaviour in relation to the pollen grain fertility in three species of *Zephyranthes*.

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Fax: +0373-70323.

Materials and methods

The bulbs of *Zephyranthes candida* Haines, *Z. grandiflora* Lindl. and *Z. flava* Haines were collected from different places of Dibrugarh and planted in the experimental garden of Dibrugarh University. For meiotic studies bulbs were dug out of beds. Flower buds of proper size were collected by cutting the bulbs longitudinally. After removing the perianths from each bud, these were fixed in Carnoy's fluid (6:3:1, ethanol, chloroform, acetic acid) for 15 min. Then they were transferred to 70 % ethanol. The anthers were squashed in a drop of 2 % acetocarmine and observed under microscope.

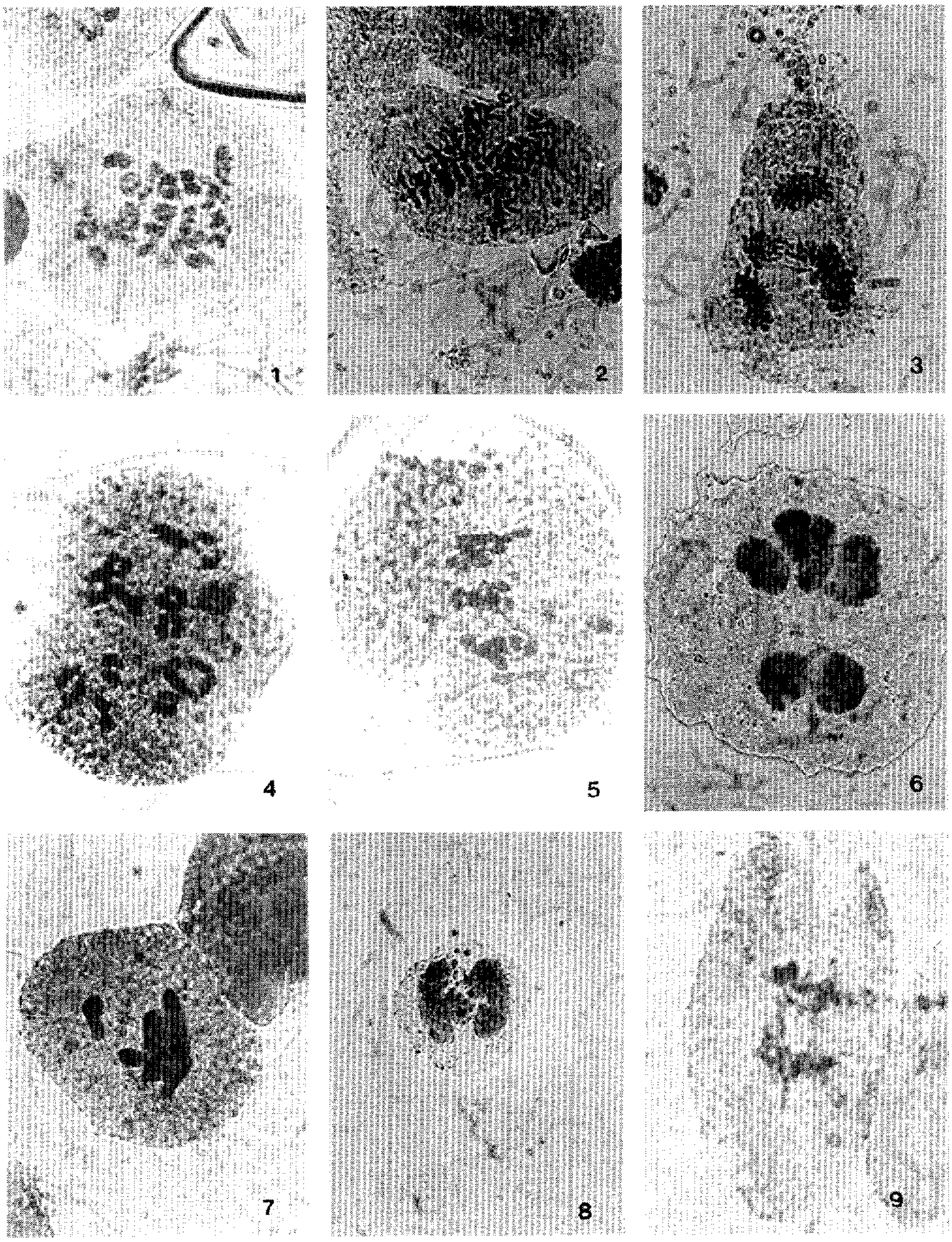
Pollen was collected from newly open florets and its fertility was examined by staining with 1 % acetocarmine. The percentage of stainable pollen grains was determined under microscope (about 200 pollen grains were counted).

Results

The occurrence of meiotic abnormality was a characteristic feature in all the three species. It was low (42 %) in *Z. flava*, in comparison with that of the other two species (Table 1). In *Z. candida* only 8 % of the cells showed bivalents. Multivalent associations like trivalents, quadrivalents were frequently observed. Nearly 40 % of the cells showed chromatin bridges and laggards in anaphase I (Figs. 1 - 3). Maximum abnormalities were observed in *Z. grandiflora* resulting from the presence of a large number of multivalent associations like trivalents, quadrivalents and pentavalents, laggard and chromatin bridges. Some of the cells showed the formation of tripolar spindle at metaphase I. Besides, some cells showed three groups of chromosomes at late anaphase I (Figs. 4 - 6). About 24 % of the cells showed unequal separation of chromosomes and polyad formation (Table 1). In *Z. flava* 42 % of the cells showed abnormalities, while the rest of them behaved normally. The abnormalities included mainly chromatin bridges, laggard, unequal separation of chromosomes in anaphase I (Figs. 7 - 9). The percentage of pollen fertility was found to be the highest in *Z. flava* (70.4 %) and relatively low in *Z. candida* (40.6 %) and *Z. grandiflora* (37.8 %) (Table 1).

Discussion

In the evolution of plants, chromosomal abnormalities play a major role on the genetic system producing isolation barriers between the species (Haque and Ghoshal 1980). When they affect the genetic system, structural changes in the chromosomes act mainly as the means of holding together certain favourable genes and therefore promote their fitness at the expense of flexibility (Darlington 1905). Many authors have reported that the chromosomal aberrations, chiefly inversion and translocation heterozygote, produce some amount of inviable gametes and thus reduce fertility. According to Darlington (1965) when the plants are heterozygous for a considerable



Figs. 1 - 9. Microphotographs of meiotic chromosomes of *Zephyranthes* (× 1000). *1 - 3: Z. candida*: *1* - diakinesis (4I+6II+4IV); 2 and 3 - chromatin bridges in anaphase I and II respectively. *4 - 6: Z. grandiflora; 4* - metaphase I (1I+6II+1III+2IV); *5* - tripolar spindle formation; *6* - pentad formation. *7 - 9: Z. flava*: *7* - laggard formation leading to unequal separation of chromosomes; *8* - abnormal diad formation; *9* - chromatin bridge formation in anaphase I.

Table 1. Frequency and percentage of meiotic abnormalities and pollen fertility in three species of *Zephyranthes*.

Species	Total PMC examined	Frequency of different meiotic stages									Meiotic abnormalities [%]	Pollen fertility [%]
		pachytene	diakinesis		M I	A I and II						
			bivalents	univalents + multivalents		normal	laggard	bridges	unequal separation	polyad		
Z. candida (2n = 19, 32, 38, 40)	70	5	6	16	8	7	10	18			63	40.6
Z. grandiflora (2n = 24)	75	8	7	18	9	13	8	5	13	4	68	37.8
Z. flava (2n = 28, 42, 48)	50		7	4	12	10	6	7		4	42	70.4

number of inversions and translocations, they produce gametes made inviable by duplications and deficiencies. The lethal gametic stage induces sterility in plants.

In the present study, all the three species show irregularities in the meiotic behaviour. In *Z. candida* and *Z. grandiflora,* maximum meiotic abnormalities are observed whereas in *Z. flava,* chromosomal irregularties are less. The abnormalities include variations in the number of bivalents, formation of univalents and multivalents, laggard and chromatin bridges formation, unequal separation in anaphase and polyad formation in telophase II. In both *Z. candida* and *Z. grandiflora,* ring and rod bivalents are observed. In *Z. candida,* the multivalent associations of chromosomes is reported by many authors. Yokouchi (1965) found tetravalents in *Z. candida* (2n = 38) and he suggests that they arose by translocation. In *Z. candida* (2n = 41), tetravalents and hexavalents are observed (Raina and Khoshoo 1972). In case of *Z. flava* abnormalities are less than in the two other species. Here, the aberration includes unequal separation and formation of laggards and chromatin bridges in anaphase I.

Haque and Ghosal (1980) have reported that the presence of anaphasic bridges with fragment in *Salvia* indicates that inversion followed by crossing over has taken place in some chromosomes. Similar nature has been observed in *Z. candida* and *Z. flava.* As reported by Darlington (1965) the treatment with heat and other agents have played an important role in meiotic irregularities as in *Lens culinaris* (Ahmed 1977).

The chromosomal abnormalities mentioned above are found to be associated with the low percentage of pollen fertility in *Z. candida* and *Z. grandiflora.* The correlation between cytological abnormalities and pollen sterility have been shown by many authors in many other plant genera (Darlington 1965). In case of *Z. flava,* pollen fertility is very high. The formation of aberrant spores, *i.e.* pentad, may be due to unequal segregation of chromosomes at telophase II. It may also be due to the failure of cytokinesis at the end of the premeiotic division (Nath and Nelson 1961).

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