Pak. J. Bot., 43(3): 1691-1694, 2011.

BREEDING AND CHARACTERIZATION OF *HOMOKARYOTIC HETEROPLASMIC* MALE STERILE LINES IN RICE (ORYZA SATIVA)

QIMING MEI[1,2] RONG XIE[3], CHENGYUAN LIU[3], XICHANG ZHOU[2] AND YINGGUO ZHU[1]

[1]*The College of Life Sciences, Wuhan University, Wuhan 430072, P. R. China.*
[2]*The Center of Gardens , Wuhan University, Wuhan 430072, P. R. China.*
[3]*Rice and Sorghum Research Institute, Sichuan Academy of Agricultural Science, Luzhou 646000 P. R. China*

Abstract

Twelve different Cytoplasmic Male-Sterile (CMS) lines were crossed with 18 rice varieties. From the hybrid with japonica rice Nongken 58, twelve homokaryotic-heteroplasmic male sterile lines were developed in B7F1 after successive backcrossing and selection for stable male sterility and desirable agronomic traits such as flowering habit and high outcrossing rate. The experimental results demonstrated that expression of the CMS factors were influenced by the corresponding nuclear genes. Three pollen abortion types, including the typical, the spherical and the stained abortion, were observed in the homokaryotic-heteroplasmic male sterile lines. Formation of the aborted pollen grains was influenced by the interaction among specific cytoplasmic and the corresponding nuclear genes. As the CMS carriers, these homokaryotic-heteroplasmic lines will have significant impact on the utilization of multiple types of CMS in hybrid rice breeding. What is more important is that these CMS lines are the invaluable materials for the investigation of the molecular mechanism of CMS formation in rice.

Introduction

Three types of cytoplasmic male sterile (CMS) lines are currently used for producing hybrid rice seeds in China. Correspondingly, these CMS plants have three types of pollen abortion including the typical, the spherical and the stained pollen abortion, when the pollen grains are aborted at mononucleate, binucleate, trinucleate pollen developmental stages, respectively (Zhu, 1979; Li, 1980). Furthermore, variation in the abortive properties occurs in the same type of CMS lines when they are placed under different nuclear backgrounds. Therefore selection of nuclear donor is very important for the development of new CMS systems. It is equally important to understand the influence of nuclear on the formation of different types of CMS.

Homokaryotic-heteroplasmic CMS lines contain the same nuclear background, therefore differences among the CMS lines are caused only by the cytoplasmic genes (Yi *et al*., 2004). Therefore, homokaryotic-heteroplasmic CMS lines are the most desirable for eliminating effect of nuclear genetic background.

The homokaryotic-heteroplasmic CMS lines found in China each only produce one type of abortive pollens (the typical, spherical, or stained abortion type). Application value of these CMS materials is very limited. Maan (1972, 1973) proposed that CMS is controlled by the interaction among multiple cytoplasmic-nuclear genes. The researches by Zhu (1979) and Li (1980) have developed the guidelines for categorization of male sterile cytoplasm involved in cytoplasmic-nuclear interaction in rice CMS systems. A hypothesis on CMS formation was formed based on the studies in our laboratory (Mei & Zhu, 1990).

In this research, 12 different CMS lines were identified according to CMS heredity, the relationship with maintainer lines, and the morphological characteristics of the abortive pollens. These CMS lines were used as female parents to cross with 18 different maintainer rice varieties, followed by continuous backcrossing. The objective was to develop homokaryotic-heteroplasmic CMS lines that produce the three types of abortive pollens with stable male sterility as well as the other desirable traits. The effect of various cytoplasmic-nuclear interactions on male sterile property of the homokaryotic-heteroplasmic CMS lines was identified. Findings from this research will serve as the basis for studying at molecular level the formation of different CMS lines; they will also be used for the production and breeding of hybrid rice.

Materials and Methods

Materials: The 12 CMS cytoplasmic donors were the wild rice abortion (WA) type Erjunan No.1 sterile line, the dwarf wild abortive type Xieqingzao CMS line, Tian-Dong wild rice type CMS line Chaoyang No.1, Nong-An wild rice type CMS line Chaoyang No.1, Gambiaka type CMS line Chaoyang No.1, Dissi type D297 CMS sterile line, Indonesia rice type Yinshui CMS line Chaoyang No.1; Ma-Xie CMS lines; Aegilops kotschy (K type) CMS sterile line, Honglian type CMS line Guangcong 41, Japan BT type CMS line Boro II, Dian-Yi type, and Tian Rui CMS 409 sterile line. Eighteen regular varieties including 'Qiuguang', 'Nongken 58','Jingxuan 2' 'Liming' 'Zhenshan 97' 'Xieqin Zao' 'Chaoyang 1' 'Congguang 41' 'Yuetai' 'Maweizhan 1' 'Maweizhan 2' 'Maweizhan 3' 'D297' 'Dianrui 409' '-32' 'K Qin' 'IR26' and 'Minghui 63' were crossed with the CMS lines.

Methods: Nuclear replacement was achieved through continuous backcrossing and selection (Xue *et al*., 1995). Pollen sterility was determined using the I_2-KI staining method. The pollen grains were stained I_2-KI and examined under microscope. Pollen grains that had irregular shape and did not stain with I_2-KI were typical abortive pollens. The spherical pollen grains with no staining with I_2-KI belonged to the spherical abortion type. Pollen grains having spherical shape and showing light color with I_2-KI staining were stained-abortion type.

Selection of homokaryotic-heteroplasmic CMS lines: For testcross Screening, 12 different CMS donor lines were crossed with 18 regular varieties which had various maintaining characteristics. In F_1, 10 seeds from each hybrid combination were grown and all the F_1 plants appeared to be phenotypically uniform. The japonica rice variety Nongken 58 was selected from the 216 test-crosses because the hybrid was able to maintain the abortive pollen type from the CMS donors. Having desirable flowering traits, Nongken 58 was used as backcross parent to develop homokaryotic-heteroplasmic CMS lines.

Qiming Mei, E-mail:mqmwhucn@yeah.net, telephone: +86(18971567229) Fax:+86(027)87874432
Rong Xie, E-mail: xr20005@yahoo.com.cn
Yingguo Zhu , e-mail: zhuyg@public.wh.hb.cn

Results

A total of 120 lines were selected from the 12 crosses between the 12 CMS lines and Nongken 58. All the F_1 progenies were male sterile with elite flowering traits. When backcrossed with the male parent Nongken 58, segregation appeared in B_1F_1. Male sterile plants with good blooming traits were selected. Single plants that looked more like the male parent were backcrossed in successive generations. After six generations of backcrossing and selection, the progenies became phenotypically similar to the male parent. Male sterility was stabilized in the progeny. The percentage of male sterile and female fertile plants reached 100%, and the population sterile plant ratio was 100%. The selection process of the homokaryotic-heteroplasmic Nongken 58 CMS lines is described in Table 1.

Identification of pollen characteristics of the homokaryotic-heteroplasmic Nongken 58 *CMS lines*: Whitish non-dehiscent anthersp formed on the plants of homokaryotic-heteroplasmic Nongken 58 CMS lines that were developed from the following CMS lines: the Hainan wild abortion (WA) type, dwarf WA type, Nong-An wild type, Gambiaka type, Indonesia rice type, Tian-Dong wild rice type, Dissi type, K52 type, and Ma-Xie type. Microscopic examination found that pollen grains were irregularly shaped and did not stain with I_2-KI. The BT type and Dian-Yi type sterile plants produced light yellow rod shaped anthers that did not dehisce either. Under microscope, pollen grains were ball shaped and lightly stained with I_2-KI. The Hong-lien type CMS sterile plants produced non-dehiscent anthers that were light yellow and rod shaped. Under microscope, pollen grains were ball shaped and did not stain with I_2-KI. Features of the aborted pollen and microscopic examination results were described in Fig. 1 and Table 2.

Table 1. Selection process of the homokaryotic-heteroplasmic Nongken 58 CMS lines (Wuhan, China).

Generations female x male	Descriptions
F_0 × Nongken 58	The 12 CMS lines were crossed with Nongken58 variety; ten seeds were harvested from each cross.
F_1× Nongken 58	In F_1, 120 lines from the 12 crosses were grown. No anther dehiscence occurred in any of the progeny plants. From each cross, three single plants were backcrossed with Nongken58 to produce the backcross seeds.
B_1F_1 × Nongken 58	In B_1 F_1, 10 lines from each of the CMS crosses were grown. No anther dehiscence occurred. The segregating population had various phenotypic properties and developmental stages. Single plants that looked more like Nongken 58 were backcrossed with male parent.
B_2F_1 × Nongken 58	In B_2F_1, 10 lines from each CMS cross still had segregating plants showing variation in developmental stages. No anther dehiscence occurred in the progenies. When examined under the microscope, spherical abortive pollen grains were produced by the Hong-lien type CMS lines; stained abortive type pollen grains were from BTCMS and Dian-Yi type CMS lines. Progenies from the other CMS lines produced the typical abortive type pollen grains. The bagged and self-pollinated plants did not set seeds. Single plants that were more like the male parent were selected and backcrossed with Nongken 58to produce backcross seeds.
B_3F_1× Nongken 58	In B_3F_1, 60 plants selected from 5 CMS lines were grown. No anther dehiscence occurred. Microscopic observation found that Hong-lien type CMS lines produced spherical abortion type of pollen grains and the BT CMS and Dian-Yi type plants produced stained abortion type of pollen grains. Pollen grains from all the other CMS lines were typical abortive type. The bagged and self pollinated plants did not set seeds. Plants from the Gambiaka and Tian-Dong wild type CMS lines were still segregating for developmental stage trait. Single plants that were more like male parent were backcrossed with Nongken 58.
B_4F_1× Nongken 58	In B_4F_1, 60 plants from five CMS lines were grown. Gambiaka progenies still had a few segregating individual plants. These CMS lines were backcrossed again.
B_5F_1× Nongken 58	In B_5F_1, all the CMS sterile line plants looked morphologically very similar to Nongken 58. No anther dehiscence occurred. When examined under microscope, pollen grains from Hong-lien CMS plants were spherical, and BT and Dian-Yi CMS plants produced stained abortion type of pollen grains. All the other CMS lines produced typical abortive pollen grains. These plants had concentrated flowering period, the fully opened flower had large opening angle, and the stigma was exposed. The bagged and self-pollinated plants did not set seeds. Cross-pollinated plants had very high ratio of seed set.
B_6F_1× Nongken 58	In B_6F_1, 100 plants were selected for each of the crosses derived from the 12 CMS lines. A total of 1200 plants were tested for male fertility. The self pollination ratio was 0 in all of the plants.
Sterile line---Maintainer lines	All the CMS lines produced seeds. At this stage, the Nongken 58 CMS lines had been successfully developed using Nongken 58 variety as the maintainer line.

Table 2. Microscopic examination of the homokaryotic-heteroplasmic Nongken 58 CMS lines pollens stained with I-KI and observation of spikelet fertility of offspring.

CMS abortive types	No. of pollen	Typical abortive	%	Spherical abortive	%	Stained abortive	%	Spikelet fertility %
Hainan wild rice abortion	1672	1628	97.37	44	2.63	0	0.00	0.00
Dwarf WA type	1881	1844	98.03	37	1.97	0	0.00	0.00
Nong-An wild rice type	1830	1816	99.23	14	0.77	0	0,00	0.00
Gambiaka type	1654	1562	94.44	92	5.54	0	0,00	0.00
Indonesia rice type	2113	2064	97.68	49	2.32	0	0,00	0.00
Tian-Dong wild rice type	2072	2026	97.79	46	2.21	0	0,00	0.00
Dissi type	1522	1497	98.35	25	1.65	0	0.00	0.00
K52 type	2020	2004	99.21	16	0.79	0	0.00	0.00
Ma-Xie CMS	1902	1854	97.46	48	2.54	0	0.00	0.00
Hong-lien type	1623	71	4.37	1552	95.63	0	0.00	0.11
BT (Boro II) type	2985	133	4.46	2852	95.54	0	0.00	0.17
Dian-Yi type	2752	14	0.00	0	0.52	2738	99.48	0.21

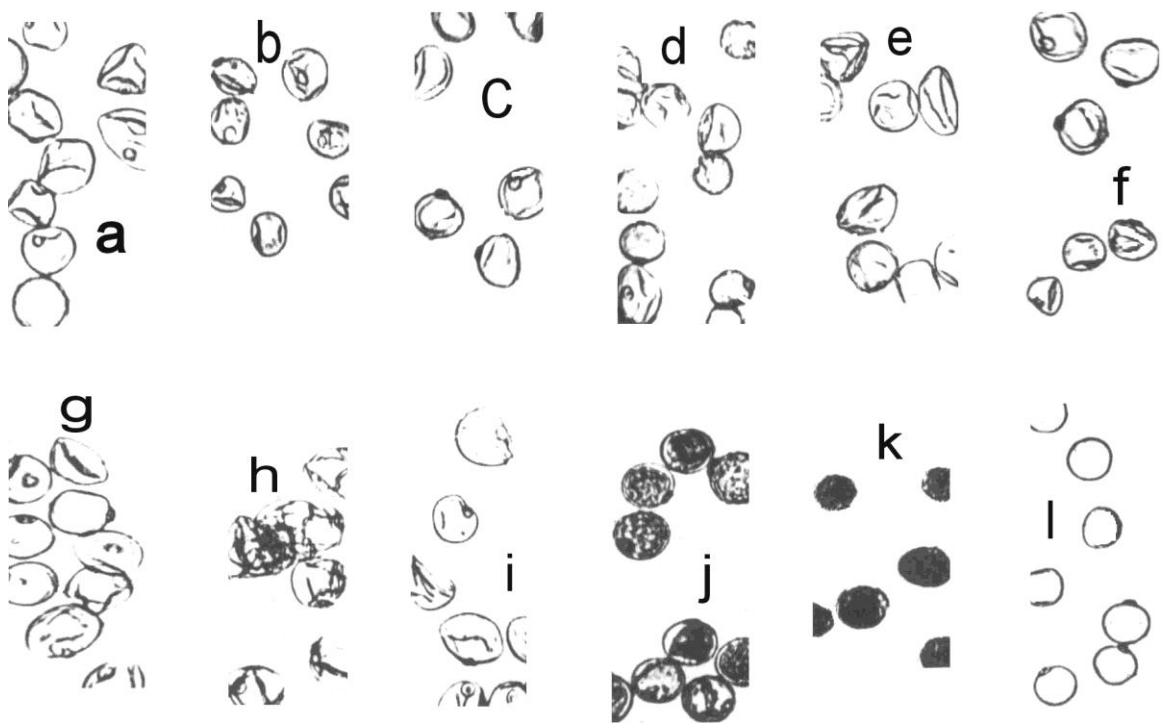

Fig. 1. Characteristics of the aborted pollens of the homokaryotic-heteroplasmic Nongken 58 CMS lines
a. Hainan wild rice abortion (WA) type; b. dwarf WA type; c. Nong-An wild rice type; d. Gambiaka type; e. Indonesia rice type; f. Tian-Dong wild rice type; g. Dissi type ; h.K52 type i. Ma-Xie CMS j. BT (Boro II) type; k. Dian-Yi type ; l. Hong-lien type
Note: 'a—i' typical pollen abortion type; 'j—k' stained pollen abortion type; 'l' spherical pollen abortion type;

Discussion

CMS is a maternal hereditary trait which is controlled by interaction between nuclear and cytoplasmic genes. CMS lines provide the bases for the wide application of hybrid vigor. The CMS mechanism in rice is controlled by a very complex genetic mechanism. Some CMS lines originated from different cytoplasm donors have similar maintaining relationship such as Dian-Yi type and BT type CMS lines. In CMS lines including Hong-lien type and the wild abortion type CMS lines, the CMS mechanisms are controlled by different cytoplasms and requires separate maintainers, moreover pollen grains also belong to different abortion types. These evidences demonstrate that cytoplasm contains many fertile and sterile genes and correspondingly, there are multiple fertile and sterile nuclear genes. The interaction between the cytoplasmic and nuclear genes resulted in various types of male sterile lines (Zhu *et al*., 1979; Li 1980; Mann *et al*., 1972, 1973).

In this study, the nucleus from a normal fertile cultivar Nongken 58 was combined with the male sterile cytoplasm from 12 CMS lines. The homokaryotic-heteroplasmic Nongken 58 CMS lines were selected from those crosses. The resultant CMS lines produced three types of aborted pollens including the typical abortion, the spherical abortion and the stained abortion. This result confirmed that, when combined with different CMS donors, one nuclear donor can lead to the formation of different types of aborted pollens in the CMS progenies.

Each factor controlling pollen abortion should act independently without interference from other members. Consequently, formation of the three types of aborted pollens is the manifestation of function from all the controlling factors. The homokaryotic-heteroplasmic Nongken 58 CMS lines produced the typical, the spherical and the stained abortion pollen grains. In these pollens expression of the male sterile factors were also influenced by the corresponding nuclear

genes from Nongken 58. The phenotypes were results of the interaction among multiple cytoplasmic and nuclear genes.

Hybrid rice is widely grown in China. Rice production in the world is changing toward growing hybrid varieties due to the high yield and resistance to biotic and abiotic stresses. The homokaryotic-heteroplasmic Nongken 58 CMS lines are carriers of different types of CMS. These genetic materials will be investigated to develop standards of biological properties of various CMS systems. Such information is very useful for using biotechnological techniques to identify and classify new CMS systems(Zhang, 1996), and for the discovery and development of CMS lines. In addition, homokaryotic-heteroplasmic lines can be used to identify the diversity of CMS heredity in rice(Guo *et al*., 2007), in order to systematically investigate the molecular mechanisms of CMS formation, and to localize and clone fertile and sterile genes (Xue *et al*., 1995; Yi *et al*., 2002, Gong *et al*., 2006). Providing this information will enhance breeding for hybrid rice using various types of genetic cytoplasmic male sterility.

References

Gong, Y.C. and C.Z. Xing. 2006. Research on Molecular Mechanism of Cytoplasmic Male Sterility in Plants. *Molecular Plant Breeding*, 4: 51-56.

Guo, H., S.X. Li., J.D. Xu., H.Y. Zhang., P.Z. Xu and X.J. Wu. 2007. SSR Marker-based Genetic Diversity of 24 Cytoplasmic Male Sterile Lines in Rice. *Hybrid Rice*., 22: 56-61.

Li, Z.B. 1980. A preliminary discussion about the classification of male sterile lines of rice in china. *Acta Agronomica Sinica*. 6: 17-26.

Maan, S. S. 1972. Inteoration systems for hybrid wheat research, *Crop. Sci*., 12: 360-367

Maan, S. S. 1973. Cytoplasmic variability in triticina. *Proc. 4th Int. Wheat Genetices Simp.,* 367-372.

Mei, Q.M. and Y.G. Zhu. 1990. Comparative study on mitochondrial dna from cytoplasmic male sterile lines of honglian type and yiebai type rice. *Journal of Wuhan Botanical Research*, 8: 25-33.

Xue, S.Y., S.N. Xiang and D.X. Xu. 1995. Breeding of CMS Lines Resistant to Rice Blast By Backcrossing Method, *Hybrid Rice*, 5-24.

Yi, P., L. Wang., C.X. Wan and Y.G. Zhu. 2002. BAC Library construction for mitochondrial genome of sterile and maintainer lines from HL Rice. *Acta Agronomica Sinica*, 28: 756-759.

Yi, P., T. Yu., Y. Liu and Y.G. Zhu. 2004. Genomic context of nucleus influences mitochondrial gene expression of HL-type rice. *Hereditas,* 26: 186-188.

Zhang, C.X. 1996. Classification and Genetics of Cytoplasmic Male Sterile in Crops. *Acta Agriculturae Boreali-Occidentalis Sinica*, 5: 83-86.

Zhu, Y.G. 1979. Studies on male sterile lines of rice with different cytoplasms. *Acta Agronomica Sinica*., 5: 29-38.

(Received for publication 28 April 2010)