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BRIEF COMMUNICATION

Cytological studies on meiosis and male gametophyte development in autotetraploid cucumber

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Abstract

With improved staining and chromosome preparation techniques, meiosis of pollen mother cells (PMCs) and male gametophyte development in autotetraploid cucumber (*Cucumis sativus* L.) was studied to understand the correlation between chromosomes behaviour and fertility. Various chromosome configurations, *e.g.* multivalent, quadrivalents, trivalents, bivalents and univalents were observed in most PMCs at metaphase I. Lagging chromosomes were frequently observed at anaphase in both meiotic divisions. In addition, chromosomes segregations were not synchronous and equal in some PMCs during anaphase II and telophase II. Dyads, triads, tetrads with micronuclei and polyads were observed at tetrad stage, and the frequencies of normal tetrad with four microcytes were only 55.4 %. The frequency of abnormal behaviour in each stage of meiosis was counted, and the average value was 37.2 %. The normal meiotic process could be accomplished to form the microspore tetrads *via* simultaneous cytokinesis. Most microspores could develop into fertile gametophytes with 2 cells and 3 germ pores through the following stages: single-nucleus early stage, single-nucleus late stage and 2-celled stage. The frequency of abnormalities was low during the process of male gametophyte development. The germination rate of pollen grains was 46.9 %. These results suggested that abnormal meiosis in PMCs was the reason for low pollen fertility in the autotetraploid cucumber.

Additional key words: chromosome configurations, *Cucumis sativus* L., microspore development.

Autopolyploid plants are usually considered superior to diploid with their respect to genetic adaptability and tolerance to environmental stresses. Cucumber (*Cucumis sativus* L., 2n=2x=14) is an important vegetable crop worldwide. Its commercial value could be enhanced by enriching its breeding and cultivation with autopolyploid. Recently some studies have been conducted on autotetraploid production in cucumber (Chen *et al.* 2004, Mackiewicz *et al.* 1996). However, the studies on genetic characters and application of the autotetraploid cucumber have been hindered because of its low fertilities. Studies of different plant species showed that the decrease in fertility is due to irregularities of chromosome behaviour during meiosis with the formation of imbalanced and non-viable gametes in polyploid (Khazanehdari and Jones 1997, Sanamyan *et al.* 2000, Zhang *et al.* 2007). However, information about this phenomenon by cucumber are lacking since cytological studies have been very difficult due to small chromosome size and their poor stainability (Dane *et al.* 1991). A few studies have been carried out on meiosis of PMCs. They provided insufficient information on the nature of microsporogenesis and male gametophyte development in diploid cucumber (Dane *et al.* 1976, Qian *et al.* 2003, Cao *et al.* 2004). No comprehensive study of meiotic chromosomal behaviour in PMCs of auto-tetraploid cucumber was carried out so far. This has hindered us to understand the relationships between chromosomes behaviour and fertility in autotetraploid. An understanding of the meiotic behaviour of the auto tetraploid may help in overcoming the low fertility. Also obtaining autotetraploid cucumber with high pollen viability and selecting lines with few meiotic abnorma-

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Abbreviation: PMC - pollen mother cell.

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lities becomes more promising. Further, possible correlations between gene expression and meiotic division of PMCs may be detected (Shymko *et al.* 2001). Therefore, the main objective of the present study was to report meiosis and male gametophyte development in autotetraploid cucumber. Autotetraploid cucumber (*Cucumis sativus* L.) lines established by Chen *et al.* (2004) have been maintained by inbreeding over the past three years. Plants of autotetraploid were grown from March to September 2007 in glasshouse at the Nanjing Agriculture University, Nanjing, China. Male flower buds, 1 - 3 mm in length, were selected at random from autotetraploid plants and fixed in Carnoy's solution [glacial acetic acid : ethanol, 1:3 (v/v)] for 24 h at room temperature. The fixed buds were then transferred to 70 % ethanol and stored at 4 °C for subsequent examination. Chromosome preparation was carried out according to Chen *et al.* (2003). For visualization of chromosome in PMCs at different meiotic stages, anthers were rinsed three times in distilled water and cut transversely in two halves with a straight razor. The pollen mother cells (PMCs) were then put into a drop of carbol fuchsin on the slide. Carbol fuchsin was prepared by the method described by Singh (2003). Residual fragments of anther walls were then removed, and a slide cover was mounted. The slide was warmed slightly over a flame before observation. When necessary for enhancing stain contrasts, anthers were discolored in 45 % acetic acid prior to slide mounting. The results were recorded and photographed using *Olympus* microscope (*BX-51*, Tokyo, Japan) possessing a camera attachment. Meiotic irregularities were expressed as percentages of PMCs with deviations in relation to the total number of PMCs studied; at least 200 cells in each stage of meiosis were counted. Stages of male gametophyte development from microspore to mature pollen grains were observed by the method as described by Chen *et al*. (2003) except for acetocarmine (1 %) staining, prepared according to Singh's (2003) method. The red coloured pollen grains were regarded as fertile. Viable and nonviable pollen grains were counted on five slides, at 10 positions per slide and at least 600 pollen grains were evaluated. Germination experiment of the pollen grains was also carried out to test the viability of pollens by the method described by Liu (1994). Germination pollens grains were then calculated on three slides, at 10 positions per slide. At least, 300 pollen grains were evaluated.

Generally, meiosis of PMCs in autotetraploid cucumber was similar to that in diploid. In the transition from interphase to diplotene, the nucleus became smaller, while the chromatin elements became more prominent. At leptotene, the nucleolus was common and stained darkly with chromonema abutting on one side forming a bouquet (Fig. 1*A*). At metaphase I, the homologous pairs of chromosome gathered on the equatorial plate, chromosomes reach their maximum contractions. Subsequently, the homologous chromosomes were divided and pulled to opposite poles by tension on kinetochore microtubules at anaphase I. A few PMCs were symmetrically separated and resulted in 14 chromosomes at each pole of the PMCs (Fig. 1*D1*). With condensation of chromatins, chromosome became more and more identifiable from prophase I to metaphase I, and illegible at telophase I in the first meiotic division (Fig. 1*E*,*F*). In the second division of meiosis, prophase II, metaphase II, anaphase II and telophase II, resemble similar stages of the first meiotic division. Normal meiosis of the PMCs led to the formation of microspore tetrads via simultaneous cytokinesis and the arrangement of microspores in the tetrads was tetrahedral (Fig. 1*J1*). However, abnormal microsporogenesis was still observed in a few PMCs. At metaphase I, chromosome configuration could be discerned. Variable chromosome configuration, *e.g.* univalents, bivalents, trivalents, quadrivalents and multivalents, *etc*., were observed in most PMCs of the autotetraploid (Fig. 1*B1-B3*). PMCs showed few chromosomes scattered outside the plates (28.6 and 38.9 % at metaphase I and metaphase II, respectively; Fig. 1*C*,*H1*). The number of chromosomes scattered in each PMC ranges from one to three. A distinct asynchrony was also observed at metaphase II (Fig. 1*H2*). Chromosome lagging resulted from the abnormal chromosome pairings and scattered chromosomes were observed in some PMCs (Fig. 1*D2*,*I2*) and chromosome bridges, asynchrony were also observed at anaphase (Fig. 1*I1*). The frequency of abnormal PMCs in each stage of meiosis of autotetraploid was counted (Table 1). Besides, multinucleoli were also observed at prophase II (Fig. 1*G*). The normal arrangement of microspores in the tetrads was tetrahedral and each microspore tetrad contained four normal microspores with similar sizes (Fig. 1*J1*). However, the percentage of PMCs with four nuclei of similar size was only 55.4 %,

Table 1. Frequency of abnormal PMCs in each stage of meiosis of autotetraploid.

Meiotic stage	Number of cells examined	Normal cells [%]	Abnormal cells [%]
Metaphase I	421	71.4	28.6
Anaphase I	334	69.7	30.3
Metaphase II	367	61.1	38.9
Anaphase II	233	56.5	43.5
Tetrad stage	211	55.4	44.6

Table 2. Types and frequency of tetrads of autotetraploid cucumber.

Types of tetrads	Number	Rate [%]
Normal tetrads	117	55.4
Tetrads + micronuclei	24	11.5
Triads	12	5.7
Triads + micronuclei	26	12.3
Dyads	3	1.4
Dyads + micronuclei	8	3.8
Polyads	21	9.9

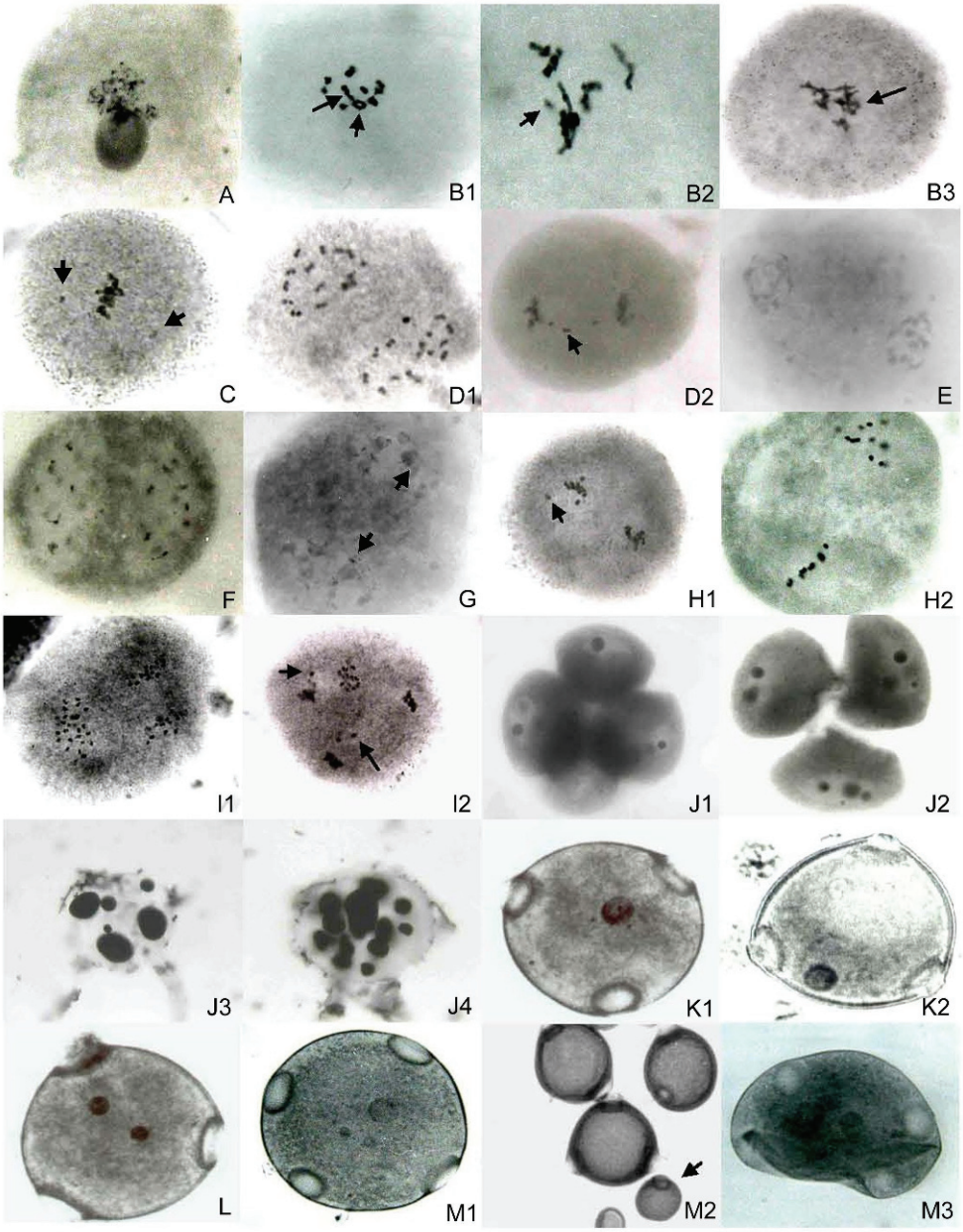

Fig. 1. Some chromosome behaviour during microsporogenesis and male gametophyte development in the autotetraploid. *A* - leptotene, chromonemata forming a bouquet shape; *B1-B3* - chromosome configuration at metaphase I (*arrows* showing chain trivalents, round quadrivalents in *B1*, *arrow* showing univalent in *B2*, *arrow* showing multivalent in *B3*); *C* - metaphase I (*arrows* showing chromosomes outside equatorial plate); *D1* - anaphase I; *D2* - lagging chromosome in anaphase I (*arrow*); *E* - telophase I, chromosome decondensed; *F* - telophase I, chromosome de-hexation; *G* - multinucleoli at prophase II (*arrows*); *H1* - metaphase II (*arrows* showing chromosome outside equatorial plates); *H2* - asynchrony at metaphase II; *I1-2* - anaphase II (*I1* - asynchrony, *I2* showing lagging chromosome); *J1* - tetrad; *J2* - triad; *J3* - triad with micronuclei; *J4* - polyad; *K1* - early uninucleate pollen; *K2* - late uninucleate pollen; *L* - two cell pollen; *M1* - mature pollen grains with 3 cells and 4 germ pores; *M2* - mature pollen grain stage (*arrow* shows abnormal pollen grains with small size); *M3* - wizened pollen grain.

while the percentage of abnormal PMCs was 44.6 % (Fig. 1*J2-J4*). Table 2 showed the types and frequency of tetrads of autotetraploid cucumber at the tetrad stage. The abnormality of meiosis is the cytogenetic factor which may influence the fertility of polyploids (Gaonkar *et al.* 1991, Costa *et al.* 2004). The high frequency of abnormalities was observed at the different stages from metaphase I to tetrad of meiosis in the present study, which was the main cause leading to low fertility in autotetraploid cucumber.

After callose dissolution, normal microspores released from microspore tetrads. They could develop into gametophytes with 2 cells and 3 germ pores after accomplishing following stages: single nucleus early stage

(Fig. 1*K1*), single nucleus late stage (Fig. 1*K2*), two celled stage (Fig. 1*L*). Mature pollen grains were two celled with 3 germ pores through which pollen tube can emerge, which was similar with diploid cucumber. However, some special patterns were observed. Few of pollen grains were observed to be of 3 cells. Grains with 4 germ pores were also observed (Fig. *M1*). In addition, we observed also few small-sized microspores (Fig. 1*M2*), and some abnormal microspores which can not develop further (Fig. 1*M3*). More than 600 pollen grains of autotetraploids were evaluated and stainability of mature pollen grains was 61.8 %. Germination experiment showed that the germination percentage of pollen grains was about 46.9 %. Usually 20 normal seeds per fruit were obtained after self-crossing. The results mentioned above confirmed the low fertility of male gametes in the autotetraploids.

From the present investigation it can be concluded that the high frequency of abnormalities in meiosis was the main cause leading to low fertility in autotetraploid cucumber. Results obtained provide a detailed cytogenetic analysis of the meiotic patterns of autotetraploid cucumber and can accelerate cucumber polyploidy breeding through selecting lines with few meiotic abnormalities.

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