Cytological Investigation of Anther Development in DGMS Line Shaan-GMS in *Brassica napus* L.

Zhao Di XIAO, Xiao Yang XIN, Hai Yan CHEN and Sheng Wu HU

State Key Laboratory of Crop Stress Biology in Arid Areas and College of Agronomy, Northwest A&F University, Yangling, Shaanxi, P.R. China

Abstract

Xiao Z.D., Xin X.Y., Chen H.Y., Hu S.W. (2013): **Cytological investigation of anther development in DGMS line Shaan-GMS in** *Brassica napus* L. Czech J. Genet. Plant Breed., **49**: 16–23.

The cytological mechanism of male sterility of Shaan-GMS, a natural mutant dominant genic male sterile (DGMS) line in *Brassica napus* L., is not well studied. Cytological observation was made on different-size buds of DGMS line 0A30A derived from Shaan-GMS line. The pollen mother cells (PMCs) of DGMS line 0A30A were degenerating at the beginning of meiosis and could not pass the anaphase I stage, with no dyads or tetrads formed, suggesting that the DNA damage checkpoint and spindle assembly checkpoint were activated in sterile anthers. During the meiosis process of sterile anthers in the sterile plants, several kinds of abnormal meiotic cells could be observed: nuclei condensed PMCs, cells with micronuclei, collapsed cells, plasmolysis cells, cells connected with nucleoplasmic bridge, and microspore analogue developed from PMCs without meiosis but enclosed by the exine wall. The results suggested Shaan-GMS to be a new type of DGMS line in *B. napus*.

Keywords: anther abortion; *Brassica napus* L.; cytological observation; DGMS

Rapeseed (*Brassica napus* L.) hybrids show an obvious heterosis. A key step in utilizing rapeseed heterosis is to use a simple and efficient pollination control system. Up to date, four pollination control systems, such as the cytoplasmic male sterility (CMS), genic male sterility (GMS), self-incompatibility (SI) and chemical hybridizing agents (CHA), have been used in rapeseed heterosis breeding (Fu 1995). Having characteristics of complete and stable male sterility, no potential negative cytoplasmic effect, broad spectrum of restoration and diverse cytoplasmic sources, GMS system has been regarded as a promising pollination control system in rapeseed heterosis utilization and a lot of hybrids based on this system have been registered in China (Li *et al*. 1985, 1990; Zhou *et al*. 2003, 2006; Shi & Dong 2004), such as Heza 3, Heza 7, Chuanyou 15, and so on.

According to the sterility controlled by dominant or recessive gene(s), GMS was classified into recessive genetic male sterility (RGMS) and dominant genetic male sterility (DGMS). DGMS mutants currently discovered in *B. napus* included the monogenic DGMS (Mathias 1985; Wang *et al.* 2003), digenic interaction DGMS (Li *et al*. 1985; Dong & Du 1993; Hu *et al.* 1999, 2004; Wang *et al.* 1999) and multiple-allele DGMS (Song *et al*. 2005).

Shaan-GMS was a newly discovered male sterile accession in *B. napus* by our group in 1994 (Hu *et al*. 1999). Genetic analysis indicated that the male fertility of Shaan-GMS was controlled by two pairs of nuclear genes (Hu *et al*. 2004). More recently, Deng *et al*. (2010) made cytological observations on heterozygous two-type line J03AB, which was derived from Shaan-GMS, by squashing and freez-

ing the anther, and showed that the pollen aborted at the mononuclear microspore stage when tetrad exine aborted after microspores were released. Exines and cytoplasm of microspores as well as tapetum cells prematurely degenerated. We know that the abortion of anther needs a course during the meiosis. To date, almost all the cytological studies just gave out a rough period or the range of developmental stages about the abortion of anther. In this investigation, we attempted to characterize the anther abortion of DGMS line 0A30A derived from Shaan-GMS in *B. napus* by cytological observation and get detail statistical data on the percentage of various cells formed at each stage of meiosis process and the kinds of cell form (normal and abnormal cell) contained in anthers of different size. The results will be helpful to reveal the possible cytological mechanism of male sterility and also lay a solid foundation for further studying the molecular mechanism on DGMS line Shaan-GMS.

MATERIAL AND METHODS

Plant material. The *B. napus* homozygous two-type line DGMS 0A30AB was used in this investigation. In this line, there are two kinds of phenotypes, male sterile plants (A) and male fertile plants (B). According to the previous investigations, the genotype of the male fertile plants (0A30B) and the male sterile plants (0A30A) in line 0A30AB was *MsMsRfrf* and *MsMsrfrf*, respectively. Here, *Ms* was the dominant male sterile gene, *ms* was its allele recessive gene, *Rf* was the dominant inhibition gene, which can inhibit the expression of the *Ms* gene and result in the restoration of F_1, and its allele recessive gene was *rf*, two genotypes *Ms_rfrf* expressed male sterility, and seven other genotypes expressed male fertility (Hu *et al*. 2004, 2006). Line 0A30AB was derived from the original homozygous two-type line DGMS 803AB (Hu *et al*. 2002), which was developed from the progeny of the cross between a spontaneous male sterile mutant Shaan-GMS (*Msmsrfrf*) in *B. napus* (Hu *et al*. 1999, 2000) and its restorer line 96-803 (*msmsRfRf*). Accession 0A30AB was sown on September 18th, 2010 in the experimental field of Northwest A&F University, Yangling. At anthesis (April 20th, 2011), individual plants in 0A30AB were checked for their fertility, the main inflorescences of each three fertile and sterile plants were collected for cytological studies.

Observation of morphological characteristics. Inflorescences for meiotic study were collected and fixed at room temperature in a mixture of ethanol and acetic acid (3:1) for 24 h, transferred to 70% ethanol and stored at 4°C until use. The cytological observation of microspore development was made by squashing and bright-field photographs were taken by an OLYMPUS BX51 microscope (Olympus Corporation,Tokyo,Japan).

Statistics of cell morphology. Buds from male sterile plants and male fertile plants of line 0A30AB were divided into six groups by their size. They are $1 < L < 2$ mm, $L = 2$ mm, $2 < L < 3$ mm, $L = 3$ mm, $3 < L < 4$ mm, $L \geq 4$ mm. Three independent slides from samples of each group were selected randomly to count different kinds of pollen mother cells (PMC), with 100 cells counted in each slide, in total, 300 cells were counted for each size samples.

RESULTS AND DISCUSSION

Comparison of morphological characteristics between fertile and sterile flowers

In flowering stage, floral organs developed normally in fertile plants (0A30B, Figure 1). Among the six anthers of one flower, four anthers were uniformly strong and long, concentrated distribution and closing to the nectary that the pistil seemed to be hidden in the anthers. While the other two weak short anthers were scattered at opposite poles and all the six anthers were full of yellow pollen grains on the surface (0A30B, Figure 1). Compared with the fertile flowers, the flowers of sterile plants displayed obvious difference. All the six anthers of sterile flowers were degraded significantly (0A30A, Figure 1), which showed that anthers with thin and short filaments, attaching closely to the bottom of the nectary, were shrivelled, dehydrated, and dried triangular, so that the pistil was obviously higher than anthers. In addition, the sterile anthers appeared pale yellow and no pollen grains in them (0A30A, Figure 1).

Cytological observations of anther development

Major cytological events during the fertile and sterile anther development were compared in Figures 2 and 3. We characterized the stamens

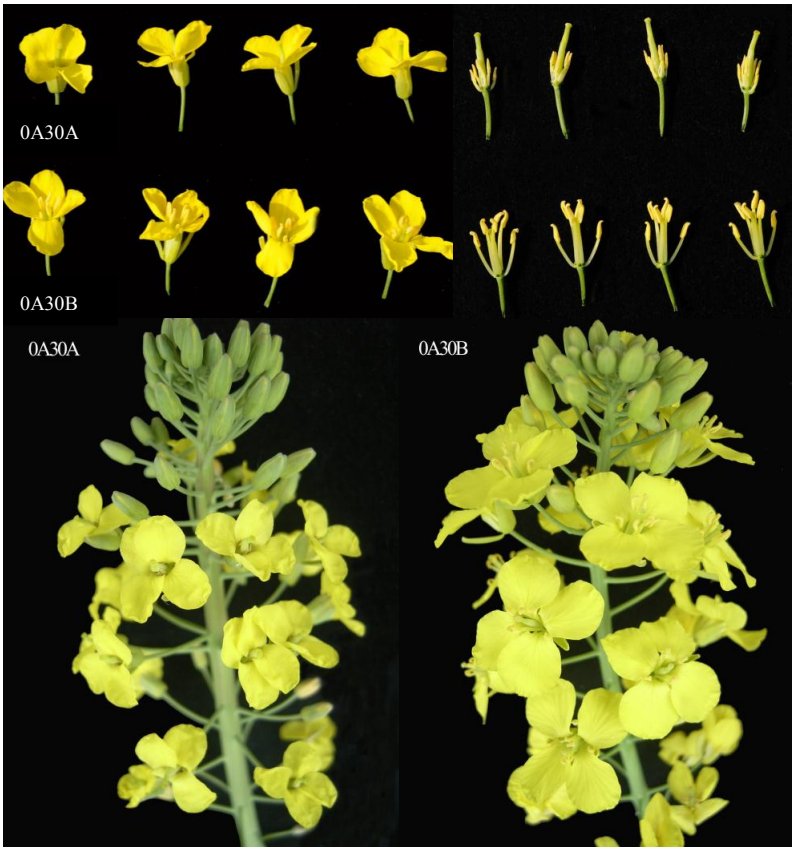

Figure 1. Comparison of morphological characters between 0A30A (sterile) and 0A30B (fertile) in flowering stage

of fertile and sterile plants by light microscopy to identify microscopic differences in cellular morphology. Based on the results of cytological observation, the meiotic process in the sterile plants was complex and abnormal. Young buds (1 < buds < 2 mm and buds = 2 mm in length), containing anthers in the ideal stage normally used for meiotic studies, showed that not all cells entered meiosis, showing distinctive features of premeiotic cells, which still contained condensed nuclei in sterile plants (Figure 3a). Buds (2 < buds < 3 mm in length), in such length, early microspore could be observed in normal buds (Figure 2e), while in sterile buds, only a small number of PMCs underwent the first chromosome segregation, and most cells were with condensed nuclei (Figure 3c–f). At about the pachytene or zygotene stage of the sterile anther, chromatin/chromosomes sometimes congregated into two or more groups of different size (Figure 3g), namely group cells (Wu & Yang 2008), and, as a result, micronuclei were formed (Figure 3g). However, in older buds (3 < buds < 4 mm in length), vacuolated microspores filled the anther locules in normal anthers (Figure 2g), but in sterile anthers, the microspore analogue named by Yu and Fu (1990) with sporopollenin deposition at the surface of pollen mother cells, which were developed directly from the PMCs without meiosis process, could be observed (Figure 3j). In addition, the protoplast of the PMC in sterile flowers was much thicker than that in the fertile ones, and shrank lately, which resulted in plasmolysis (Figure 3h). Moreover, cells connected with nucleoplasmic bridge (Figure 3i), which was the result of the emergence of dicentric chromosomes triggered by translocations (Fenech & Crott 2002; Thomas *et al.* 2003), DNA mis-repair, and telomere end-fusions (Fenech 2006) could also be observed. During the last stage (buds > 4 mm in length), the PMCs began to form the pollen wall alike on their surface at the mononuclear microspore stage equivalent to the normal anther. Gradually, the cell wall of most microspore analogues was degrading, therefore, some debris looking as lipid drops was attached to the cell wall (Figures 3j, k). Finally, the cytoplasm of microspore analogue almost degraded thoroughly, with only empty shells or degraded cell wall left (Figure 3l). Among all the sizes of

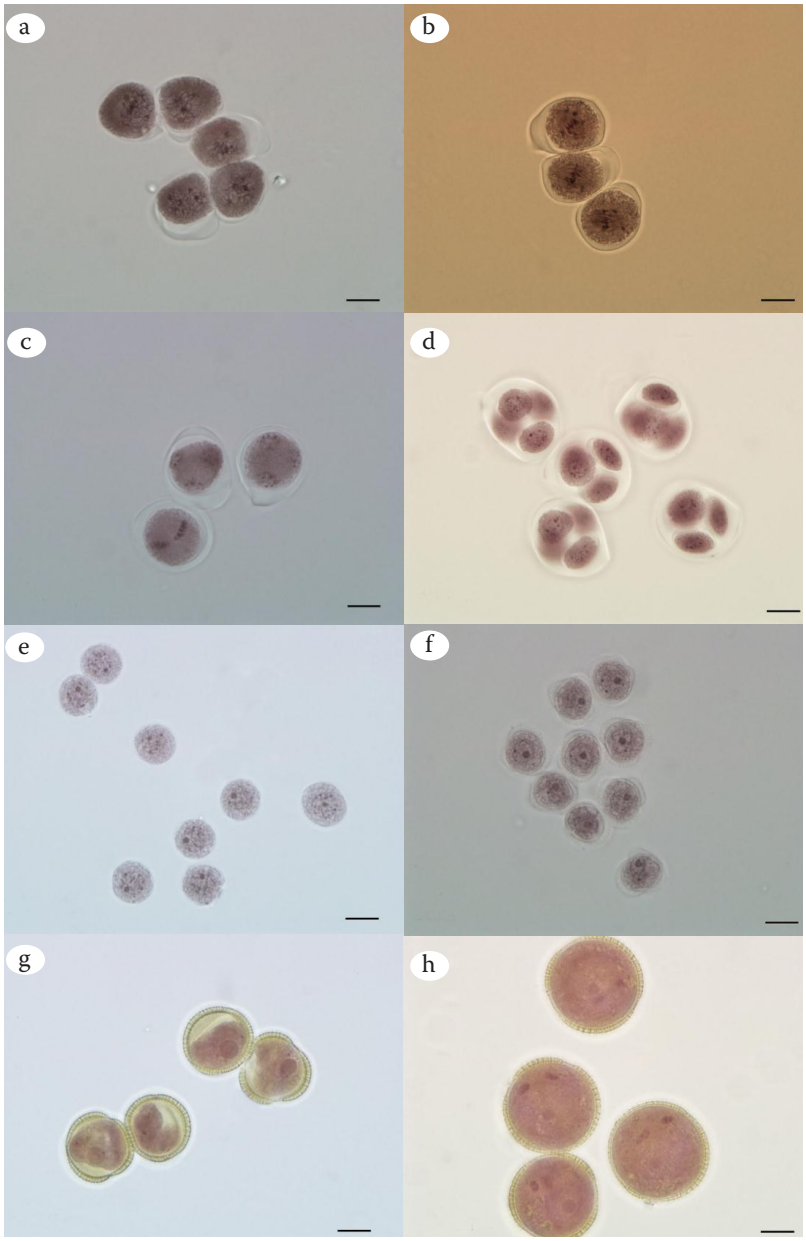

Figure 2. Major cytological events during anther development in fertile plants of 0A30AB in *B. napus* a – premeiosis stage, b, c – meiocyte stage, d – tetrad stage, e, f – early microspore stage, g – vacuolated microspore stage, h – late or engorged pollen stage; scale bars denote 10 μm

buds, neither dyad nor tetrad was discovered in the sterile anthers.

Cellular events observed in DGMS line 0A30A revealed that the PMC morphology in meiotic division is abnormal in sterile anthers. However, the cytological characteristics of the anther abortion in DGMS line 0A30A were different from those in Yi-3A and its derived line Rs1046A (Wu & Yang 2008). The PMCs both in Rs1046A and 0A30A were degenerating at the beginning of meiosis and could not succeed in finishing the whole meiosis process as normal PMCs, but they stopped at different stage, the former at about the pachytene or zygotene stage, the latter stopped at about the anaphase I or telophase I stage. The anther's cross-section analysis revealed that the tapetal cells in sterile anther of 0A30A plants commenced degradation at about the metaphase I stage (the results not shown here). The degradation of the tapetal cells in the DGMS line Yi-3A began at the microspore analogue stage, which is equivalent to the vacuolated microspore stage in the normal anther (Yang *et al.* 1999). So, the degradation of tapetal cells in DGMS line 0A30A was initiated earlier than that in Yi-3A. The above results showed that the characteristics of anthers abortion in DGMS line 0A30A differed from those of DGMS line Yi-3A (Rs1046AB).

A previous report of Deng *et al.* (2010) on the cytological observation of male gametogenesis

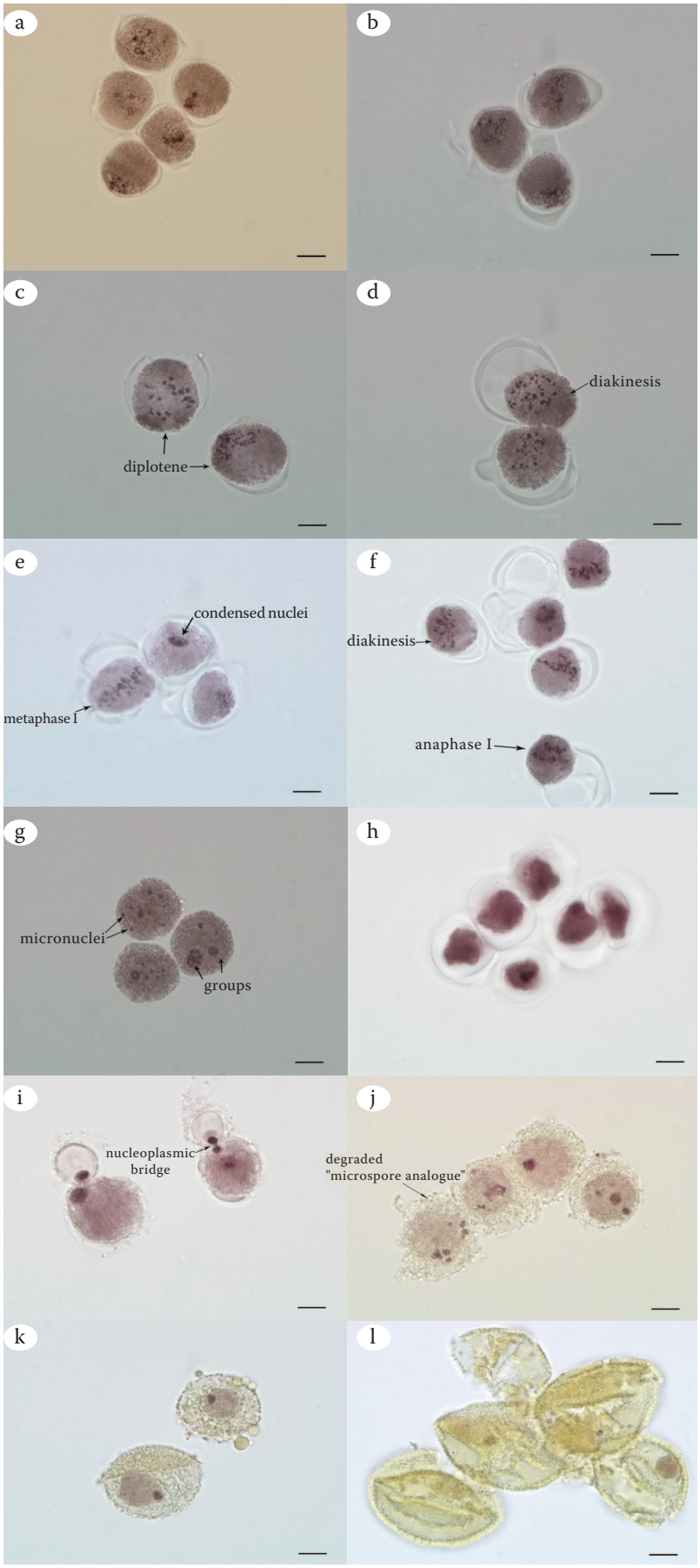

Figure 3. Major cytological events during anther development in sterile plants of 0A30AB in *B. napus* a–d: premeiosis stage, e–f: meiocyte stage, g–i: deformed cell morphology, j–l: microspore analogue; scale bars denote 10 μm

development of heterozygous two-type line J03AB derived from DGMS Shaan-GMS in *B. napus* showed that the sterile anther conducted meiosis, resulting in the formation of tetrads of haploid microspores. Afterwards, the released haploid microspores aborted, and exines of microspores could not form normally, then the cytoplasm of microspores as well as tapetal cells prematurely degenerated. Compared with their results, there was an obvious difference in the cellular morphology in the development of both pollen and tapetum in our experiment. The possible reasons for such different results are that the material sources of Shaan-GMS used are different in both experiments and/or the time of collecting buds for meiotic study in Deng *et al*. (2010) was not appropriate. The material J03AB (the genotype was *Msmsrfrf* (J03A) × *msmsrfrf* (J03B)) used by Deng *et al*. (2010) was a heterozygous near-isogenic line, which was obtained by successive backcrossing after crossing Shaan-GMS with double low quality breeding line 220. The material 0A30AB (*MsMsrfrf* × *MsMsRfrf*) we used was a homozygous two-type line. Though the fertility of DGMS line Shaan-GMS is stable at various genetic backgrounds, it is still influenced slightly by low temperature or insufficient daylight time in a few genetic backgrounds, which results in a few fertile flowers appearing in the main inflorescences of male plants (Hu *et al*. 1999). So, if these fertile flowers in the sterile plants were taken to make cytological observations, then, different results will be obtained.

In summary, the abortion of anther development in DGMS line 0A30A derived from Shaan-GMS occurred at the beginning of meiosis and stopped at about the anaphase I or telophase I stage, and the tapetum was degenerating at about metaphase I. The abortion of anther development in 0A30A is different from that of DGMS Rs1046AB (or Yi-3A), suggesting 0A30A to be a new type of DGMS.

The statistical analysis of cell morphology

The detailed statistical results of each kind of cellular events of different size buds are shown in Table 1. Young buds (1 < buds < 2 mm in length) containing anthers at the beginning stage of meiosis showed that some cells were abnormal, which contained condensed nuclei and degraded cells in sterile anthers. In 2 mm length buds, cells at the second division of meiosis and tetrads could be observed in the normal anthers, while most cells such as cells at pachytene/zygotene, condensed nuclei PMCs and group cells were observed in the sterile anthers, no PMCs entering into metaphase I. In buds (2 < buds < 3 mm in length), the number of cells at the stage of pachytene decreased; however, the number of cells at the stage of diplotene and diakinesis increased, a few cells had processed into metaphase I or anaphase I. Group/micronuclei cells were also increased in sterile anthers. In buds (3 mm in length) of sterile plants, PMCs at the stage of diakinesis, metaphase I or anaphase I were decreased, in contrast, condensed nuclei and collapsed cells were increased. In buds (3 < buds < 4 mm in length), nucleoplasmic bridge could be observed, nuclei condensed cells were in preponderance, and a few cells had processed into the microspore analogue in sterile anthers. In buds (≥ 4 mm in length), microspore analogues were only contained and some of them were collapsed

Table 1. Different cell events occurred at the stage of meiosis in buds of four size groups in sterile plants (%)

Stage of anther development or major defects	Bud size range (mm)			
	1 < L < 2	L = 2	2 < L < 3	L = 3
Leptotene	39.67	–	–	–
Pachytene/zygotene	46.00	27.33	15.00	14.00
Diplotene	–	2.67	4.67	–
Diakinesis	–	6.00	10.33	4.33
Metaphase I	–	–	2.33	3.00
Anaphase/telophase I	–	–	8.00	4.67
Nuclei condensed PMCs	7.33	49.00	35.67	44.00
Group/micronuclei cell	–	9.34	14.00	11.67
Uncertain cell	–	1.67	6.33	–
Collapsed cell	7.00	4.00	0.33	3.67

Table 2. Comparison of major cytological events in buds of different size between fertile and sterile plants in 0A30AB

Bud size range (mm)	0A30B	0A30A
1 < L < 2	leptotene to metaphase I	leptotene, pachytene
L = 2	metaphase II, telophase II, tetrad	pachytene, diplotene, diakinesis
2 < L < 3	early microspore	pachytene, diplotene, diakinesis metaphase I, anaphase I
L = 3	early microspore (thicker wall)	pachytene, diakinesis
3 < L < 4	vacuolated microspore	microspore's analogue
L ≥ 4	mature pollen	microspore's analogue

in the same sterile anther. As mentioned above, cells in anthers of buds > 3 mm in length were not classified as statistical objects.

Major cell events and defects in both fertile and sterile buds of different size are summarized in Table 2. In the 1–2 mm length buds in fertile plants, microspores were developed from leptotene to metaphase I stage, in the sterile plants, microspores were from leptotene to pachytene stage. In 2 mm length buds in the fertile plants, microspores were at the metaphase II, telophase II, tetrad stages, while in sterile plants, they were at the pachytene, diplotene and diakinesis stages. In the 2–3 mm length buds, early microspores were observed in the fertile anthers, however cells were still at the stage of pachytene, diakinesis, metaphase I, anaphase I in sterile anthers. In buds larger than 3 mm in length, vacuolated microspores to mature pollens were observed in the fertile anthers, however in the sterile anthers, the PMCs began to form the pollen wall alike on their surface, which were microspore analogues. In addition, droplet-like particles which might be the product of the degraded pollen wall alike were attached to the surface of microspore analogue (Figures 3j, k). Among all the different sizes of sterile buds, no dyads or tetrads could be found.

The results in Tables 1 and 2 indicated that, in the sterile plants, the percentage of cells at the stages of leptotene, pachytene, diplotene, diakinesis was reduced gradually with the process of meiosis, whereas the percentage of nuclei condensed cells and micronuclei cells was increased. Based on the statistical results, in general, the development of sterile anthers was delayed compared to fertile anthers, and the latest PMCs of sterile anthers stopped at the anaphase I stage when no dyads or tetrads were formed.

The development of sterile anther was delayed compared to fertile anther, suggesting that there existed some regulatory mechanisms in the meiosis program to monitor the defects in chromosome and cause the cell cycle arrested. Like mitosis, a stage termed premeiotic interphase also exists in meiosis, and the cell genome is replicated before entering into meiosis in this stage. The process of meiosis can be divided into four stages, G1, S, G2 and M. The conversion from one stage to the next stage is regulated strictly in meiotic cells. The checkpoints play important roles in regulating the conversion correctly, these include three main checkpoints in meiosis, DNA damage checkpoint, DNA replication checkpoint and spindle assembly checkpoint (SAC) (Wang *et al*. 2009; Wang 2010). With the increasing number of cells with condensed nuclei, it implied that DNA damage checkpoint was activated at the cell with damaged DNA. Only these repaired cells could enter into the next stage of meiosis. However, when they failed, they fell into cells with condensed nuclei or micronuclei. That PMCs of sterile anthers stopped at the metaphase/anaphase I stage and no dyads or tetrads were formed suggested that the cell cycle had been interrupted. SAC could be activated when any of the centromere did not accurately connect with the spindle that resulted in the cell cycle arrested at that time. Because of the failure of assembling the centromere to the spindle microtubule, SAC could not work properly, which induced the microspore sterility (Wang *et al*. 2009).

In summary, the delaying development of sterile anther in DGMS line 0A30A compared to fertile anther might be related to DNA damage checkpoint and SAC.

Acknowledgements. This work was supported by the earmarked fund for China Agriculture Research System (CARS-13), National Key Technology R&D Program (2010BAD01B02) and a grant of Northwest A&F University to S.W. Hu.

References

Deng Y.J., Hu S.W., Tian B.M., Dong C.H., Liu S.Y. (2010): Cytological observation of male gametogenesis development of digenic dominant Shaan-GMS in *Brassica napus*. Chinese Journal of Oil Crop Sciences, **32**: 001–005. (in Chinese)

Dong Y.L., Du H. (1993): Study on a dominant genetic male sterility discovered in *Brassica napus* L. Southwest China Journal of Agricultural Sciences, **6**: 6–10. (in Chinese)

Fenech M. (2006): Cytokinesis-block micronucleus assay evolves into a "cytome" assay of chromosomal instability, mitotic dysfunction and cell death. Mutation Research, **600**: 58–66.

Fenech M., Crott J.W. (2002): Micronuclei, nucleoplasmic bridges and nuclear buds induced in folic acid deficient human lymphocytes: Evidence for breakage-fusion-bridge cycles in the cytokinesis-block micronucleus assay. Mutation Research, **504**: 131–136.

Fu T.D. (1995): Breeding and Utilization of Rapeseed Hybrid. Hubei Science and Technology Press, Wuhan. (in Chinese)

Hu S.W., Yu C.Y., Zhao H.X., Ke G.L. (1999): The genetic of a new kind of male sterility accession "Shaan-GMS" in *Brassica napus* L. Acta Botanica Boreali-Occidentalia Sinica, **19**: 63–67. (in Chinese)

Hu S.W., Yu C.Y., Zhao H.X. (2000): The discovery of a new kind of male sterility accession in *Brassica napus* L. and a primary genetic study. Acta Agriculture Boreali-Occidentalis Sinica, **9**: 90–94. (in Chinese)

Hu S.W., Yu C.Y., Zhao H.X., Lu M. (2002): Development of GMS homozygous two-type line 803AB from dominant GMS Shaan-GMS in *Brassica napus* L. Acta Agriculturae Boreali-Occidentalis Sinica, **11**: 25–27. (in Chinese)

Hu S.W., Yu C.Y., Zhao H.X., Zhang C.H., Lu M. (2004): Identification and genetic analysis of the fertility restoring gene for dominant male sterility accession "Shaan-GMS" in *Brassica napus* L. Journal of Northwest Sci-Tech University of Agriculture & Forestry, **32**: 9–12. (in Chinese)

Hu S.W., Fan Y.F., Zhao H.X., Guo X.L., Yu C.Y, Sun G.L., Liu S.Y., Wang H.Z. (2006): Analysis of *MS2Bnap* genomic DNA homologous to *MS2* gene from *Arabidopsis thaliana* in two dominant digenic male sterile accessions of oilseed rape (*Brassica napus* L.). Theoretical and Applied Genetics, **113**: 397–406.

Li S.L., Qian Y.X., Wu Z.H. (1985): Genetic study on genic male sterility and its utilization in *Brassica napus* L. Acta Agriculturae Shanghai, **1**: 1–12. (in Chinese)

Li S.L., Zhou X.R., Zhou Z.J., Qian Y.X. (1990): The inheritance and utilization of dominant genic male sterility in *Brassica napus* L. Crop Research, **4**: 27–32. (in Chinese)

Mathias R. (1985): A new dominant gene of male sterility in rapeseed (*Brassica napus* L.). Zeitschrift für Pflanzenzuchtung (Plant Breeding), **94**: 170–173.

Shi H.J., Dong Y.L. (2004): Studies on F_1 seed production for Chuanyou 15 using genetic 3-line. Southwest China Journal of Agricultural Sciences, **17**: 12–15. (in Chinese)

Song L.Q., Fu T.D., Yang G.S., Tu J.X., Ma C.Z. (2005): Genetic verification of multiple allelic gene for dominant genic male sterility in 609AB (*Brassica napus* L.). Acta Agronomica Sinica, **31**: 896–902. (in Chinese)

Thomas P., Umegaki K., Fenech M. (2003): Nucleoplasmic bridges are a sensitive measure of chromosome rearrangement in the cytokinesis-block micronucleus assay. Mutagenesis, **18**: 187–194.

Wang D.J., Tian J.H., Wang H., Hu X.P., Li D.R., Guo A.G. (2003): RAPD markers linked to mono-dominant GMS in rapeseed. Acta Botanica Boreali-Occidentalia Sinica, **23**: 1556–1560. (in Chinese)

Wang T.Q., Tian Z.P., Huang Z.S., Wei Z.F., Shao M.B. (1999): Selection and breeding of dominant nucleic male sterile line with low erucic and glucosinolate content (Qianyou 2AB) in B. *napus*. Guizhou Agricultural Sciences, **27**: 14–18. (in Chinese)

Wang X. (2010): Study on the mechanisms of spindle assembly checkpoint. Journal of Qiqihar Medical College, **31**: 1442–1443. (in Chinese)

Wang Z., Jiang X.H., Zhang H., Shi Q.H. (2009): Spindle assembly checkpoint. Chinese Journal of Cell Biology, **31**: 145–150. (in Chinese)

Wu J.Y., Yang G.S. (2008): Meiotic abnormality in dominant genic male sterile *Brassica napus* L. Molecular Biology, **42**: 572–578.

Yang G.S., Qu B., Fu T.D. (1999): Anatomical studies on the anther's development of the dominant genic male sterile line Yi-3A in *Brassica napus* L. Journal of Huazhong Agricultural University, **18**: 405–408. (in Chinese)

Yu F.Q., Fu T.D. (1990): Cytomorphological research on anther development of several male-sterile lines in *Brassica napus* L. Journal of Wuhan Botanical Research, **8**: 209–216. (in Chinese)

Zhou X.R., Li S.L., Zhou Z.J., Zhuang J., Gu L.D. (2003): Breeding of dominant genic male sterility (GMS) hybrid variety Heza No. 3 with low erucic acid and low glucosinolate in *Brassica napus* L. Journal of Shanghai Jiaotong University (Agricultural Science), **21**: 304–308. (in Chinese)

Zhou X.R., Zhuang J., Sun C.C., Li S.L., Sai X.F., Hu G.B., Wang W.L., Li Y.L., Gu L.D., Qian X.F. (2006): Breeding of a dominant genic male sterility (GMS) hybrid variety Heza 7 of *Brassica napus* L. with double low (erucic acid and glucosinolate) content in rapeseed. Acta Agriculturae Shanghai, **22**: 6–9. (in Chinese)

Received for publication February 13, 2012
Accepted after corrections September 12, 2012

Corresponding author:
Sheng Wu Hu, PhD., Northwest A&F University, State Key Laboratory of Crop Stress Biology in Arid Areas and College of Agronomy, Yangling, Shaanxi 712100, P.R. China
e-mail: swhu83251@nwsuaf.edu.cn