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Development of germplasm of yellow seeded winter oilseed rape (*Brassica napus* L. var. *oleifera*)

Iwona Bartkowiak-Broda, Aleksandra Piotrowska, Blazej Hernacki, Teresa Cegielska-Taras, Krzysztof Michalski
Plant Breeding and Acclimatization Institute (PBAI), Department of Genetics and Breeding of Oilseed Crops, Strzeszynska 36, 60-479 Poznan, Poland; e-mail: ibart@nico.ihar.poznan.pl

Introduction

One of the most important goals in quality breeding of oilseed rape is reduction of dietary fibre in seeds combined with the increase of oil and protein content.

The most efficient strategy to reduce the fibre content is the development of yellow seeded cultivars which have thinner seed coat than black seeded cultivars. Meals produced from yellow seeds would contain less hulls and as a result lessfibre.

Yellowseedness results from disturbances in metabolism of phenylpropanoids which consequently leads to decreased share of pigments concentrated mainly in seed coat and the elements of cell wall construction responsible for thickness of the coat. The management of phenylpropanoids, different in yellow seeded plants, has important, both positive and negative, impact on the whole plant metabolism. In addition, it contributes to the reduction of agronomical value of seeds, however to a very different degree, what makes further breeding possible. The selection of *B. napus* lines with stable expression of yellowseedness is complicated by the fact that complementary alleles of genes responsible for this character should be present on both genoms $A^yA^yC^yC^y$.

Origin of *Brassica napus* yellow seeded lines

The origin of oilseed rape with yellow seeds obtained in our Department is a natural mutant with brighter seeds, found in double low rapeseed breeding material, crossed with spring line of *B. napus* with segregating seed colour (Piotrowska*et al*. 2003). The spring line, obtained from Canada Agriculture Research Station, originated from the cross between *Brassica napus* × *Brassica rapa* (Fig. 1).

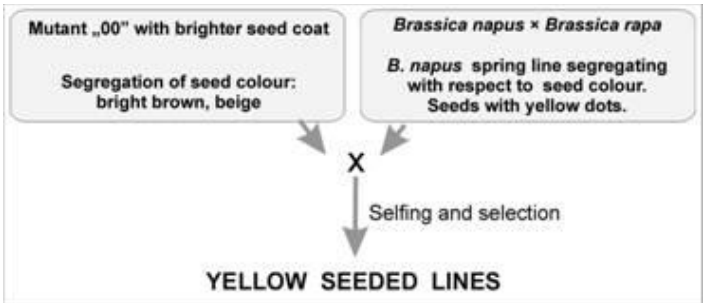

Fig. 1. The origin of yellow seeded lines

Table 1
Characteristics of yellow seeded lines — means for 142 lines selected in F_4 and F_5 progeny of hybrids between yellow seeded and black seeded lines

Trait	Mean		Minimum	Maximum	Coefficient of variability
	cv. Lisek	Yellow lines			
Oil content (%)	45,8	50,9	44,6	54,2	4,6
Protein content (%)	17,1	19,4	16,9	22,4	6,2
Total glucosinolatecontent ($\mu M \cdot g^{-1}$ of seeds)	9,0	7,9	3,1	14,9	29,3
ADF (%)	22,1	13,0	10,3	17,7	13,1
NDF (%)	28,8	19,4	16,6	25,6	9,6
Yield of seeds ($dt \cdot ha^{-1}$)	36,8	25,5	13,3	35,7	19,7
Yield of fat ($dt \cdot ha^{-1}$)	16,8	13,1	5,9	19,3	22,5
Colour of seeds		4,0	1,7	5,0	20,9
1000 seed weight (g)	4,0	4,4	3,5	5,2	8,2

ADF — acid detergent fibre; NDF — neutral detergent fibre
Scale of colours: 1 — brown colour, 2 — brown colour with yellow overcolouring, 3 — yellow and brown colour, 4 — yellow colour with brown overcolouring, 5 — yellow colour

The selected yellow seeded lines have been improved in respect to quality traits by crossings with double low black seeded lines and cultivars, followed by selection and inbreeding.

The investigations of developed lines with stable expression of yellow seed character revealed significant reduction of ADF and NDF fibre and an increase in fat and protein content (Table 1).

Analysis of segregating populations

Table 2
Segregation of seed colour in F_3 progeny of hybrids between yellow- and black seeded doubled haploid (DH) lines. Testing of accordance of seed colour segregation at the ratio 1:15.

	Combination	Colour of seeds						Total	χ^2 calculated
		5	4	3	2	1	B		
1	DHY 38 × DHB	3	8	7	21	9	6	54	0,0049
2	DHY 114 × DHB	4	3	8	15	11	11	52	0,0205
3	DHY 129 × DHB	0	0	3	12	5	16	36	1,4519
4	DHY 134A × DHB	2	1	3	10	2	19	37	0,0162
Total 1–4		9	12	21	58	27	52	179	0,2715
5	DH H_6-105 × DHY	8	12	10	15	0	24	69	2,513
6	DH W-40 × DHY	0	3	0	7	15	30	55	2,6776
7	DH O-120 × DHY	6	6	17	22	0	14	65	0,5426
Total 5–7		14	21	27	44	15	68	189	0,2571
Total 1–7		23	33	48	102	42	120	368	0,0116

$\chi^2_{0,05;1}$ = 3,841 — critical value
Y — yellow colour
B — black colour

Scale of colours
1 — brown
2 — brown with yellow overcolouring
3 — yellow and brown
4 — yellow with brown overcolouring
5 — yellow

Table 3
Segregation of seed colour of DH lines developed from F_1 hybrids between yellow- and black seeded DH lines. Testing of accordance of seed colour segregation at the ratio 1:15.

	Combination	Colour of seeds						Total	χ^2 calculated
		5	4	3	2	1	B		
1	DHY 114 × DH H_6-105	0	1	1	1	0	0	3	0,5556
2	DH H_6-105 × DHY 114	0	2	1	1	6	10	20	0,4800
3	DH W-40 × DHY 129 I	0	1	1	11	10	21	44	1,9636
Total 2–3		0	3	2	12	16	31	64	3,2667

On the basis of seed colour in F_1 hybrids and in the segregating progeny F_3 it was stated that yellow seed colour is determined by mother genotype and at least two pairs of alleles with probably epistasis effect. Black colour is dominating. In progeny F_3 the ratio 1:15 = yellow seeds: brown-yellow + brown + black seeds has been statistically confirmed.

Mapping population

Mapping population consisting of 195 lines obtained from hybrids of two yellow seeded (DHY114 and DHY 1291) and two black seeded (DHB H5 105 and DHB W-40) DH lines.

DH Z 114 × DH H5 105 = 71 lines
DH H5 105 × DH Z 114 = 21 lines
DH W-40 × DH Z 129I = 32 lines
DH Z 129I × DH W-40 = 71 lines

The aim:

- Analysis of seed colour segregation
- Identyfication of molecular markers linked to genes responsible for yellow seed colour
- Identyfication of QTLs of yellowseedness
- MAS (marker assisted selection)

Markers used for analysis of mapping population:

- 100 starters RAPD, in it:
  — 89 standard starters for rapeseed
  — 11 starters described as generating markers linked to oilseed rape seed colour (Somers *et al.* 2001)
- 30 pairs of AFLP starters, including 15 pairs described as generating markers linked to oilseed rape seed colour (Subharwal *at al.* 2004; Yan *et al.* 2007; Negi *at al.* 2000)
- SSR.

Characterization of yellow seeded lines by molecular markers and identification of main genes or QTLs linked to the seed colour or fibre content will allow to apply marker assisted selection in future breeding of yellow seeded oilseed rape varieties.

It is important that primers used until now did not generate exactly the same marker poly morphism as that described for other genetic sources of yellow seeds. This suggests distinctness of our yellow seed source.

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