

Full Length Research Paper

Cytological evidences of pollen abortion in *Ornithogalum caudatum* Ait

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Ornithogalum caudatum Ait exhibits strong physiological adaptability; however, it suffers a serious menace of pollen abortion, as almost no seed is formed in the plants. In the current study, chromosome karyotype of root-tip cells (RTC) during mitosis, and chromosome behavior of pollen mother cells (PMC) during meiosis in *O. caudatum* were investigated. We recorded 18 euchromosomes with variable number (28~36) of B chromosomes in the somatic cells genome. In leptotene of meiotic prophase I, the synapsis between euchromosomes was normal, but there were several darker granular nodular bodies in the cells, which could be the precursor for B chromosomes. Some of the cells were observed as duplication loop in zygotene, indicating that the PMCs had gone through a chromosome structure variation with duplication, which could reduce the fertility of pollens. Numerous chromosome bridges, fragments and lagged chromosomes were observed in meiosis anaphase I. It reflects that these cells might have undergone paracentric inversion, which is one of the important reasons of pollen abortion. In meiosis anaphase II, the sister chromatids separate unusually forming the triad, which leads to gamete sterility. These findings conclude that pollen abortion in *O. caudatum* mainly results from the variation in chromosome structure, which could be related to the B chromosomes in somatic cells.

Key words: Chromosome structure variation, B chromosome, root-tip cells, pollen mother cells, gamete sterility.

INTRODUCTION

Genus *Ornithogalum caudatum* Ait, with common name as Star of Bethlehem or Christmas Star, is a member of the Liliaceae family. It is native to southern Africa, and called as false sea onion or pregnant onion (Tilton and Horner, 1980; Kariukia and Kako, 1999). It is occasionally grown as a window or greenhouse plant, with an alias of

Urginea maritima. Now, it is widely cultivated as a medicinal value just like aloe vera (Tang et al., 2002; Chen et al., 2010). Recently, saponins isolated from *O. caudatum* are being used to cure lung cancer, breast cancer, and their anticancer activity has been documented. The root system of *O. caudatum* is developed and the lamina is strong, with many physiological adaptations, such as drought, freezing and stress tolerance etc. However, pollen abortion is serious in *O. caudatum*, therefore, only the developed bulbs are its main propagulum.

Phenomenon of pollen abortion in *O. caudatum* is very common, and it could be related to abnormality of chromosome pairing in meiosis; further, B

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Abbreviations: **m**, Metacentric; **sm**, submetacentric; **st**, subtelocentric.

chromosomes may affect the pollen fertility (Wiegas, 1980; Parker et al., 1990; González-Sánchez et al., 2004; Kumar and Srivastava, 2010). The B chromosomes are accessory genomic elements to the so-called A or standard genome. They have been found in many species of plants and animals. Populations usually show a polymorphism for the number and morphology of B chromosomes (Camacho et al., 2000; Basheva et al., 2010). The B chromosome also called as supernumerary chromosome, additional chromosome or extra chromosome, is a kind of particular chromosome outside the genome chromosome. Genetic pattern of B chromosome is transmitted in a non-Mendelian fashion due to their unstable mitotic and / or meiotic behavior, and is irregular, its genetic viability is generally inert, and B chromosome is smaller than euchromosomes. The B chromosomes have a special distribution pattern of heterochromatin, and have some impact on the adaptability and fertility in plants (Camacho et al., 2000; Jiménez et al., 2000; Bidau et al., 2004; Perfectti et al., 2004; Basheva et al., 2010). In this study, we report the results of studies based upon B chromosomes characteristic and behavior in somatic cell and two kinds of chromosomes structure variation, such as duplication and paracentric inversion in meiosis, which may lead to the pollen abortion in *O. caudatum*.

MATERIALS AND METHODS

Plant materials

This study was carried out from 2009 to 2010 in the province of Anhui, located in the eastern China. The materials used for this study were fresh young root tips and pollen mother cells (PMC) in anthers of *O. caudatum*.

Karyotyping of root-tip cells

Somatic chromosomes were studied in the root tip cell (RTC) meristems of growing plants, which were pretreated in ice-cold distilled water (0°C) for 24 h. Then fixed in Carnoy's solution (absolute ethyl alcohol and glacial acetic acid in 3:1,v/v) at 4°C for 24 h and stored in 70% aqueous ethanol (Ostergren and Haneen, 1962). Root tips were macerated and dissociated with 1 M HCl at 60°C for 5 min, stained according to the improved carbol fuchsin technique before squashing (Darlington and La Cour, 1976).

Slides were made permanent using Canadian balsam as the mounting medium. At least 10 metaphase cells were drawn using a Sony camera lucida and selecting the five best for measurements. The nomenclature used for the description of chromosome morphology is that proposed by Levan et al. (1964). The karyotype classification followed was that from Stebbins (1971). Idiograms were drawn based on mean centromeric index and arranged in the order of decreasing size.

Chromosome characterization of PMCs

The pollen mother cells (PMCs) were studied using the same method as for RTC except acid dissociation. They were characterised by terminal and interstitial knobs at the pachytene

(Jagathesan and Sreenivasan, 1971) and were identified through geimsa banding technique (Mirzaghadri, 2010). A double stain that differentially stains the filled and unfilled pollen grains was used to assess the pollen fertility (Alexander, 1969).

RESULTS

B chromosomes and karyotype analysis

Total number of chromosomes in *O. caudatum* species is not fixed, but they range from 46 to 54. Generally, each cell includes 18 large chromosomes and 28 to 36 small ones (Figure 1). The difference between chromosome numbers is caused by the unequal number of these small chromosomes. These small chromosomes are significantly shorter than the 18 large ones. The number and morphology are multifarious in different cells, and there is no congruent relationship among the 18 large chromosomes, so the probability of satellite is excluded. With the definition and characteristics of B chromosomes by Beukeboom (1994), Camacho et al. (2002) and Jones and Houben (2003), we consider that these small chromosomes are B chromosomes.

Karyotype formulae and parameters for the studied species are summarized in Tables 1 and 2, and karyogram is shown in Figure 1, which illustrate the karyogram of mitotic metaphases. As a whole, karyotypes of the analyzed species showed a predominance of submetacentric (sm) chromosomes. The karyotype formula is $2n = 18 = 4 m + 14 sm + (28 \text{ to } 36) Bs$. The morphologic difference of all 18 euchromosomes was inconspicuous; the ratio of the longest and the shortest chromosome was 1.35. In general, the karyotype was moderately symmetrical in the Stebbins 2A category, and there was no satellite chromosome in the genome.

B chromosomes in meiosis

During PMC meiosis, the characteristics of behavior and change of B chromosomes were significantly different than that of A chromosomes. Generally, B chromosomes were small granular corpuscle, and most of them showed the lagged chromosome phenomenon. For this, some examples at different stages of meiosis with typical pictures of A and B chromosomes are shown in Figure 2 and described subsequently.

Figure 2A to E shows that the synapsis of A chromosomes are normal from leptotene period to early diplotene in meiosis prophase I. There is no obvious B chromosome, but many nodule shapes. Deep dye granular corpuscles can be observed on the fibers of A chromosome, which might be the precursor of B chromosomes. Figure 2E and F shows the diplotene period, where the synapsis of A chromosomes reached the maximum, and simultaneously, the cross knots are obvious. Here, B chromosomes begin to come off from A

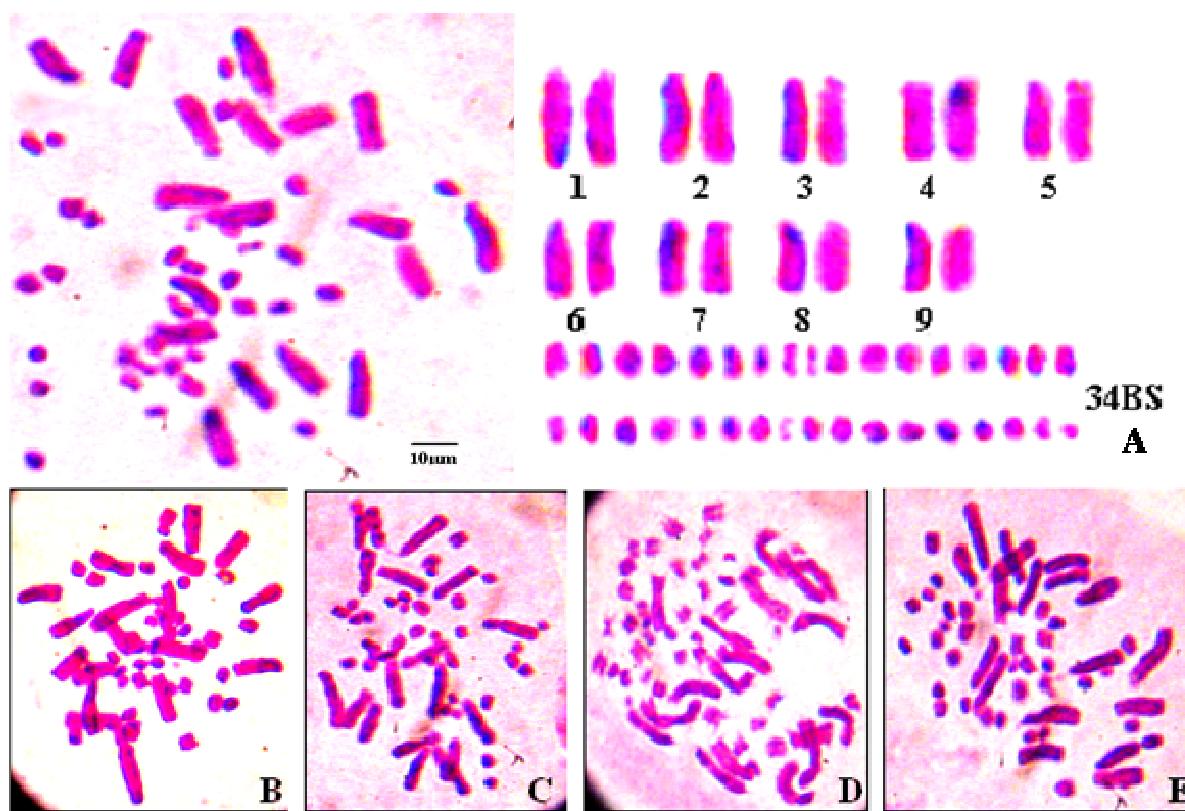


Figure 1. Chromosome karyotype picture of *Ornithogalum caudatum* Ait. A. $2n = 18 + 34$ BS; B. $2n = 18 + 28$ BS; C. $2n = 18 + 30$ BS; D. $2n = 18 + 32$ BS; E. $2n = 18 + 36$ BS.

Table 1. Characteristics of chromosomes in *Ornithogalum caudatum* Ait.

Sample	Relative length (%)			Index of relative length	Centromere index (%)	Arm ratio (long/ short)	Type of chromosome
	Short	Long	Total				
1	1.38	2.63	4.01	1.15	34.41	1.91	sm
2	1.35	2.54	3.89	1.12	34.70	1.88	sm
3	1.30	2.46	3.76	1.08	34.57	1.89	sm
4	1.33	2.17	3.45	0.99	38.55	1.63	m
5	1.21	2.50	3.68	1.06	32.88	2.07	sm
6	1.19	2.15	3.34	0.96	35.63	1.81	sm
7	1.13	2.11	3.24	0.93	34.88	1.87	sm
8	1.14	1.89	3.03	0.87	37.63	1.66	m
9	1.09	1.87	2.96	0.85	37.58	1.72	sm

chromosomes gradually, pairing one another, forming independent bivalents as A chromosomes. Figure 2G and H shows that B chromosomes move closer to the equatorial plate as A chromosomes in meiosis metaphase I, there is obvious cytological difference between A and B chromosomes.

A chromosome in meiosis

We observed a clear loop structure in a pachytene cell (Figure 2D), which was formed out of one chromosome from a pair of synapsis A chromosomes, which could have resulted from the deletion or duplication of

Table 2. Comparison of karyotype in *Ornithogalum caudatum* Ait.

Karyotype formula	Chromosome length ratio (L/S)	Proportion of AR>2	Index of asymmetry (%)	Karyotype type
$2n = 18 = 4\text{ m} + 14\text{ sm} + (28\text{--}36)\text{ Bs}$	1.35	0.11	64.80	2A

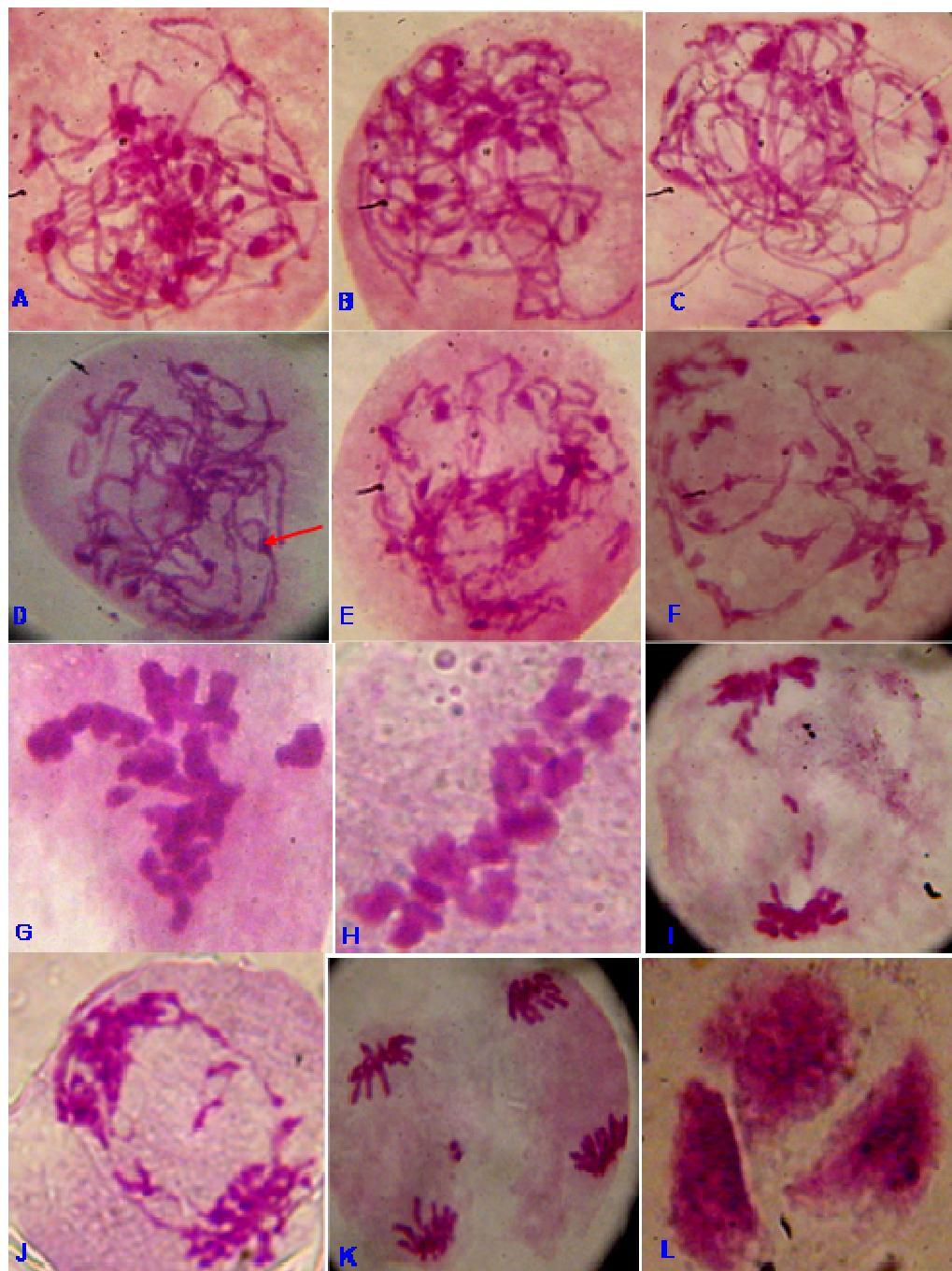


Figure 2. *Ornithogalum caudatum* Ait PMC meiosis chromosome characteristics at different periods. A: leptonema; B: zygote; C: pachytene; D: pachytene (arrow indicates the duplication loop); E: early diplonema; F: diplonema(arrows indicate cross knots and B chromosomes); G: diakinesis; H: metaphase I; I: anaphase I (arrow indicates lagging chromosomes); J: Late anaphase I (arrows indicate lagging chromosome fragments and chromosome bridges); K: anaphase II (arrow indicates lagging chromosome); L: telophase II (triad).

chromosome structure variation. In our opinion, if it belongs to deletion, then the plant with this kind of chromosomal aberration should be aborting fully, and it should be so with the pollens; but in fact, in addition to the fertility of pollens in *O. caudatum*, its floral organs and other parts of the nutritive organs are very developed, a variety of physiological adaptation are also very strong. Therefore, we believe, deletion mutation in *O. caudatum* chromosomes may be impossible, this type of variation should belong to duplication.

Several chromosome bridges and chromosome fragments were observed in some cells during meiosis anaphase I of PMCs (Figure 2I and J), suggesting that these PMCs have undergone paracentric inversion. Because inversion can induce delete or duplicate chromatids, and the spores grown from them would be abortive. In addition, the lagged B chromosomes can still be observed in meiosis anaphase II (Figure 2K), and we also found triad in some PMCs (Figure 2L), the chromosomal inversion and the triad can be the most important cytological evidence of pollen abortion in *O. caudatum*.

DISCUSSION

The role of B chromosomes in plants is obvious during the growth and development processes; and the harmfulness is mainly due to variation in the number of B chromosomes. Our study shows that in addition to containing the 18 A chromosomes, there are 28 to 36 B chromosomes in the somatic cells of *O. caudatum*. But there were not so much B chromosomes in meiosis stages of PMCs, which could relate to chromosome loss because of pair error among B chromosomes during meiotic process. This phenomenon is similar to that described earlier in the B chromosomes of rye (Wiegas, 1980; Houben et al., 2001). In maize, B chromosomes also affect the pollen viability; they could make A chromosomes, which show more complex genetic behavior, the frequency of univalent in PMCs is increased significantly in meiosis metaphase I (Rusche et al., 1997; González-Sánchez et al., 2004; Masonbrink and Birchler, 2010). Such behavior of B chromosomes may cause pollen abortion. The phenomenon of pollen abortion is common in *O. caudatum*, suggesting that pollen abortion of *O. caudatum* may be related to the B chromosomes, which can lead to pollen sterility directly. It also affects the pollen abortion through A chromosomes indirectly, such as causing the structure variation of A chromosomes.

In this study, we found that B chromosome of *O. caudatum* showed the characteristics and behavior as defined by Beukeboom (1994). However, the cytologic features and genetic behaviors were significantly different to A chromosomes, for example, B chromosomes showed heterochromatinization activity during leptotene, zygotene and pachytene period, and showed lagging chromosomes in meiosis anaphase I and II, etc. These

features were similar to the B chromosomes of rye. The triad structure was also reported in the study of B chromosomes during meiosis telophase II in garden pea (Berdnikov et al., 2003; González-Sánchez et al., 2003; Teruel et al., 2007). Triad and anaphase bridges structure are important cytological evidence in pollen abortion in *O. caudatum*; however, the causality between chromosome structure variation and B chromosome needs to be explored.

Duplication loop, anaphase bridges and lagging chromosomes were found in meiosis anaphase I and II of *O. caudatum*, which indicated that duplication and inversion occurred between two homologous A chromosomes. This event produces some distorted chromosome fragments, such as chromatid without centromere, chromosome pieces in the inversion heterozygote. Therefore, we can speculate that these chromosome fragments will enter the spores randomly in meiosis anaphase II, and continue to pass on, and would develop into B chromosomes eventually. Our results support the hypothesis that A chromosomes develop from the B chromosomes (Jamilena et al., 1994; Lamb et al., 2005; Basheva et al., 2010).

From leptotene to early diplotene period in meiosis prophase I, there was no significant number of B chromosomes. Instead, we observed many nodule shaped, fast color granular corpuscles on the fiber of A chromosomes. The B chromosomes appeared after early diplotene period in PMCs, and especially, it was very easy to differentiate the B and A chromosomes in this period. Therefore, we speculate that B chromosomes could be derived from the nodular corpuscles of A chromosomes. The color differences between A and B chromosomes during meiosis prophase I, suggests that B chromosome in *O. caudatum* has heterochromatin composition. However, in other periods of meiosis, B chromosomes do not show the heterochromatin characteristics, and are homologous chromosomes synapsis as A chromosomes, forming the bivalents and assigned to each pole as A chromosomes. In addition, a loop caused by the chromosome structure variation was observed in the pachytene period, and due to the strong physiological adaptation of *O. caudatum*, we eliminated the possibility of "deletion". However, to explain the loop and B chromosome in *O. caudatum*, the reasonable justification could be that one of two synapsis A chromosomes missed a piece of fragment, and produced one B chromosome from this.

Several factors contribute to pollen abortion in plants, for example, drought in rice (Nguyen et al., 2009), tapetal expanding abnormally at dyad stage in maize (Zhang et al., 2009), nutrient metabolism in *Lycium barbarum* L. (Qing et al., 2009), abnormal microspore development in Ougan mandarin (Hu et al., 2007).

In the current study, the main reason for pollen abortion in *Ornithogalum caudatum* Ait could be related to the variation in chromosome structure, which could be

further related to the B chromosomes in somatic cells.

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