Development of New Type of CMS Source from Dongxiang Wild Rice (Oryza rufipogon)

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Abstract: This study was conducted to develop and characterize a novel new cytoplasmic male sterile (CMS) source which was identified from Dongxiang wild rice by crossing Dongxiang wild rice as female with Zhongzao 35, an indica inbred variety, as male and continuous backcrosses with Zhongzao 35. Observation under optical microscope manifested that this novel CMS belongs to typical abortion type with fewer number of pollens compared with CMS-WA. Sequential planting showed that this novel CMS has complete and stable male sterility. Testcross experiment showed that all the 24 testers including B and R lines of CMS-WA and CMS-HL and other indica inbred varieties are the maintainers with complete maintaining ability, suggesting that this novel CMS has fertility restoration totally different from CMS-WA and CMS-HL and belongs to a novel type of CMS. So far, we only discovered a unique fertility restoration source for this novel CMS. Inheritance analysis showed that the fertility restoration for this CMS showed that it was governed by three pairs of independent dominant genes, which is the first report that fertility restoration in CMS rice is controlled by three pairs of independent dominant genes. Prospect for use of this novel CMS system in hybrid rice breeding was also discussed.

Key words: Dongxiang wild rice; novel CMS; fertility restoration

Cytoplasmic male sterility (CMS) and nucleus controlled fertility restoration are widespread plant reproductive features that provide useful and efficient tools to exploit heterosis in crops. In three-line system of hybrid rice, more than 60 types of CMS were identified in China. However, based on their inheritance, morphology of abortive pollens, and restoration-maintenance relationships all the CMS identified so far are classified into three types, namely, CMS-BT (Boro II), CMS-WA (wild abortive), and CMS-HL (Honglian) (Li et al., 2007). Searching for new types of CMS has become one of the greatest efforts of hybrid rice breeders because of the possibility of genetic vulnerability due to homo-plasmic CMS as experienced in maize (Hooker, 1974). Here we report the identification and characterization of a novel source of CMS from Dongxiang wild rice (Oryza rufipogon) and the inheritance of its fertility restoration.

MATERIALS AND METHODS

Rice materials

Plant materials used in this study included Dongxiang wild rice, a group of indica inbred varieties, including R or B lines of CMS-WA and CMS-HL, an F2 population generated from a cross between the novel CMS line and the identified fertility restoration source, and a top-cross F1 population produced from the novel CMS line / the fertility restoration source // R463.

Development of novel type of CMS from Dongxiang wild rice

To search for new source of male sterile cytoplasm, a cross between Dongxiang wild rice as female and Zhongzao 35 as male was made and subsequent backcrosses were made by using Zhongzao 35 as recurrent pollinator parent. The novel CMS line at BC4F1 was planted and a sequential planting experiment was conducted for the novel CMS line at BC2F1 to study the genetic stability of the male sterility.

Testcross experiments

Testcrosses were made by using the novel CMS line as female and a group of test varieties, including B lines and R lines of CMS-WA and CMS-HL and indica inbred varieties, as pollinator, to characterize the novel CMS line in terms of maintenance-restoration relationship. To evaluate the restoring ability of the identified restoration source for the novel type of CMS, spikelet fertility of the testcross between the novel CMS and the identified restoration source was measured by counting filled and unfilled grains from bagged panicles covered before flowering and panicles without bagging at harvest time (about 30 d after flowering).

Observation of pollen fertility
Pollen fertility was observed under optical microscope through 1% I$_2$-KI staining method by collecting four anthers from the top, middle and bottom spikelets from each single plant before flowering. The percentage of stainable pollen was computed by counting the number of deeply stained pollen over the total number of pollen observed. The plants with 0% stainable pollen rate were categorized as sterile class, and all the others with > 0% stainable pollen rate were regarded as fertile (including partial fertile) class.

**Inheritance analysis of fertility-restoring (Rf) genes for novel CMS**

An F$_2$ population from the cross of the novel CMS line and the fertility restoration source, and a top-cross F$_1$ population from the novel CMS line / fertility restoration source // R463 were employed for inheritance analysis of the Rf genes. Spikelet fertility of the testcross was measured by counting filled and unfilled grains from bagged panicles covered before flowering and panicles without bagging at harvest time (about 30 d after flowering).

**RESULTS**

**Identification of novel CMS source from Dongxiang wild rice**

To search for new source of CMS rice, a cross was made by using Dongxiang wild rice as female and Zhongzao 35, an indica inbred cultivar, as male, and subsequent backcrosses were made by using Zhongzao 35 as recurrent pollinator parent. Male sterility was observed in several lines at BC$_3$ generation, then, single plants from one of the male sterile BC$_3$ lines were selected to backcross with Zhongzao 35. All 10 BC$_4$ lines performed 100% pollen sterility which was maintained for all the lines at BC$_5$ to BC$_6$. One of 20 male sterile BC$_6$ families was selected phenotypically for similarity with recurrent parent and further advanced. The developing process of the new type of CMS was showed in **Fig. 1**.

**Characterization of male sterility for novel type of CMS**

Pollen sterility for the novel type of CMS line at BC$_6$ line was observed under microscope in the early planting season in 2011. Sequential plantings were carried out in the BC$_7$ generation for pollen observation at 15 day interval starting from March 23 to July 21, 2012, in the experiment station of Rice Research Institute, Jiangxi Academy of Agricultural Sciences, Nanchang, China.

**Male sterility and its stability**

The results showed that pollen sterility of the novel CMS line was 100% at both BC$_6$ and BC$_7$ generations. Moreover, the BC$_7$ sterile plant rate was all 100% for each planting group consisting of 200 or 220 single plants. These results indicated that the male sterility of this novel CMS was complete and stable from generation to generation and under different environments.

**Pollen abortion type**

**Fig. 2** showed that the novel CMS line belongs to typical abortion type but with fewer abortive pollens compared with CMS-WA. Moreover, this novel type of CMS has 100% of typical abortive pollens.

**Fertility restoration of novel CMS line in relation to CMS-WA and CMS-HL**

In order to test the sterility maintenance and fertility restoration,
testcrosses were conducted by using the new CMS line (at BC,F ) as female and 24 varieties as testers, including nine CMS-WA R lines (Minghui 63, Minghui 86, R998, R402, R463, R207, R1067, 11SN2199 and Guanghi 308), five CMS-WA B lines (Jin 23 B, Rongfeng B, Wufeng B, Tianfeng B, and Mianxiang 1B), two CMS-HL R lines (9311 and Miyang 23), one CMS-HL B line (Yuetai B), and seven indica inbred varieties (Nantehao, Liangang B, Wanniangongni, Nanjing 11, Hong 410, Luhongzao and Guichao 2). Observation of pollen viability showed that all the 24 testcrosses had zero percentage of stainable pollen. It was more noteworthy that even no partial sterility was observed. In other words, either R lines or B lines of both CMS-WA and CMS-HL or the indica inbred varieties were the maintainer lines with complete ability to restore fertility to this new type of CMS. Therefore, it could be concluded that this new CMS has totally different maintenance-restoration relationship from CMS-WA and CMS-HL.

Identification of fertility restoration source for novel CMS line and its inheritance

By testcross with more rice varieties, so far we have identified a unique fertility restoring source for the novel CMS line. Both pollen viability and seed-setting rate under natural condition and bagged condition were normal for the testcross generations (Table 1).

To characterize the inheritance of the Rf genes, an F population generated from a cross between the novel CMS line and the identified fertility restoration source, and a top-cross F population generated from the novel CMS line / fertility restoration source // R463 (R463 is a fertility restorer for CMS-WA which showed complete maintaining ability to the novel CMS line) were investigated for pollen viability. The results showed that the ratio of fertile to sterile plants in the F population and the top-cross F population fitted to segregation ratios of 63:1 and 7:1, respectively (Table 2), revealing the fertility restoration of the novel CMS was controlled by three pairs of independent dominant genes. These results also suggested that this novel CMS follows sporophytic instead of gametophytic inheritance as showed in the expected proportion of sterile plants existed in the F and the top-cross F populations.

**DISCUSSION**

**Novel sources of CMS in rice**

It is estimated that more than 60 types of CMS lines based on the origins of the cytoplasm, including WA-type, Dian1 type, Honglian type, Gambiaka type, K-type and Maxie type, have been developed in China from the 1970s to the mid-1980s (Li et al, 2007).

However, all these CMS lines can be categorized into three types, namely, CMS-BT, CMS-WA and CMS-HL, based on their inheritances, morphology of abortive pollens and restoration-maintenance relationships. A CMS-CW type (W1-A) is obtained from a backcross between the Chinese wild rice, strain W1 (Oryza rufipogon), and japonica cultivar Reimei (Ishimine and Shinjyo, 1978), which is reported to have normal pollen but incapable of germinating, hence failing in pollination and fertilization. In addition, CMS-CW is also classified as the fourth type of CMS line which has a different fertility restoration from CMS-BT. The fifth type of novel CMS was found from Oryza perennis by nucleus substitution with IR64, and has been designated as IR66707A, which has a different fertility restoration from CMS-WA (Dalmacio et al, 1995). Recently, the sixth type of novel source of CMS (designated as CMS-FA) identified from Oryza rufipogon is reported to have different fertility restoration from both CMS-WA and CMS-HL (Wang, 2006). Here we identify the seventh type of CMS from Dongxiang wild rice (Oryza rufipogon), which is also totally different from CMS-WA and CMS-HL in terms of restoration-maintenance relationship.

**Inheritance of fertility restoration for CMS rice**

The inheritance of fertility restoration in the CMS-WA system has been extensively investigated. A majority of studies reported digenic inheritance with two independent genes and the chromosomal locations of the two Rf genes (Rf3 and Rf4) have also been mapped (Sattari et al, 2008). In the case of CMS-HL, fertility can be independently restored by either of two genes, Rf3 or Rf6 (Liu et al, 2004). CMS-BT is restored by the nuclear restorer gene Rf-1, which is initially identified in Chinsurah Boro II and as a single locus in the nuclear genome (Shinjyo, 1969; Wang et al, 2006). The fertility restoration for the CMS-CW type is reported to be controlled by a single gene designated as RfCW (Fuji and Toriyama, 2005).

There has no report about the inheritance of fertility restoration for IR66707A. However, it is reported that fertility of the new CMS source, CMS-FA, which shows a fertility restoration different from both CMS-WA and CMS-HL, is restored by one pair of dominant gene (Wang et al, 2008a). Here we report that the fertility restoration for another new source of CMS identified from Dongxiang wild rice (also from O. rufipogon) is governed by three pairs of independent dominant genes. Though CMS-FA (Wang, 2006) and the one

| Table 1. Stainable pollen rate and seed-setting rate of cross between novel CMS line and fertility restoring source. |
|---|---|---|---|
| Materials Pollen staining (%) | Seed-setting rate (%) |
| | Natural condition | Bagged condition |
| F | 94.0 ± 3.65 | 87.24 ± 5.47 | 84.63 ± 5.32 |
| CK | 94.4 ± 3.48 | 85.57 ± 3.64 | 82.24 ± 2.80 |

a stands for the cross between the novel CMS line and the fertility restoring source. b stands for Rongyou 463, a rice hybrid widely commercialized in China.

| Table 2. Segregation of sterile and fertile plants in populations. |
|---|---|---|---|---|
| Population | Fertile plants | Sterile plants | Ratio of fertile to sterile plants | Expected χ² (P ≥ 0.05) |
| F | 4363 | 38 | 63:1 | 1.646 | 3.84 |
| Top-cross F | 208 | 34 | 7:1 | 0.399 | 3.84 |
we report here are both sourced from cytoplasm of *O. rufipogon* and have totally different fertility restoration from CMS-WA and CMS-HL, they have different inheritance of fertility restoration. Therefore, it could be inferred that the sources of their cytoplasm controlling the CMS are different. This is the first report that the fertility restoration is controlled by three pairs of independent dominant genes in CMS rice.

**Prospect for use of novel CMS in hybrid rice**

One of major factors constraining development of hybrid rice by means of CMS/Rf system is to develop the maintainer lines and subsequent nucleus substitution of the original CMS line with the maintainer lines by repeated backcross. However, in the process of maintainer line improvement, it is inevitable to use elite inbred varieties with breeding target traits, such as grain quality, pest resistance, and so on, as donor parent to develop new maintainer lines. Unfortunately, the availability of rice germplasm desirable for being as maintainer lines for CMS-WA and CMS-HL is very limited (Liang et al., 2002; Li et al., 2010). In view of the CMS-FA, the frequency of varieties with maintaining ability was 55.5% (122 of 220 rice varieties) (Wang et al., 2008b). However, as for the novel CMS identified from Dongxiang wild rice, all the tested 24 varieties including B lines and R lines of CMS-WA and CMS-HL as well as indica inbred varieties are capable of completely maintaining the male sterility without partial fertility observed. In fact, Chen et al. (1995) found out that all of more than 2000 varieties used for screening the fertility restoration for Guojiyouzhan A (a pollen-free CMS line also identified from Dongxiang wild rice) were the maintainers with complete fertility maintaining ability. In combination of our results with the report by Chen et al. (1995), most rice varieties could be potential maintainers with complete maintaining ability for the CMS identified from Dongxiang wild rice. Therefore, we anticipate that this wide spectrum of varieties with complete maintaining ability could greatly facilitate developing the maintainer lines and respective A lines for the type of this novel CMS and increasing the breeding efficiency. In other words, this novel CMS system of rice could not only diversify the CMS system but also promote the development and production of hybrid rice.

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