Development of novel yellow-seeded *Brassica napus* germplasm through interspecific Cross *B. juncea* × *B. napus*[1](G3)

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Abstract

The yellow seeded trait is a challenge of the rapeseed breeding at present because of lack of yellow-seeded germplasm in *Brassica napus*. The yellow-seeded *B. juncea* landrace Sichuan Yellow as the gene donor was crossed with the double-low *B. napus* cultivar and the yellow-seeded plants were found in the BC_1F_2 population. The novel yellow-seeded *B. napus* lines with over 46% oil content have been developed after successive selfing of four generations.

Key words: *Brassica napus*; *Brassica juncea*; Seed coat color; Interspecific cross; Germplasm

Introduction

As in other angiosperms, fertilization in *Brassica* results in the formation of the seeds from the ovules. This remarkable transformation involves the activation and coordination of the distinct developmental pathways leading to an embryo, endosperm and seed coat. The seed coat (testa) consists of several layers of specialized maternal cell types in *Brassica* (Wan et al, 2002). Differentiation of the seed coat from the ovule integuments includes some of the most dramatic cellular changes observed during seed development.

Compared with black seeds, yellow seeds of *Brassica* have a significantly thinner seed coat, thereby leading to a lower hull proportion in the seed and, consequently, higher oil content. Some other advantages of yellow seeds include more transparent oil, and higher protein and lower fiber content of the meal (Rakow & Raney, 2003). *Brassica napus* is worldwide the most important oilseed crop species. However, no yellow-seeded forms were discovered in natural germplasm of *B. napus*. It is an important objective for rapeseed breeders to develop yellow-seeded cultivars for *B. napus*.

Majority of the Chinese *B. juncea* accessions are yellow-seeded while there are brown-seeded *B. juncea* accessions. Some studies on inheritance of seed coat color in *B. juncea* carried out in Europe and in India revealed that the brown seed coat in *B. juncea* is controlled by two independent segregating dominant genes with duplicate effect (Liu, 2000; Vera et al, 1982; Yan, 2004) and the yellow seeds will be produced when both the loci are in a homozygous recessive condition, and the maternal genotype influences the expression of the trait. The two loci were located at A and B genomes of *B. juncea* (Liu et al, 1997). These genetic characteristics and difficulties in breeding rapeseed through the interspecific cross of *B. juncea* with *B. napus* (Liu et al, 2001, 2002, 2004; Rashid et al, 1994) make it difficult to transfer the yellow seed coat trait from *B. juncea* into *B. napus.* However, we found two plants bearing yellow or mottled seeds in a BC_1F_2 progeny derived from the high oil interspecific cross yellow-seeded *B. juncea* × *B. napus* and further developed yellow-seeded *B. napus* lines with content and double-low or low-erucic quality.

Material and Methods

Plant materials The yellow-seeded *Brassica juncea* inbred was developed by successively selfing a landrace Sichuan Yellow for seven generations. The black-seeded *B. napus* inbred 1047, which is glossy from the fourth-leaf stage on, was kindly provided by Dr. Mou Jianguo at The Second Agricultural Institute of Chengdu. A inbred line of the Canadian spring rapeseed cultivar Stellar produced the mottled seeds when grown in Changsha.

Breeding procedure The Sichuan Yellow inbred was crossed with the Stellar inbred as the male parent in a greenhouse. The resultant F_1 plants, which were highly sterile, were repeatedly pollinated with pollen of the inbred 1047. The harvested BC_1F_1 seeds were grown in a field and produced 46 plants which were all glossy. These BC_1F_1 plants were self-pollinated by bagging. The BC_1F_2 progeny, grown in a field, segregated with 178 glossy and 172 waxy plants. One yellow-seeded plant was found from each of two types of plants different in glossiness. The seeds of these yellow-seeded plants were showed by the near-infrared analysis to have intermediate contents of erucic acid and glucosinolates (Table 1). The seeds from both yellow-seeded plants were sown, but only the seeds from the waxy plant produced 25 BC_1F_3 plants from which 12 yellow-seeded plants were selected. The seeds harvested from these yellow-seeded individual plants were separately determined for contents of oil, fatty acids and glucosinolates. The bright yellow seeds were chosen by hand for growing into twelve BC_1F_4 families. The total and yellow-seeded plants were counted in each family and percentage of yellow-seeded plants calculated. The bright

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yellow seeds of the individual plants selected from two families with highest percentage of yellow-seeded plants were chosen by hand for growing into BC_1F_5 families.

Quality analysis The seeds harvested in May, Changsha were used for quality analysis by the near-infrared method using NIRS 3700 analyzer (FOSS).

Results

Seed quality of the parents ant their progenies at early generations As shown in Table 1, the Sichuan Yellow inbred had a high oil content of 44.00% while the double-low Stellar inbred had a poor performance with oil content of 35.42% when grown as a winter rapeseed in Changsha. The glossy inbred 1047 was intermediate in oil content between the parents used in the original cross. The BC_1F_2 progeny segregated remarkably in erucic acid and glucosinolates contents. The BC_1F_3 progeny derived from the waxy yellow-seeded BC_1F_2 plant varied in seed coat color and contents of oil, erucic acid and glucosinolates from which 12 plants were selected for advancing to next generation with high oil content, low erucic acid content and relatively low glucosinolates content.

Table 1. Seed quality in the parents and the yellow-seeded progenies from the cross *B. juncea* × *B. napus*

generation	No. plants determined	Glucosinolates content(μmol/g)	Erucic acid content (%)	Oil content (%)
Sichuan Yellow	3	87.77	45.73	44.00
Stellar	3	22.31	1.61	35.42
1047	3	66.76	0.00	39.86
BC_1F_2	1	61.82	8.17	39.65
BC_1F_3	12	54.88-126.26	4.77-23.24	35.55-44.72

Percentage and seed quality of the yellow-seeded plants in the BC_1F_4 families The bright yellow seeds of the 12 plants selected in the BC_1F_3 progeny were separately grown in plots, forming 12 families which varied remarkably in agronomic characters. The plants were individually scored foe seed coat color at maturity. The results showed that all plants produced yellow seeds in one of BC_1F_4 families (No. 12) although no yellow-seeded plants appeared in another BC_1F_4 family (No. 1). The yellow-seeded plants percentage varied from 0% to 100% between the BC_1F_4 families (Table 2). The determination of the seeds harvested from the plants producing plump, bright yellow seeds showed that while the families No. 4 and 7 were low in erucic acid content and generally low in oil content the two highest-percentage families No.8 and 12 segregated in erucic acid content and produced bright yellow-seeded plants with high oil content in seeds (up to 45.91%) and low erucic acid content in oil (Table 2).

Table 2. Percentage and seed quality of the yellow-seeded plants in the BC_1F_4 families

Family No.	Number of yellow-seeded plants	Percentage of yellow-seeded plants	Seed quality of the yellow-seeded plants			
			Number of plants analyzed	Glucosinolates content(μmol/g)	Erucic acid content (%)	Oil content (%)
1	0	0.0	0			
2	17	70.8	10	71.60-85.04	2.17-26.05	37.72-42.34
3	3	25.0	3	68.70-79.15	5.01-29.16	41.39-41.51
4	4	25.0	4	60.26-70.59	1.87- 2.26	34.53-38.75
5	2	10.0	2	30.17-88.60	22.66-28.16	41.80-43.92
6	9	30.0	5	78.19-99.56	13.43-29.06	35.26-41.86
7	14	60.9	14	49.53-98.50	2.01-12.04	34.65-41.99
8	22	78.6	20	62.79-103.87	2.05-23.02	36.37-45.91
9	2	6.9	2	69.73-106.50	2.39-13.53	42.69-43.32
10	7	46.7	7	69.55-102.72	10.23-27.58	32.57-41.78
11	5	50.0	5	73.34-93.33	8.56-22.84	32.66-40.74
12	10	100.0	7	51.35-93.26	1.70-21.86	36.39-42.68

Percentage and seed quality of the yellow-seeded plants in the BC_1F_5 families The seeds of the pure yellow-seeded plants selected from the 2 highest-percentage BC_1F_4 families were sown together with those of the parental lines of *B. napus*. All the progeny plants were individually scored for seed coat color. Seed quality of the yellow-seeded plants in the BC_1F_5 families and the parental lines were determined. As shown in Table 3, the percentages of yellow-seeded plants of eight BC_1F_5 families derived from the BC_1F_4 family No. 8 varied from 33.3% to 91.4% while those of four BC_1F_5 families from the family No. 12 varied from 62.1% to 100.0%. The percentage of yellow-seeded plants averaged 64.11% and 85.28% over these 8 and 4 BC_1F_5 families, respectively. Comparison of Tables 2 and 3 revealed that the BC_1F_5 families derived from the BC_1F_4 family No. 12 produced higher percentage of yellow-seeded plants than the families derived from the BC_1F_4 family No. 8 as in the previous generation, indicating it is efficient to select for yellow seed coat trait on the basis of percentage of yellow-seeded plants in rapeseed. The yellow-seeded individual plants with oil content of 42%--46% and erucic acid content of less than 3% were selected from such BC_1F_5 families as No. 8-1, 8-2 and 12-1. The seeds (Fig. 1) of the selected plants contained glucosinolates of 43.0-83.7μmol/g which were higher than those of the two *B. napus* parental lines Stellar and 1047.

Table 3. Yellow-seeded plants percentage and seed quality of the BC_1F_5 families derived from the 2 highest-percentage BC_1F_4 families

Family No.	Percentage of yellow-seeded plants(%)	Seed quality of the yellow-seeded plants			
		No plants analyzed	Glucosinolates content(μmol/g)	erucic acid content (%)	oil content (%)
8-1	33.3	8	55.1-83.7	1.4-4.0	30.9-45.9
8-2	50.0	36	43.0-81.6	1.2-4.7	33.3-43.7
8-3	61.5	30	72.0-105.8	10.7-29.9	36.7-44.1
8-4	55.6	27	62.6-109.9	10.7-30.7	34.6-44.6
8-5	63.3	28	75.8-103.4	11.4-32.0	31.9-43.8
8-6	91.4	26	55.1-90.9	10.3-34.3	37.7-43.9
8-7	73.0	32	62.4-91.6	11.2-33.7	36.8-46.5
8-8	84.8	30	66.6-105.3	14.9-35.1	38.6-46.1
12-1	83.3	15	53.2-70.5	1.1-3.2	36.2-42.3
12-2	62.1	27	40.0-65.8	1.3-3.3	31.7-43.7
12-3	95.7	39	42.4-85.5	11.7-35.1	36.5-46.7
12-4	100.0	21	57.3-72.4	11.5-32.6	39.3-44.3
Stellar	0.0	5	42.5-46.7	0.6-2.9	35.70-39.9
1047	0.0	5	60.3-70.6	0.3-0.7	33.30-41.4

Fig.1. **A**. Seed color of the yellow-seeded *B. napus* line through the cross *B. juncea* × *B. napus* and its parents Sichuan Yellow (upper left), 1047(upper right) and Stellar (down right) **B**. Stereoscan photograph of the seeds harvested from the yellow-seeded *B. napus* plants in the BC_1F_5 family No. 8-1

Discussion

The hybrid F_1 plants from an interspecific cross between *Brassica juncea* and *B. napus* are highly sterile, only some 20% of pollen grains being stainable by aceto-carmine (Liu et al, 2002). These hybrid F_1 plants have to be backcrossed to one of the parental species to obtain enough seeds (Liu et al, 2001). The expression of yellow seed coat trait is delayed for one or two generations when the F_1 hybrid plants from the cross yellow-seeded *B. juncea* × *B. napus* were backcrossed to the black-seeded *B. napus* accessions. However, the yellow-seeded individual plants must be recovered by selfing if the genes controlling yellow seed coat trait are transferred into *B. napus* through allosynapsis, which was also supported by Rashid et al (1994).

The yellow-seeded plants selected from some BC_1F_5 families had desirable seed quality characters, but poor agronomic performance. Their agronomic characters such as loose branches, late flowering, and shrunken seeds should be improved in order to develop yellow-seeded *B. napus* cultivars. The novel yellow-seeded accessions are being evaluated through analysis of molecular markers for elimination of the undesired chromosomal segments introgressed from *B. juncea* as much as possible as well as used as parents crossed with superior commercial cultivars.

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