

# Breeding of high erucic acid recessive genic male sterile line 303AB for industrial purpose

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## Abstract

An interspecific cross between *B. napus* L. (a recessive genic male sterile line) and *B. campestris* var. *purpuraria* L. was done. After selfing or sib-mating for five generations, high erucic plants were selected, and one new recessive genic male sterile (RGMS) line was bred, whose erucic acid content reached 58% with the maximum of over 60%.

**Key words:** *B. napus* L., *B. campestris* var. *purpuraria* L., interspecific cross, high erucic acid, recessive genic male sterile (RGMS)

## Introduction

Erucic acid as a long-chain fatty acid, can not be absorbed and decomposed in human body, and its nutrition value was low. However, erucic acid and its derivative products play important roles in industry, such as steelcasting, lubrication, plastic, paint, ink, cosmetic, and food, etc. Demand for erucic acid is increasing rapidly with the development of world industry<sup>[3]</sup>.

In 1980s, the objectives of improving rapeseed quality focused on the low erucic acid and glucosinolate contents. Breeding and commercial use of many single or double-low varieties accelerated the process of high quality and industrialization of rapeseed. Breeding high erucic acid rapeseed for industrial purpose is the constituent parts of rapeseed industry development. Because we didn't pay enough attention to this, high erucic acid germplasm was lost severely. We are extremely short of breeding materials with the erucic acid content of over 55%. It is necessary to accelerate studies on high erucic rapeseed germplasm, enrich high erucic gene pool, and to provide new high erucic materials with rapeseed breeding. Meanwhile, it is important for breeding high erucic acid rapeseed hybrid varieties that improve fertility and adaptability of high erucic acid rapeseed to strengthen industry competitive power.

Cross breeding is the conventional method of improving erucic acid content of rapeseed. On the one hand, breeding materials with higher erucic acid content were obtained by an interspecific cross. For example, a few varieties such as HERO, Mercury, Neptune, Venus and Castor, bred by Manitoba University of Canada, had erucic acid content of 53.0%–54.1%<sup>[4,5,6,7]</sup>. By an inter-varietal cross, the cultivar Gaojie No.1<sup>[8]</sup>, which had erucic acid content of 60%, was bred by Agriculture Science Academy of Jiangshu. On the other hand, stable high erucic rapeseed varieties can be obtained by a wide cross between *Brassica napus* and *B. campestris* or *B. juncea*. For example, a new *Brassica napus* variety MianYou 13<sup>[9]</sup> was bred by the wide cross between *B. napus* and *B. juncea* in Mianyang institute of Agriculture Science. In this study, we took the method of the wide cross between *B. napus* and *B. campestris*. We crossed a high erucic acid RGMS line with a high erucic acid *B. campestris* to develop high erucic RGMS lines and hence new combinations.

## 1 Materials and methods

### 1.1 Materials

The sterile plants in the RGMS high erucic line Mian 1AB-3 as the maternal (whose erucic acid content was 54.0%), were crossed with *B. campestris* var. *purpuraria* L.(whose erucic acid content was 51.5%).

### 1.2 Experimental method

F<sub>1</sub> progeny from the cross between *Brassica napus* and *Brassica campestris* were treated with 0.1% colchicines and open-pollinated. The plants in F<sub>2</sub> progeny were Individually selfed by bagging. We determined erucic acid content and selected for high erucic acid content each generation on the basis of yield and resistance evaluation. We selected some advanced high erucic plants and segregating sterile plants to do pair-wise sib-mating. We did selfing, sib-mating and erucic acid testing for generations so that the agronomic characteristics, fertility and erucic acid content were stable. Fatty acid composition was determined by gas chromatography according to the agriculture standard NY/T91-1998. Oil content was determined by nuclear magnetic resonance.

## 2 Result and analysis

### 2.1 Breeding procedure of high erucic RGMS

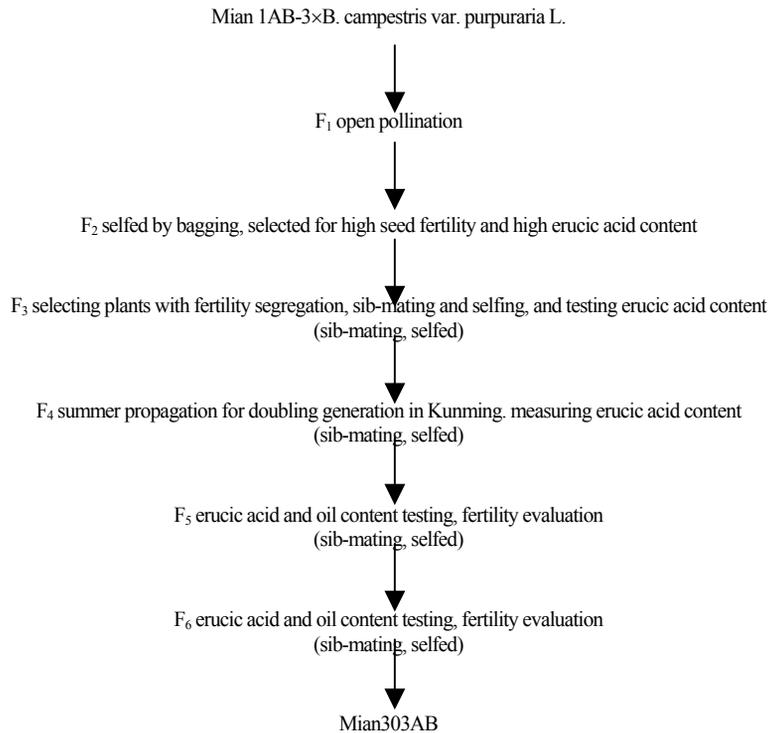


Fig. 1 Breeding procedure of Mian 303AB

Breeding process for Mian 303AB was shown in Fig.1. In spring of 2001, we obtained F<sub>1</sub> progeny from the cross between the sterile plants of high erucic acid RGMS line Mian 1AB-3 and *Brassica campestris* var. *purpuraria* L. and sowed seed in autumn. Seedlings were treated with 0.1% colchicines. In spring of 2002, plants were selfed by bagging but we didn't get the selfed seed because of the abnormal seeds. We harvested the spontaneous pollinated seeds from the F<sub>2</sub> progeny and sowed them in autumn. In spring of 2003, we selected plants which had normal flower and plant-leaf type were inclined to that of *Brassica napus*, to do selfing by bagging. Erucic acid content of individual plant seed well were measured. We selected 4 individual plants, which seed well and had 58% erucic acid content, to sow in autumn. In spring of 2004, we investigated and found in the field in the initial flowering that, a individual plant fertility were segregated, and the sterile plants rate were 8.33%. Then we selected advanced plants to do 3 paired sib-mating and 17 plants selfing, sowed seeds in Kunming in autumn and measured erucic acid content. In spring of 2005, we selected good stable agronomic traits of individual plant with fertility segregation to do paired sib-mating and selfing, and tested erucic acid and oil content after harvest. Individual plant satisfied with our demands were sowed in autumn. In spring of 2006, the breeding lines, which had 50% fertility segregation rate, high oil contents, stable erucic acid content of over 58% and stable agronomic traits, were sib-mated. A GMS line was obtained and named 303AB.

## 2.2 Selecting for economic traits

Because the progeny population segregation from the cross between *Brassica napus* and *Brassica campestris* were complex, we not only selected high erucic acid content, but also selected economic traits. There were a selecting thought for progeny selecting that we attached to selecting seed-fertility plants had abundant and dense pods when attached to selecting short and ramifying-well traits, so as to obtain high erucic acid GMS line with short and ramifying-well plants and abundance and dense pods. The selecting thought was shot through the whole process of progenies selecting. In the medium-grade of soil, the density of 303AB was 120000 plants per hm<sup>2</sup>, the plant height could reach 1.6 to 1.7 m, the first ramification number reached 9.5~12, pods per plant reached 450~500, seed number per pod was 15~18, 1000-seed weight was about 3.5 gram, the erucic acid content reached 58%, oil content reached 40%, oil content of part of materials reached 45%.

## 2.3 Fertility performance

The segregation ratio of sterile plants of sib-mating progenies of Mian 303AB was about 1:1, the segregation ratio of fertile plants of selfing progenies was 3:1, the fertility segregation regulation was the same as that of Mian 1AB-3. We did the testcross between Mian 303AB and 30 high erucic acid lines of *Brassica napus*. The fertility performance was that plants were fertile fully and didn't come about fertility segregation. This above indicated that Mian 303AB was belonged to RGMS line, whose sterile gene originated from Mian 1AB-3.

## 3 Discussion

Taking the technology route of the wide cross between *Brassica napus* and *Brassica campestris*, we bred high erucic acid RGMS line Mian 303AB, which had 58% of erucic acid content, especially, the maximum value reached 61.87%. It was

proved that it was effective to breed high erucic acid *Brassica napus*, and feasible to bred high erucic acid RGMS line. We need to further study the reason of improving erucic acid content of *Brassica napus*. It is possible that the erucic acid gene of *Brassica campestris* was integrated into *Brassica napus*, or micro-effective multi-gene were recombined.

Study has been proved that there were at least 5 erucic acid allele genes in *Brassica campestris* L. and *Brassica napus* L., that was e, E<sup>a</sup>, E<sup>b</sup>, E<sup>c</sup>, E<sup>d</sup>, controlled 0, 10%, 15%, 30% and 3.5% of erucic acid content, respectively<sup>[10,11]</sup>. High erucic acid mutation materials were found in different species of *Brassica* genus. Erucic acid content of one E gene reached over 16%<sup>[12]</sup>. It is the important way for improving erucic acid content of *Brassica napus* that how to obtain E gene controlled higher erucic acid content and transfer it into existing high erucic acid *Brassica napus*.

Qicunkou thought that erucic acid content of *Brassica campestris* had distinct differences between varieties and regions in our country. Oil cabbage in winter rapeseed region of the South, had the erucic acid content of 50%, and was stable between varieties, its genotype was E<sup>c</sup> E<sup>c</sup>, but had differences between varieties. Chinese cabbage in spring rapeseed region of the North, had the erucic acid content of 30%, its genotype was E<sup>b</sup> E<sup>b</sup>. It is very important to make full use of rich *Brassica campestris* resources in the south for breeding the high erucic acid rapeseed varieties<sup>[13]</sup>.

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