

# Floral morphology and pistil fertilization ability of female sterile mutant FS-M<sub>1</sub> in *Brassica napus* L.

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## Abstract

A comparison between female mutant FS-M<sub>1</sub> and its wild type Ningyou 10 was carried out on ability of pollen germination on stigma and pollen tube development inside style by means of microscopic histological dissection and their floral morphology. Results showed that the floral morphology of FS-M<sub>1</sub> differ in some aspects to that of wild type. Pistil length of FS-M<sub>1</sub> was shorter than that of wild type. The 6 stamens of FS-M<sub>1</sub> were almost equal to each other in length, while that of wild type composed of 4 longer one and 2 shorter one. There were two extra crescent-like organ developed at the ovary base of FS-M<sub>1</sub> with missing nectars. Ovary shape and length of FS-M<sub>1</sub> was more flat and shorter respectively than that of wild type. Stigma end of FS-M<sub>1</sub> shaped a fork-like looking, was differ obviously from wild type. The numbers of papillae cells of FS-M<sub>1</sub> was sparse and its size was larger than that of wild type. Number of pollen grains observed on stigma of FS-M<sub>1</sub> was less than that on wild type. The papillae cells of FS-M<sub>1</sub> was drying and shriveling gradually in 2-3 days after flowering. There were few germinated pollens observed on surface of stigma of FS-M<sub>1</sub> after hand-pollination. But no pollen tube found inside the style. Nevertheless there were pollen tubes existing inside the style of FS-M<sub>1</sub>, resulting in a normal seed-set when hand-pollination was made after excision of the stigma or part of the style. Results from above indicate that losing function of the papillae cells of FS-M<sub>1</sub> resulting from its abnormal development is possibly a key factor causing the female sterility.

**Key words:** *Brassica napus* L.; Mutant; Female Sterility; Floral Organ; Pistil; Fertilization Ability

## 1 Introduction

Female sterile mutants have been found in many crops, such as wheat, rice, ramie, piuns tabulaeformiscar and so on. Many studies on the biologic characteristic, mechanism, inheritance and applicable capacity of the sterility phenomenon have been performed. In 1996 the female sterile mutant FS-M<sub>1</sub> in *Brassica napus* L was firstly found. Former studies on FS-M<sub>1</sub> showed that pod set ratio of open- pollinated, artificial-pollinated, and constrained self-pollinated was about 3% respectively, and seeds per pod was about 1.3-2.07. Pod set of wild type and pollination by using pollens of mutant was normal. It indicates that pollen of FS-M<sub>1</sub> develops well. In this research we studied floral morphology and pistil fertilization ability of female sterile mutant FS-M<sub>1</sub>.

## 2 Materials and methods

Female sterile mutant FS-M<sub>1</sub> and its wild type "Ningyou 10" were *Brassica napus* L. with yellow seed, low erucic acid. They were bred by Jiangsu Academy of Agricultural sciences. In autumn of 2003 these materials were planted in nursery garden. Plots of 2 rows and 3m length with rows spacing of 40cm were used.

Floral morphology study: Choose the bud of FS-M<sub>1</sub> and Ningyou 10 before it flowers at flowing time. Then observe and photo the ovaries using microscopy (Olympus SZX12).

Embryological study: At flowering time FS-M<sub>1</sub> and Ningyou 10 were artificial-pollinated with pollens of wild type or mutant. Pollinated ovaries were fixated with glutaraldehyde (4%) after being pollinated 4, 8, and 12h later. Whole transparency embryo structure was observed by means of microscopic histological dissection after being treated with Yanglusheng's method. Pollen germination on stigma and pollen tube development inside style were investigated using fluorescence microscopy after being stained with Wangxiaoli's method.

## 3 Results

### 3.1 Floral morphology of FS-M<sub>1</sub>

Floral morphology of FS-M<sub>1</sub> differs in some aspects to that of wild type, Ningyou10 (Fig. 1). Calyx of mutant looks like that of wild type in morphology, so does petal. The 4 longer stamens of FS-M<sub>1</sub> were longer than the 2 shorter stamens, only about 0.8mm, while that discrepancy of wild type reaches to 1.5~2.0mm. Pistil of FS-M<sub>1</sub> was shorter remarkably than stamens in length. No nectar was observed, but there were two extra crescent-like organ developed at the ovary base of FS-M<sub>1</sub>. Stigma end of FS-M<sub>1</sub> shaped a fork-like looking, differs obviously from that of wild type. Pistil length of FS-M<sub>1</sub> was shorter than that of wild type. Ovary shape of FS-M<sub>1</sub> was more flat than that of wild type and ovary length of FS-M<sub>1</sub> was shorter than that of wild type (Fig. 2). Ovules of FS-M<sub>1</sub> developed well. The numbers of ovules of FS-M<sub>1</sub> was same to that of wild type (Fig. 3).

### 3.2 Studies on female sterile mechanism of FS-M<sub>1</sub>

#### 3.2.1 Shape of pre-pollinated papillae cells and development of pollinated papillae cells

Papillae cell of FS-M<sub>1</sub> is plump and erecting on the stigma before flowing. The numbers of papillae cells of FS-M<sub>1</sub> was

sparse and its size was larger than that of wild type (Fig. 4). The papillae cells of FS-M<sub>1</sub> was drying and shriveling gradually in 2-3 days after flowering. On contrary the papillae cells of FS-M<sub>1</sub> was erecting in 7 days after flowering. Number of pollen grains observed on stigma of FS-M<sub>1</sub> was less than that on wild type after washed with NaOH (1%), (Fig. 5).

### 3.2.2 Development of ovaries of FS-M<sub>1</sub> by open-pollination

Ovaries of FS-M<sub>1</sub> halted to develop at the ending of flowering and became yellow and shrinking, falling off at last. Ovaries of wild type became long and wide and developed rapidly by contraries.

### 3.3 Germination of pollens and elongation of pollen tube of FS-M<sub>1</sub>

Part of stigma of FS-M<sub>1</sub> and wild type was excised after artificial-pollinated 4h later and observed with microscope. The result showed that pollens germinated on the surface of stigmas of the FS-M<sub>1</sub> and wild type (Fig. 7). Pollen tube could be seen stabbing into the papillae cells on the stigma of wild type, but this didn't occur on the mutant (Fig. 8).

Stigma of FS-M<sub>1</sub> was excised and pollinated then. Pollens germinated on the surface of excised stigma and pollen tube elongated among style, arriving megaspore at last (Fig. 10). It indicated that function of ovary and megaspore of FS-M<sub>1</sub> was normal.

### 3.4 Seed set of FS-M<sub>1</sub> after excision of stigma

Seed set ratio of FS-M<sub>1</sub> was about 98.5% and seed of per pod was 12.7 when the stigma was excised and hand-pollination was made by its own or wild type pollens. While that of wild type was about 99% and 13.5 respectively. It indicated that stigma and ovule of FS-M<sub>1</sub> developed well.

In a word, losing function of the papillae cells of FS-M<sub>1</sub> resulting from its abnormal development is possibly a key factor causing the female sterility.

## 4 Discussion

Female sterility of FS-M<sub>1</sub> is caused by its abnormal development of pistil which differs from other female sterile mutant. Pollens germinated on the surface of FS-M<sub>1</sub>, but pollen tube couldn't stab the papillae cells on the stigma. Papillae cell of stigma is composed of cutin, wax and protein membrane from outer to inner. Protein membrane composed of protein and lipid captures pollens and take part in hydration and inter-recognition. Papillae cells take important part in pollens germination. Studies by Jensen on pistil of cotton showed that papillae cells decomposed and cytoplasm degenerated, releasing water for pollens germination and aryl compound to induce pollens germinating. Studies on pollination of *nigra* and *oleracea* by electric microscope showed that papillae cells took an important part on the germination and recognition of pollens. On the base of well development of pollens and succeed pollination made by excising stigma, it can be concluded that papillae cells of FS-M<sub>1</sub> accept pollens but lost the function of inducing pollen tubes stabbing into papillae cells and extending among styles. The dry and shriveled papillae cells are a proof of the hypothesis. Thus it can be seen that the losing of special functional genes of papillae cells causes the female sterility of FS-M<sub>1</sub>. We had failed to improve the pods set ratio by using difference concentrations of NaCl, C<sub>6</sub>H<sub>14</sub> and CHCl<sub>3</sub> to saturate the stigma of FS-M<sub>1</sub>. Intensive studies would be performed, such as, the change of chemical substance (protein, enzyme, etc) on the surface of stigma.

Missing function of the specific gene of papillae cells could also be the main cause of the changes of floral morphology of FS-M<sub>1</sub>. It will be testified later that the possibility of the crescent-like organ formed at the ovary basal is resulted from the distortion of the nectars or not. And intensive study on female sterility of FS-M<sub>1</sub> will be performed further.

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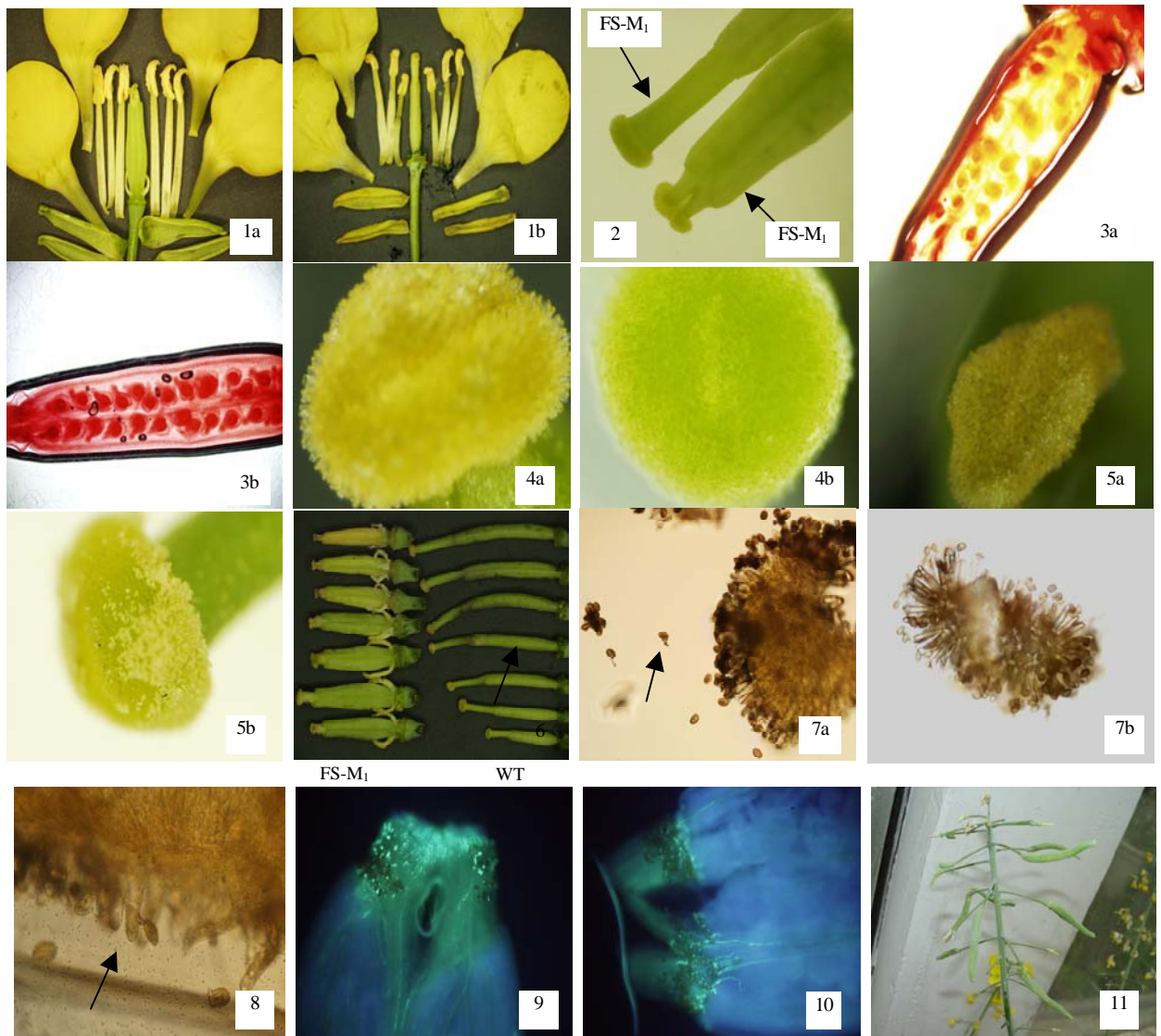


Fig. 1: Floral morphology of FS-M<sub>1</sub> (1a) and Wild type (WT,1b)(70×); Fig. 2: Pistils of FS-M<sub>1</sub> and WT(230×); Fig. 3: Showing a normal ovule developed (after treated with transparent agent) of FS- M<sub>1</sub>(3a) and WT(3b) (270×); Fig 4: Papillae cell. Showing sparse and larger papillae cell of FS- M<sub>1</sub>(4a) compare to WT(4b) (950×); Fig 5. Showing pollen grains adhering to the surface of stigma of FS- M<sub>1</sub> and WT(900×); Fig 6: Showing ovary developed of FS- M<sub>1</sub> and WT after free-pollination at flowering(75×); Fig 7: Showing the germinated pollen grains on stigma of FS- M<sub>1</sub> and WT(600×); Fig 8: Showing germinated pollen grain is stabbing a papillae cell on the stigma of WT. This phenomena not appeared on FS- M<sub>1</sub> (1000×); Fig 9: Showing germinating pollen grains on stigma of FS- M<sub>1</sub> after of style excision and hand-pollination(200×); Fig 10: Showing pollen tube inside the style of FS- M<sub>1</sub> after style excision and hand-pollination(200×); Fig 11. Showing pod-set of FS- M<sub>1</sub> (0.25×).