

# Studies on the sterility of stable CMS LINE 991A in *Brassic napus*

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## Abstract

991A is a new stable CMS line in *B.napus*, bred by successive directional selection. Its sterility is stable from initial flower date to final flower date, whether in low temperature of below 10°C or in higher temperature condition at Changsha in the spring of 2001-2004. The results are as follows: 991A didn't produce trace-pollens, or produced a very small yellow spots on anthers of partial flowers of 10.3% of plants only in 2001. But the spots on anther of 991A was unable to scatter pollen grains. While the low temperature sensitive CMS line D6A produced trace-pollens or mass pollens each year. Exchanged the maintainers of 991A with D6A, were formed two F<sub>1</sub> (991A/D6B) and (D6A/991B). The rate of flowers with trace-pollens of (991A/D6B) and 991A were 2.6%, 2.5%, respectively in the spring of 2001. That of (D6A/991B) and D6A were 5.35%, 11.57%, respectively. Two BC<sub>1</sub> [(991A/D6B)/D6B] and [(D6A/991B)/991B], were formed by backcross. The rate of flowers with trace-pollen of [(991A/D6B)/D6B] is 5.01%, which is close to that of D6A (5.8%) in the spring of 2002. But that of [(D6A/991B)/991B] is 2.31%, while that of 991A is 0.0%. The results showed that the sterile stability of 991A is controlled by interaction of genes in cytoplasm and nucleus. It has the same Restorer and maintainer as D6A. Hybrid seed production test of 991A is carried out in Hunan Province. The rate of sterile plant of hybrid seed of 991A/C14910 and 991A/P1 were 2.0%, 1.4%, respectively, while that of D6A/C14910 and D6A/P1 is 15.9%, 13.7%, respectively. It showed that 991A is suited for hybrid seed production in Hunan climate condition.

**Key words:** *Brassic napus*, cytoplasm male sterile (CMS), stable sterile, trace-pollen

## Introduction

Cytoplasmic male sterile (CMS) three-line method is one of the most important approaches in the heterosis utilization in *Brassic napus*. However, the main problem of the CMS system applied in production upon to now is that most of CMS lines such as Polima CMS lines and Shan 2A have trace pollens which can influence the purity of hybrid seed under the condition of low temperature. Therefore, breeding stable CMS line which is not sensitive to low temperature is very meaningful to avoid the risk of seed production.

A new stable CMS line 991A which is not sensitive to low temperature has been bred in our breeding practice, which is reported as follows.

## Materials and Methods

### Materials

CMS line D6A bred from the separating offspring of Qingyou2 which is temperature-sensitive and has a lot of trace-pollens under low temperature, and its maintainer D6B, inbreeding line P13 and 18 restorers of D6A are all from Institute of Hunan Yahuaseeds.

### Methods

**Breeding for 991A and 991B:** A stable sterile line without trace pollen under the condition of continuous low temperature even below 5 °C was found in Spring of 2000 in Changde of Hunan province from the back-crossings of P13 with D6A, which was named as 991A. Its maintainer 991B was bred from the back-crossings of P13 with D6B.

**Fertility investigation:** The fertilities of D6A and 991A were investigated from 2001 to 2004 in Changsha. Thirty plants of every line were observed. The fertility is identified as the following standards: 1. completely sterile flowers: the length of stamens is shorter than 1/2 length of pistils and there is no pollen in anthers; 2. partially sterile flowers: the length of stamens is as long as 2/3 length of pistils and there are trace-pollens in anthers and the anthers can spread pollens; 3. fertile flowers: the length of stamens is longer than or as long as that of pistils and there are a lot of normal pollens in anthers.

**Research on the exchange of nucleus and cytoplasm:** The maintainer of 991A and D6A were exchanged each other and the following combinations were gained: two F<sub>1</sub>: (991A×D6B), (D6A×991B); two BC<sub>1</sub>: [(991A×D6B)×D6B], [(D6A×991B)×991B]. The fertility of all combinations were also investigated.

**Testing for the restorers of 991A:** 991A and D6A were test-crossed with 18 restorers of CMS line. The fertilities of F<sub>1</sub> of these test-crossing combinations were investigated for their restoring rates.

**Hybrid seed production:** Hybrid seeds of combination 991A/C14910 and D6A/C14910, 991A/P1 and D6A/P1 were produced under natural isolated condition at Changsha (2003). The rates of sterile plants of all hybrid combinations were observed, and the hybrid seed purity was evaluated.

## Results

### Fertility performance of 991A and D6A:

2001: 1) D6A: there were a lot of trace pollens from 9 to 23 March.

2) 991A: some trace pollens (small yellow spots on anthers, 1mm long or so) were found but could not be spread on the anthers in only 10.3% of total plants from 10 to 16 March. Nevertheless, no trace pollen was found on other plants in total flower stage.

2002: 1) D6A: there were a few of trace pollens from 16 to 20 March.

2) 991A: there was no trace pollen in total flower stage.

2003: 1) D6A: there were some trace pollens from 14 to 19 March.

2) 991A: there was no trace pollen in total flower stage.

2004: 1) D6A: there were some trace pollens from 29 February to 5 March.

2) 991A: there was no trace pollen in total flower stage.

### Effect of nucleus and cytoplasm on trace pollen

After the maintainers of D6A and 991A were exchanged each other, the combination  $F_1$  and  $BC_1$  displayed different fertility. The results of fertility investigation (Table 1) to two  $F_1$  and two  $BC_1$  generations compared with the fertility of D6A and 991A were as follow:

*Two  $F_1$* : It showed that the sterile degree of combination (991A×D6B) (which sterile degree was 97.4%) was almost as high as that of 991A (97.41%) in the spring of 2001. The sterile degree of combination (D6A×991B) (which sterile degree was 94.65%) was between that of D6A (88.43%) and 991A. But the lasting period of trace-pollens occurring for combinations 991A/D6B and D6A/991B were the same (10 days), that of 991A was the shortest (6 days) and D6A was the longest (14 days).

*Two  $BC_1$* : The sterile degree of combination [(991A×D6B)×D6B] (which sterile degree was 94.99%) was near that of D6A but apparently lower than that of 991A (100.0%) in the spring of 2002. That of [(D6A/991B)/991B] (97.69%) was between that of D6A and 991A. The lasting period of trace-pollens occurring for combination [(991A/D6B)/D6B] was as long as that of D6A (5 days), and longer than that of [(D6A/991B)/991B]. With regard to that 991A and 991B, and D6A and D6B, have the same nucleus respectively, the above results may mean that the nucleus of 991A can apparently decrease the influence of trace-pollen, and the cytoplasm of 991A is less sensitive to low temperature than that of D6A.

**Table 1 The fertility of different generations (Changsha, 2001,2002)**

Material	occurring stage of trace pollen	total flowers	rate of flowers with trace-pollen/%	sterile degree/%
(991A/D6B)	9-19 March, 2001	11376	2.60	97.4
(D6A/991B)	9-19 March, 2001	8304	5.35	94.65
991A	10-16 March, 2001	8164	2.59	97.41
D6A	9-23 March, 2001	8515	11.57	88.43
(991A/D6B)/D6B	16-21 March, 2002	6406	5.01	94.99
(D6A/991B)/991B	16-18 March, 2002	6810	2.31	97.69
991A	no trace pollen, 2002	6552	0.0	100.0
D6A	16-21 March, 2002	7218	5.80	94.20

*The restorers and maintainers of 991A*: From the above results, it can be found that 991B can keep the sterility of D6A and D6B can keep the sterility of 991A, which means that D6A and 991A have the same maintainers. Furthermore, all  $F_1$  generations of test-crossing combinations of 991A with 18 restorers of CMS line D6A are all fertile, which means that D6A and 991A have also the same restorers.

*Hybrid seed production test*: The rates of sterile plants of 991A/C14910 and 991A/P1 were 2.1%, 1.4%, respectively, while D6A/C14910 and D6A/P1 were 15.9%, 13.7%, respectively. The hybrid seed purity of the combinations produced by 991A was higher than that of combinations produced by D6A.

## Summary and Discussion

According to the fertility investigations of  $F_1$  and  $BC_1$  generations through exchanging maintainers of 991A and D6A, it is thought that the sterile stability of 991A is the result of interaction of nucleus and cytoplasm. Some variations may occurred in cytoplasm. Therefore, it may be more effective through adopting the methods of "one-cytoplasm-and- multi-nucleus" (a maternal plant cross with more paternal plants) or "one-nucleus-and-multi-cytoplasm" in the process of breeding stable CMS lines.

991A is a new stable CMS line insensitive to low temperature. It has the same maintainers and restorers as D6A.

Hunan Province is located in the middle reaches of the Yangtze River. Cold waves arise frequently, and air temperature is changeable. 991A is suited for hybrid seed production in Hunan climate condition. 991A and 991B are fine basic materials for breeding new stable CMS lines, and some trans-breeding works are being undertaken.

## References

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