Reasearch on creating new germplasm of high oil content in *B. napus*

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Abstract

Microspore culture of four (F_1) hybrid lines of *B.napus* was made and 1003 DH plantlets were obtained. By selection of 2 successive years for oil content, 16 new accessions were obtained whose oil content varied from 45% to 50.5% with adequate traits, days to flowering and maturing. This result suggested that microspore culture or doubled-haploid breeding would play an important role in creating new germplasm of *B.napus*, especially in polygenic traits such as oil content.

Key words: Microspore culture, *Brassica napus*, Oil content

Introduction

The rapeseed (*B. napus*) is now one of the most important sources of edible vegetable oil for China. The total planting acreage, total production and yield have increased significantly since high quality rapeseed breeding initiated in China in early 1980s. The seed oil content, a key quality trait has not been emphasized by most of rapeseed breeders because of the demanding need of reducing the contents of glucosinolate and erucic acid in the seeds (Liu, 1992). In fact, breeding for higher oil content is an important way to increase rapeseed economic value and farmer's income by increasing oil production in their limited land.

Oil content of dry seed weight of rapeseed cultivars in China is 38-42% which is lower than that of Australia and Europe cultivars ranging from 42% to 44%. There are several reasons that lead to the low oil content in China. First, high oil content is not included in the breeding goals. Second, most of the breeding programs do not have simple, fast, and accurate methods to determine oil content. Third, high oil content germplasms are rare for the most of breeding programs.

The technique of microspore culture is very effective for crop improvement and variety development, especially in rapeseed. Double haploid breeding and microspore culture have been well developed and utilized in plant breeding programs (Li and Guan, 1994; Lichter, 1982; Simmonds and Keller, 1999). Many oilseed rape cultivars were developed by microspore culture in the world, but reports on using microspore culture to develop high oil content oilseed rape have not seen. In present study, a set of high oil content DH rapeseed lines were obtained through microspore culture of F_1 plants and single plant selection for two consecutive years.

Materials and Methods

Four single crosses were made between four parents with different oil content in the spring of 2000. They were Huayou3/Legency; Huayou3/Stellar, Huayou6/Legency and Huayou6/Stellar in 2000. DH cultivars Huayou3 and Huayou6 were developed by anther culture by the rapeseed research center of the Institute of Cash Crops, YAAS, China. Their oil contents were 43.0% and 42.5% respectively. Legency and Stellar were spring rapeseed cultivars from Canada. They are late maturity cultivars in Yunnan environment comparing to local cultivars. Four hybrids were planted in the experimental field of YAAS and their flower buds were used in microspore culture in the fall of 2000.

The protocol of microspore culture was based on the previous reported method (Cun et al., 2003). For microspore isolation, 20 flower buds with a size of 3-4mm were collected from the field at 8-10am. Those flower buds were surface sterilized with commercial 20% sodium hypochlorite for 10-15 minutes and washed three times with distill water. The flower buds were then smashed with 10-50 ml glass injectors in filter-sterilized 0.5ml B5-13 medium (B5 medium with 13.0% sucrose and buffered at pH5.8). The solution was filtered through a 46-um nylon mesh into a centrifuge tube. The crude microspore suspension was centrifuged to select microspores at late single-nuclear and early double-nuclear stages. The microspores were washed with B5-13 medium three times and NLN-13 medium (NLN medium with 13.0% sucrose and buffered at pH6.0) one time. The microspores were re-suspended in 4 ml NLN-13 medium supplemented with 300-500 mg/L colchicine and 0.5 g/L activated charcoal, and incubated in the dark at 31 °C for 24-30 hours.

The microspores were washed with NLN-13 medium to wipe off colchicine, divided into 6 Petri dishes, and incubated in the dark at 31 °C for additional 4-10 days until the clusters of 4-10 cells could be seen under microscope. The Petri dishes were incubated in the dark at 25 °C until torpedo embryos were developed. The embryos were transferred to B5-13 medium supplemented with 1μmol ABA and incubated in the light at 17-23 °C for 15-25 days. Mature embryos were transferred to B5-1 solid medium (B5 medium with 1% sucrose and 1% agar) and allowing the formation of seedlings. Plantlets were transferred to greenhouse 25-35 days later, and then transplanted in the experimental field in 15-25 days. DH seeds were harvested separately.

DH seeds were sowed in the experimental field in Jingling, Kunming in October, 2001. Each family was sowed in 6 rows with 6 hills per row. The plants were sinned to 2 plants per hill at the 5-leave stage. More than 36 plants were kept for each family. One to five plants of every family with uniform agronomic traits were selfed with pollen bags and seeds from individual plants were harvested in bulk. The seed oil contents were tested and those with high oil content were advanced to next round selection.

The oil content test followed method described in Guan's book (Guan, 1985). The seeds from individual plants were cleaned. 10 g seeds were used in the analysis. Each sample tested three times. Two similar results from three results were averaged to get the oil content of the sample.

Results

Microspore culture and DH plant development

1500 embryos were obtained from the four crosses in January to March in 2001. 1003 plants geminated from those embryos including 405 plants from the cross Huayou3/Legency, 214 plants from the cross Huayou3/Stellar, 321 plants from the cross Huayou6/Legency and 63 plants from the cross Huayou3/stellar. Total of 692 plants were harvested, including 313 plants from the cross Huayou3/Legency, 156 plants from the cross Huayou3/Stellar, 178 plants from the cross Huayou6/Legency and 45 plants from the cross Huayou3/stellar. The results showed that the embryo inducing rates in microspore culture and embryo germination rates were different among four crosses which indicated significant genotype effects on the efficiency of microspore culture and embryo germination (Table 1).

Table 1 Regeneration of embryos derived from in vitro microspores of *B.napus* (2001)

Crosses	No. total of embryo	ratio of embryogenesis	Regenerating plants	single plants
Huayou3/Legency	540	75	405	313
Huayou3/Stellar	345	62	214	156
Huayou6/Legency	510	63	321	178
Huayou6/Stellar	105	60	63	45
Total	1500		1003	692

Selection for high oil content germplasm

2000 individual plants/families were harvested in May, 2002. 200 lines with the life cycle around 180 days and advisable economic traits were selected. The oil contents of 16 of these lines were above 45% with a highest one of 48.1%.

Table2 Oil content of single plants(2002)

Coding	Resourse	Oil content	Coding	Resourse	Oil content
33-8	Huayou3/Legency	47.61	45-14	Huayou3/Legency	45.41
33-39	Huayou3/Legency	47.49	45-25	Huayou6/Legency	45.74
33-57	Huayou3/Legency	45.46	45-57	Huayou6/Legency	46.17
33-62	Huayou3/Legency	45.57	45-78	Huayou6/Legency	45.75
33-65	Huayou3/Legency	47.95	45-82	Huayou6/Legency	46.47
33-79	Huayou3/Legency	45.74	45-87	Huayou6/Legency	47.89
43-19	Huayou3/ Stellar	46.44	45-105	Huayou6/Legency	48.10
43-28	Huayou3/Stellar	47.26	45-163	Huayou6/Legency	47.48
Control 1	Huayou3	40.60	Control 2	Huayou6	41.20

The oil contents of 15 selected lines among 16 lines from 2002 were increased by 0.12%-2.42%. The oil contents of three lines among 16 lines were higher than 49% with highest one of 50.52% (Table 3).The increasing oil content in the selected lines indicated that it is highly possible to increase oil content of rapeseed by making a cross between high oil content lines, fixing desirable gene combination with microspore culture, and continuously selecting for high oil content individuals.

The 16 lines of above 45% oil content from 2002 were evaluated in two locations, Jiangchuan and Jinning of Yunnan province in 2003. Selections were applied among and within families based on oil content, economic traits and their uniformity. Seeds from high oil production area Jiangchuan had consistent higher oil content comparing to Jinning. This result indicated that the oil contents of these lines were stably inherited.

Discussion

Rapeseed oil is a traditional cooking and industrial oil. Due to increasing price of oil price and decreasing of fossil energy resources, rapeseed oil as a potential biodiesel material has drown widely attention for many countries. As a result, increasing rapeseed oil content became a major breeding goal for rapeseed breeding programs. Traditional methods for high oil content rapeseed breeding were through pedigree selection, hybrid breeding, yellow-seed selection and mutation breeding etc (zhou, 1992). It takes many years to develop a high oil content rapeseed cultivar by using these methods.

Table 3 Oil content of single plants grown in different regions (2003)

Coding	Oil content(Jinning)	Oil content(JiangChuan)	Coding	Oil content(Jinning)	Oilcontent(Jiangchuan)
33-8	48.20	48.32	45-14	47.29	48.21
33-39	48.61	49.15	45-25	45.19	46.32
33-57	46.31	47.05	45-57	46.29	46.23
33-62	47.10	47.87	45-78	47.24	47.98
33-65	49.72	49.88	45-82	47.15	48.28
33-79	47.25	48.45	45-87	49.43	50.02
43-19	46.62	47.88	45-105	50.52	50.85
43-28	48.87	49.82	45-163	48.37	49.65
Huayou3	40.64	42.23	Huayou6	41.18	42.87

In present study, four single crosses were made between four genotypes, microspore were obtained from those F_1 plants and cultured to form DH plants, DH plants were selfed to produce seeds, oil content of seeds from individual DH plant was analyzed, high oil content germplasms were obtained within three year. This method of breeding for high oil content is of high efficiency. The results of present study showed that the frequency of producing high oil content individuals was highly related to their parent genotypes. Among 16 lines with above 45% oil content, 8 of them are from the cross Huayou3/Legency, 7 of them are from the cross Huayou6/Legency. One of them are from the cross Huayou3/Stellar, and none of them is from the cross Huayou6/Stellar. This indicated that the variation of oil content in the progeny was highly correlated with its genetic background. In the 16 lines with above 45% oil content, seeds harvested from Jinning having higher oil content also produced higher oil in Jiangchuan. This indicates that the heterosis of the high oil content can be fixed in the progeny of a hybrid of different genetic background materials. This can be achieved using gamete level selection through microspore culture and DH haploid breeding.

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