Research and Commercial Application of the Complete Dominance Yellow-seeded Gene in Brassica Napus L.

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ABSTRACT

A lot of materials of yellow-seeded B. napus have been bred from the different genetic sources since 1982. Among them one yellow-seeded hybrid *YuHuang No 1* have been registered in 2001, the yellow-seeded ratio of the variety is almost 100% and the yellow-seeded degree is above 80%, its seeds have thinner seedcoat, lower cellulose and polyphenol content, higher oil (>44%)and cake protein (>49%) content. It will be planted 200,000 ha in autumn of 2002.

A series of experiments have been done from 1996 to 2002. The preliminary results showed: •the different yellow-seeded genes exist in the different yellow-seeded lines, these genes showed dominance, partial dominance and recessive; •the seed color is a quality-quantitative character;•one complete dominant yellow-seeded gene have been found; •the melanin might be the main factor to influence the color of seedcoat and the lower lignin content might be the reason for the lower hull percentage in yellow-seeded; •one mutation line with yellow seed and low glucosinolate had been found from B. oleracea var. aceaphala and some new yellow-seeded B. napus lines with yellow-seeded genes both in AA and CC genome have been gotten;•one different DNA segment (661bp) raleted with yellow-seeded gene have been cloned and the sequence been analysed by the differential display reverse transcription PCR.

Key words: complete dominant yellow-seeded gene – *YuHuang No 1* – melanin – B. oleracea var. aceaphala –DNA segment

INTRODUCTION

The breeding and study on yellow-seeded B. napus began in 1975 (Liu,1979) in China. In 1990's five varieties of yellow-seeded B.napus had been registered in China, some had been used in production. From 1982 we began the breeding of yellow-seeded rapeseed (*B.napus* L.). Now we have gotten 16 yellow-seeded lines of Brassica napus L. from the different genetic sources. Among them, GH01 ([(Tower×81008)×81008]×Fei-yie which derived from *B.napus*×B.juncea) and GH03 (irradiated progenies of 84-57N-9 (B. napus)) are best. The hybrid Yuhuang No. 1 of GH01 crossed with P54 (black-seeded line) have better yield characters and uniform growth, the seeds are bright yellow. Its oil content is 42.5-44.5% and cake protein content is 49.1%; the seedcoat ratio(14.13%), seedcoat cellulose(32.9%) and tannin content (47.26mg/g) are lower 22.6%, 42.5% and 42.1% than black-seeded check variety respect respectively. The seed yield of Yuhuang No.1 were 2100kg/ha.—2369kg/ha. in the varieties region experiment and in the production experiment. The variety have been registered in 2001, and have been harvested 200,000ha. at more than 100 places in 18 provinces in 2003.

The genetic rule and physiological mechanisms on the seed color of yellow-seeded rapeseed have been studied in the paper.

MATERIALS AND METHODS

•16 yellow-seeded rapeseed lines (*B. napus* L.) and 20 black-seeded lines from different genetic sources were used to analyze the genetic rules and diversity of testa pigments content, oil and protein content, and RAPD markers. These yellow-seeded lines were bred from *B.napus*×yellow-seeded *B.juncea; B.napus*×yellow-seeded *B.rapa*; the mutant of the irradiated black-seeded B. napus; *B.napus*×yellow-seeded *B.carinata*; (yellow-seeded *B.campestris*×yellow-seeded *B.oleracea)* ×*B.napus*. •three pairs of different genetic near-isogenic lines of black- and yellow-seeded *B.napus* were used in the study on the dynamic changes in the different developmental stages of embryo about the contents of four types of pigments, the activity of their related enzymes including polyphenol oxidase (PPO), phenylalanin ammonia- lyase (PAL) and tryosinase,

and the contents of related amino acid, two of them were used to obtain cDNA fragment related to the *B. napus* seed color. • a new yellow-seeded and low slucosinolate gene source in B. oleracea var. aceaphala had been used to compose new yellow- seeded B.napus lines.

RESULTS

1) **Yellow-seeded material creating**. The three approaches were applied in breeding yellow-seeded materials in last ten years: •Composite crossing between the yellow-seeded lines which came from different genetic sources; •some lines with better yellow seeds and better yield were planted together, let them cross each other by open-pollinated, after two years, select better plants inbred again, the pedigree select was used in last generations; • In 1992, we found a new yellow-seeded and low slucosinolate gene source in B. oleracea var. aceaphala and crossed it with yellow-seeded B. rapa•embryo tissue culture•double chromosome and got regenerative plants B. napus with yellow seeds•crossed with another yellow-seeded B.napus•microspore culture for pollen of F1•double chromosome•a new line with yellow-seeded genes from A and C genome. Now we have gotten more than 1000 inbreeding yellow-seeded lines (B.napus), they are of high yield, or high oil, and/or high protein, or bright yellow seed, some of them are canola. We have bred some black- and yellow- seeded near-isogenic lines also.

2) **Genetics on yellow-seeded genes.** •the F_0, F_1 and F_2 of 7×7 diallal cross between seven yellow-seeded lines and the F_0, F_1 and F_2 of 6×7 incomplete diallal cross between yellow- and black-seeded lines (*B. napus L.*) had been observed, the different yellow-seeded genes exist in the different yellow-seeded lines, these genes showed dominance, partial dominance and recessive; •most of the F1 of GH01, from *B.napus*×*B.juncea*, crossed with 30 black-seeded lines showed yellow seeds, especially in the F1 crossed with black-seeded lines A220,T25,T44,P54, the seeds were bright yellow just like their parent GH01. Among 515 plants of F2 of GH01×A220, 371 plants were yellow and 144 plants black, which suited the model of one complete dominance gene. However, among yellow-seeded plants, the color showed continuate variety, which means there are some modified genes affect the seed color, the seed color is a quality-quantitative character.

3) **Study on mechanisms for the synthesis of pigments**. During the early and mid stages in seed development the contents of polyphenols, flavonoid and anthocyanin in black seeds were much higher than those of yellow ones. However, in the late stage only the content of melanin in black seeds was more than 6 times higher than that of yellow one, and the differences in lignin content and hull percentage between yellow- and black-seeded lines was up to highly significant level. It was indicated that the mature seed color was mainly determined by melanin, and the lower lignin content might be the reason for the lower hull percentage in yellow-seeded lines. The changes in the activity of PPO, PAL and tyrosinase were similar to polyphenols, anthocyanin and melanin, respectively. It was speculated that the synthesis of pigments would be influenced by the activity of their related enzymes. Results also indicated that the contents of total phenylalanine and tyrosine in black- seeded were higher than that in yellow ones, but free amino acid contents were lower than that in the latter. According to the experimental results, it was speculated that the free tyrosine and the first three types of pigments contributed to the increase of melanin content in mature seeds.

4) **Study on the molecule biology of yellow-seeded genes** •Based on 97 RAPD markers which were amplified with 21 random primers the fourteen yellow-seeded lines could put into 2 clusters corresponding to genome difference. • Using mRNA differential display technique, a repetitive and steady differential cDNA fragment had been obtained after the screen of 90 pairs of random primer and anchor primer combination. Designed two nesting primers and extended the fragment by PCR-Walking, a cDNA fragment of 661bp was cloned and sequenced, it is a function unknown cDNA fragment related to the seed color of *B.napus* after the sequence analysis. Here is the sequence:

ATTCGGTAGGATCCCGCAGAACGAGGCCAGGATCCTGGCCGTCCAAGACGCGTATCAATT
GTGAACTTTTGAGTTTTCTCTCCCTCTCATCACCTCCCCTTCCTTATATGATTGAATACGCT
TCACATTGAATAGCACACAAAGTAATAAAAAAACACATGATAGAGATGATTAGGTTTACAGT
ATTATCATTCTTTGTTGTTTTCCTTCTCTTATTCGCTTGCAATGAGTCTTCGGCTAAGACTG
CTAAGTATGATAAGTCAGATGAGTCGGACGAAAACGACGATCTCGCCGCTGTACCGTCAT
GTTGTGGGTTTTCATCGCCTCTTCTGATCAAGAAAGATCAATGGAAACCAATCTTCGCGAA
CAAGTTCGGACAGATCTCAACCGTTCAAATCGGCGATGGATGCGGCGGGATGGGCCCTTA

CAAAATACATTCCATAACGCTGGAACCAAACGCGCTTATGCTCCCTCTTCTTCTTCATTCCG
ACATGGTCTTCTTTGTCGACTCTGGTACATATCTATAATCTGCTTCAGTGTCATGTATTCAG
GGTCGGATCATAGACTTTGACACCTAAATTTTTAAGAAGTAATGTATAGTTTTTTTTCTGAAA
ACCAAGTAATTTATATAGTTATCGTTTACCAAAAAAAAAAAAAGCTT

DISCUSSION AND CONCLUSION

•A material with yellow-seeded genes of complete dominance has been found and applied commercially; •the seed color is a quality-quantitative character; •the melanin and lignin might be the main factor to influence the color and hull percentage of seeds respectively; •some new yellow-seeded genes in CC genome have been gotten from one mutation B. oleracea var. aceaphala and been transferred to yellow-seeded B. napus lines; •one cDNA segment (661bp) related with yellow-seeded genes have been cloned and sequenced.

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