Developing high-oil content homozygous two-type line 20228AB of recessive genic male sterility in *Brasscia napus* L.

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Introduction

Rapeseed (*Brassica napus* L.) was one of the most important oil crops widely planted, which plays an increasingly important role in the oil crop market in China. A primary objective in the breeding of oilseed *Brassica napus* is to increase the oil yield per unit area, which is determined by the seed yield potential and oil content in the seeds. Over the past twenty years, average unit yield of winter rape increased from 1000-1200 kg/hm^2 to 1700 kg/hm^2, and many high-yielding hybrid varieties even could reach 3700 kg/hm^2. Compared with rapid increase of the yield, oil content in seed just only increased 3-4 percent, and was about 40%. So, increasing oil content to enhance oil yield per unit area is the main objective of rapeseed breeding.

A recessive genic male sterility (RGMS) system, 20118AB, has been developed from DH (doubled haploid) population in *Brassica napus* canola variety line. Genetic analysis indicated that was controlled by two recessive sterile genes (*a* and *b*) interacting with one inhibitor gene (*rf*), and the later was epistatic to the former two genes controlling male sterility and made the fertility normal. 20118AB line is composed of 50% male sterile plants (i.e. 20118A, Genotype: *aabbRfRf*) and 50% male fertile plants (i.e. 20118B, Genotype: *AabbRfRf* or *aaBbRfRf*), and maintained by harvesting the progeny from 20118A plants through sib-mating (i.e. 20118A male sterile plants pollinated by 20118B male fertile plants). Full male sterile group (Genotype: *aabbRfrf*) could be obtained from a cross (20118A×male fertile plants with the genotype *aabbrfrf*), which has a broad spectrum of restoration with almost all normal rapeseed lines restoring the fertility of F_1 hybrids. So, 20118A could be used to production of hybrids by sterility line, temporary maintainer line and restore line. Hybrid combinations with high heterosis were easy to be obtained because of the wide spectrum of restorers. Several hybrid cultivars, such as Huyouza No. 1, Xiangnong 03 and Huyouza No.4, have been registered in China recently, and widely planted in the middle and lower reaches of the Yangtze River.

By germplasm innovation, many lines in *Brassica napus* with oil content 45-50% had been developed, which will provide possibility to develop high-oil content hybrid parents for increasing oil content of hybrid cultivars. In the paper, a novel homozygous two-type line 20228AB with high oil content had been developed from 20118AB with conventional breeding method.

Materials and Methods

Plant materials: The RGMS line 20118AB of *Brassica napus* was used as material, 20118AB line with oil content 40.36% has been maintained by full sib-mating (20118A×20118B) for 8 generations; Donor material is conventional rapeseed (*Brassica napus.* L) cultivar Huyou 15 approved by nation, oil content 44.56%; The temporary maintainer line M-6477 (Genotype: *aabbrfrf*) is to use production of full sterile line for hybrid combination, oil content 47. 64%。

Fatty acid composition and glucosinolate content analysis: Glucosinolate contents of the population were evaluated by NIRs (Bruker, Vector 22/N, Uppsala, Sweden), and fatty acid composition was analysed by gas chromatography (HP6890).

Analysis of morphology: Growth feature, leaf shape, leaf color, plant appearance, the lenth of pods, seed coat color, etc. be observed

Yield determination: The yield of two hybrid combinations are from statistical analysis of Shanghai regional rape test in 2009 based on multi-site tests.

Results

Development of high-oil content of homozygous two-type line 20228AB

A cross (20118B×Huyou 15) was made and selfed, and then the male sterile plants from F_2 population was backcrossed with Huyou 15, and selfed. By quality analysis, the male sterile plants from the generations with high-oil content were continuously backcrossed with Huyou 15 four times by BCF_2 population. The male fertile plants from the generations with high-oil content was selfed, 2 among 8 genotypes showed 3:1 segregation ratio, one genotype 16:1, and the others full fertility. Sib-mate was made between the male sterile and male fertile plant from the segregation population. The generations could show 3:1 or 1:1 segregation ratio, the group from sib-mating showing 1:1segregation ratio could be a new homozygous two-type line (fig.1).

The morphology and quality traits of homozygous two-type line 20228AB

The agronomic characters of 20228AB were similar to Huyou 15's because of continuous backcross. Plants are semi-upright or creeping growth in seedling stage. The color of leaf and stalk are green, flower yellow. The color of seed is black and brown. Plant high is about 160 cm. Plants have over 8 branchs at podding stage.

By the analysis of quality, oil content of 20228AB is 47.3%, erucic acid content 0.17% and glucosinolate content 20.34μmol/g.

Utility of homozygous two-type line 20228AB

Two hybrid combinations (Huyouza No.7 and Huyouza No.8) were developed with homozygous two-type line 20228AB in 2007(Fig.2). The yield of Huyouza No.7 increased 8.9% than Huyou 15(CK) in 2009 Shanghai rapeseed regional test, and Huyouza No.8 20.5%.

By the analysis of quality, oil content of Huyouza No.7 is 47.76%, and Huyou No.8 50.12%, their erucic acid content and glucosinolate content conform to the national double low standard. So, it was believed to have the potential to produce more commercial hybrids because of 20228AB with the high general combining ability and high-oil content (Table 1).

Discussion

A key problem in utilizing heterosis in rapeseed breeding is that of producing hybrid seeds economically. A recessive genic male sterility (RGMS) system 20118AB has some advantages, such as stable and complete male sterility, rich sources of cytoplasm, and high yield of producing hybrid seeds. So, more attention has been paid on it. However, it is difficult to develop new homozygous two-type line and temporary maintainer line by the conventional breeding method because of the male sterility controlled by three genes.

To obtain high-oil content homozygous two-type line, an effective backcross was studied in this paper. The results showed that it was feasible to develop high-oil content homozygous two-type line by the continuous backcross of BCF_2 population. The male sterility of 20118AB was controlled by three genes, and oil content of seed by quantitative trait genes, so sample size for segregation population should be properly increased in order to obtain the objective. By the analysis of quality, high-oil content homozygous two-type line could be effectively obtained through increasing the pressure of choice for oil content. Oil content was controlled by nuclear gene, and yet affected by cytoplasmic gene, so genetic effects of cytoplasmic gene were noted in the course of backcross.

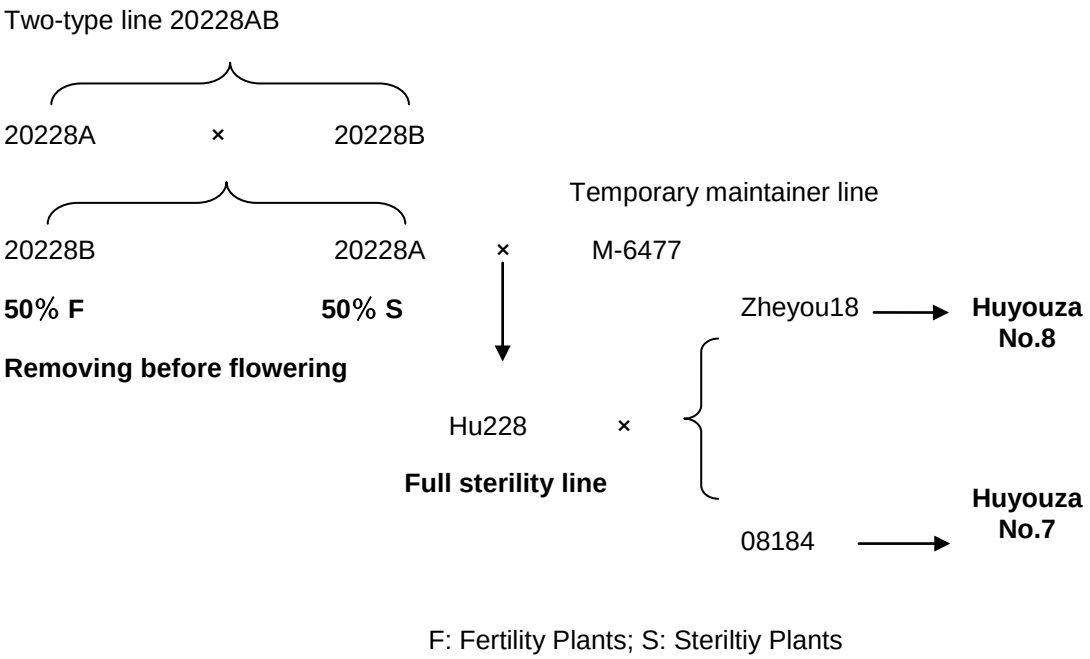
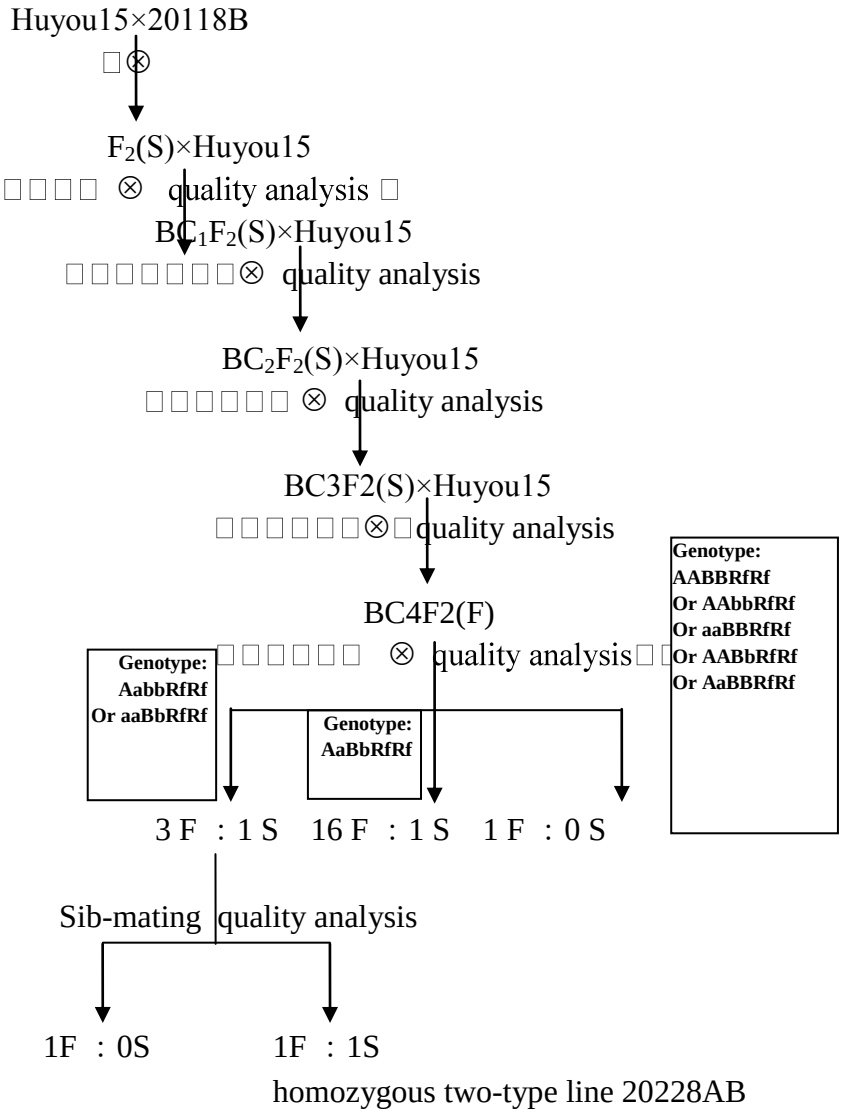


Fig.2 Breeding of two hybrid combinations by using 20228AB as sterility line



F:Fertility Plants; S: Steriltiy Plants

Fig.1 Breeding of homozygous two-type line 20228AB of recessive genic male sterility in *Brassica napus* L.

Table 1 The behaviors of Huyou No.7 and Huyou No.8 in 2009 Shanghai rapeseed regional test

Cultivars	yield (kg/hm^2)	more than CK (%)	Erucic acid content (%)	Glucosinolate (μmol/g)	Oil content (%)
Huyou No.7	2622.0	8.9	0	21.75	47.76
Huyou No.8	2902.5	20.5	0.01	18.69	50.12