

Discovery and identification of a novel double-low cytoplasmic male sterile 681A and three-line system in *Brassica napus*

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ABSTRACT

A novel double-low stable CMS line has been discovered and identified in *Brassica napus*. This CMS system shows great promise for the development of higher oil quality and yield hybrid cultivars. Results showed that the majority (83.4%) of the tested materials are completely male sterile (MS) B-lines, while only 35 of 279 testcrosses (12.2%) possess complete male fertility restorability (MFR). The CMS and B-line are thus designated as 681A and 681B, respectively. We also found certain materials that can be the better restoring sources if the materials originated from *B. juncea* or *B. juncea* × *B. napus* crosses, such as 714R, which has the best MFR. Unlike most of the other CMS systems which were developed from allocytoplasm, the new CMS 681A would not exhibit incompatibility between the allocytoplasm and the mitochondrial nuclear genome and other problems since it originated from a newly released double-low *B. napus* cv. Xiangyou 13. Results also revealed that the relationship of both MS maintaining and MFR of their testcross progenies performed very unique. B- and R-lines, especially R-lines of pol-CMS can be directly utilized in this new CMS system. The R gene of 714R can also be easily transferred to other *B. napus* cultivars for R-line breeding. Genetic study showed that all 78 hybrid F₁ plants of 681A × 714R were fertile. Segregating ratio of F₂ and BC₁ testcrosses was 3F:1S and 1F:1S, which both perfectly fit the X²-test for a single dominant gene for male sterility restoration. Furthermore, the results suggested that the 681A CMS regulating gene may be located at a single locus, the same as pol-CMS and MFR of 681A is controlled by a dominant gene which belongs to the spontaneous inheritance.

Key words: Cytoplasmic male sterility (CMS) Double-low rapeseed *B.napus*

INTRODUCTION

Significant hybrid vigor was recorded in rapeseed. The hybrid F₁ seed production can be simplified by using CMS. Several CMS systems were developed through alloplasmic substitutions in this crop. Ogu-CMS originated from a radish variety has been transferred to rapeseed, but the restorer gene has not been demonstrated for this system in *B.napus*. Nap-CMS from a spring cultivar and a winter cultivar of *B.napus*; and Pol-CMS from a winter cultivar of *B.napus* and new CMS materials have been developed in rapeseed. However, Pol-CMS is still the relatively stable system for hybrid seed production although it conferred both a slightly temperature-sensitive MS and a more or less negative on the yield. Most of the hybrids, 13 of 22 registered were developed from Pol-CMS system. The bottleneck has been that MS of all the reported CMS lines is not always completely stable, especially at the high or low temperatures(20-26 or 10-12). It can impact in the hybrid purity leading to the yield reduction eventually. The agronomic traits of CMS lines are also poor and yield vigor is not as high as other crops. Most of the alloplasmic CMS systems are associated with floral abnormalities resulting from nuclear-cytoplasmic incompatibilities and all the current CMS lines are not double-low materials. It is thus necessary to search, create and develop new more stable CMS sources with higher yield and quality (double-low), good agronomic traits, and stresses tolerance potential. We discovered a potential CMS material from a single heterozygous nuclear plant with mutant cytoplasm from a newly released double-low *B.napus* c.v. Xiangyou 13.

MATERIALS AND METHODS

The varieties of rapeseed (*B.napus* and *B.juncea*) used in this study were provided by Oil Crops Institute, Hunan Agricultural University. A potential new CMS material was discovered from a single heterozygous nuclear and cytoplasm mutant plant of a newly released double-low, high yield *B.napus* cv. Xiangyou 13 in 1991. The new CMS line and B-lines and R-lines (714R developed from *B.juncea* × *B.napus*) were testified for selection through selfing, testcross and backcross between 1992-1998. The novel CMS line and B-line have been designated as CMS 681A and 681B, respectively. The experiments were conducted at Research Station, Hunan Agricultural University, Changsha.

The stability of 681A and its B-lines, R-lines; the genetic characteristics of MFR, combining ability between 681A and R-lines (published elsewhere), oil contents and quality(double-low) and agronomic traits such as plant height, fruit numbers per plant, primary stem branching characteristics and floral organ size; the relationship of MS maintenance and MFR comparison between 681A and Pol-CMS were systemically investigated. The statistical analysis (X^2 -test) was performed for the determination of MFR gene(s) according to the Mendelian independent segregation inheritance law and the progeny segregation ratios from both F_2 and BC_1 testcross populations.

Total of 279 test-crosses were made between 681A and R-lines excluding 127 B-lines producing F_1 seeds which were then selfed and backcrossed to produce F_2 and BC_1 test-cross seeds. The progenies of cross or test-cross were testified between 681A × Pol-CMS B, and 681A × Pol-CMS R, or Pol-CMS × 681B, and Pol-CMS × 714R. A test-cross was also made between (Pol-CMS × 714R) F_1 and F_1 (681A × Pol-CMS R) to determine if this CMS regulating gene(s) locate at the locus or loci same as Pol-CMS does.

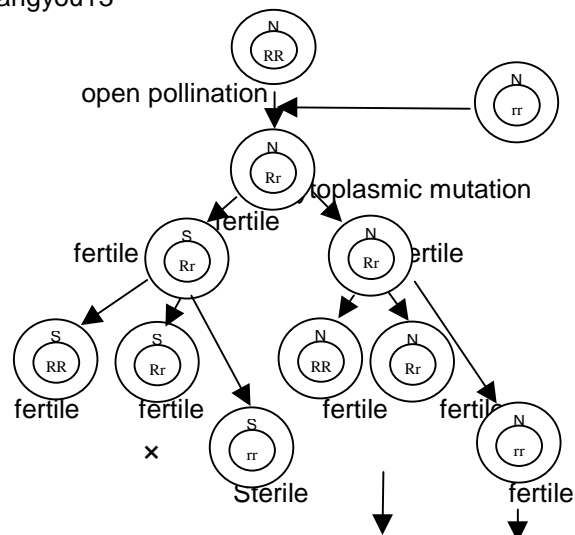
The double-low(classification standard: erucic acid <1% and glucosinolates <30 μ mol/g) and oil contents analysis methods were followed accordingly to Guan(1985).

RESULTS AND DISCUSSION

A single cytoplasmic male sterile mutant plant with heterozygous nuclear was discovered from a double-low, higher yield *B.napus* c.v. Xiangyou 13 in 1991. Both the sterile progenies and the fertile progenies produced from the same mutant plant were then crossed to each other for testifying, respectively. The result showed that only one single male fertile plant, 92-572 was identified to possess the maintaining ability for the sterile plant, 92-782 in 1992. Both the sterile and the fertile plants were further coded as 93-681 and 93-766 in 1993. The former one has thus been designated as 681A and accordingly the latter one as 681B. The R-line 714 for 681A has also been developed and identified successfully. Here is the discovery and identification detail procedure of 681A and 681B(Fig.1).

A single mutant plant found in Xiangyou 13 in Yongzhou, southern Hunan, China

cv. Xiangyou13



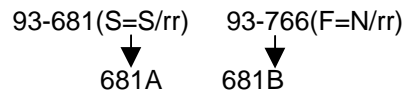


Fig.1. The development procedure of CMS 681A and 681B.

Note: Cytoplasm fertile(N) or sterile (S) and gene RR, Rr and rr indicate fertile, fertile and sterile in the nuclear, respectively.

According to the above analysis, the inheritance mechanics of male sterility and fertility is that 1). The male fertility is encoded by a dominant nuclear restorer gene(R) in this new CMS system. The plant is fertile when homozygous(RR) or heterozygous(Rr) gene in the nuclear, no matter the cytoplasm gene is fertile or sterile; 2). The male fertility is also determined by the gene type of the cytoplasm when the nuclear gene is homozygous recessive (rr). The plant is sterile while the cytoplasm gene is sterile; otherwise, the plant is male fertile if the nuclear gene is rr. Thus, plant is sterile only when mitochondria carry the MS gene while the cytoplasm gene is rr. The origin of 681A cytoplasm might be from a Japanese variety Chikuzen. Both the cytoplasm and nuclear of Xiangyou 13 are fertile. However, the heterozygous nuclear has both functional genes(N and S) for encoding the expression of the fertility and sterility while is pollinated from out pollen grains which could have the sterile-inducing gene(S). The mutant cytoplasm of the heterozygous nuclear of Xiangyou 13 produce two types via fertile to sterile progenies.

The result showed that there is a good stable maintaining effect between 681A and 681B, and good MFR effect between 681A and R-lines. A complete sterility maintaining plant from the same heterozygous mutant plant progeny number averaged to be 83.4%. Of 35 R-lines (12.2%) of 279 test-crosses revealed very strong complete MFR, while majority could only be the good B-lines for this new CMS system. We also found that the most of the varieties originated from *B.juncea* or between *B.juncea* and *B.napus* can be the better restoring sources for 681A. For instance, the best R-line 714 has been developed from a cross of *B.juncea* × *B. napus*.

The 681A plant and flower organ size are relatively smaller. Plants are 681A are shorter than those of R-line 714, fruit number per plant of 681A is also less than that of R-line; and both the petal and the stigma of 681A is smaller than that of B-line and R-line, respectively. However, both the primary branch number has no difference. Both 681A and 714R belong to double-low (erucic acid 0.3%, glucosinolates 25-28 $\mu\text{mol/g}$) materials. The oil contents of both range from 38-40%.

Total of 78 hybrid F_1 plants (47 from the greenhouse and 31 from the field) of 681A X 714R were identified to be fertile at both the greenhouse and the field cultivation conditions. Of 71 male fertile plants and 26 male sterile plants were obtained from F_2 segregating progenies; 20 male fertile plants and 17 male sterile plants were further produced through the test-cross (BC_1) with 681A. The result indicated that the male fertility restoration is controlled by a single dominant gene. A segregating population with 82 male fertile plants and 23 male sterile plants was produced from F_1 (male fertile plant emasculated) of Pol-CMS × 714R pollinated by the F_1 of 681A X Pol-CMS R. Results showed that CMS 681A and Pol-CMS have the same maintaining and restoring relationship.

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