Genetic and molecular analysis of specific-origin yellow-seeded winter rapeseed (*B. napus* L. var. *oleifera*)

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Abstract

Double low (00) lines of winter yellow-seeded *B.napus*, displaying stable expression of genes for yellowseedness have been developed. Investigated lines revealed significant reduction of A(cid)D(etergent)F(ibre) and N(eutral)DF, an increase in fat and protein content and variability in yield of seeds. The genetic determination of seed colour and fibre content was investigated. Statistical analyses show high heritability of these traits. Molecular analyses are conducted on two high polymorphic mapping populations of DH-lines by RAPD and AFLP primers to complete characterizations of these lines and identify main QTLs linked to yellow colour and fibre content.

Key words: *Brassica napus* – yellowseedness – canola quality – dietary fibre – molecular markers

Introduction

Many applications of rapeseed oil and high level of its production generate large quantities of rapeseed meal and mill cake remaining after its extraction. These in turn can be valuable feedstuff (Bartkowiak-Broda *et al.* 2008). However, large fibre and polyphenols content make assimilation of protein by animals difficult and result in lower rapeseed meal energy value (Slominski *et al*. 1999; Smulikowska *et al*. 2006).

The most efficient strategy to reduce fibre content is the development of yellow-seeded cultivars. The colour is a visual marker of fibre content. In addition, negative correlation between the presence of ADF, NDF (Acid- and Neutral Detergent Fibre) and both protein and oil in seeds are observed (Rashid and Rakow 1999; Potapov and Osipova 2003; Piotrowska *et al*. 2003).

Until now many different sources of yellow-seeded rapeseed have existed. In this paper the results of investigations concerning the source of yellowseedness developed in our Institute are presented.

Origin and characteristic of *Brassica napus* yellow-seeded lines

A breeding program focused on creation of yellow-seeded winter rapeseed in Department of Genetics and Breeding of Oilseed Crops in Poland develops new original source of yellowseedness derived exclusively from *Brassica napus.* Therefore obtained lines of winter yellow-seeded *B.napus*, showing stable expression of this trait during subsequent generations, are also characterized by canola quality. The origin of developed oilseed rape with yellow seeds is a spontaneous mutant with brighter seeds, found in breeding materials of double low winter rapeseed, crossed with spring line of *B.napus* segregating in yellow spotted seed-coat (Piotrowska *et al.* 2003). The second compound was gained from previous hybrid of *B.napus* × *B.rapa* (received from Canada Agriculture Research Station).

The best lines were self-pollinated and crossed with high yielding black-seeded varieties, to create new stable yellow-seeded lines with good quality features value (Piotrowska *et al.* 2003).

The earlier investigations give us clear evidence that developed lines are characterized by lower fibre, higher oil and protein content (exceed even by a few percent) in comparison to black seeded rapeseed. The average seed yield of first yellow-seeded lines was on the level of 80% of standard black seeded cultivar Bojan (Ochodzki *et al*. 2003). This difference could be partially explained by smaller average weight of 1000 seeds in the beginning of selection. Another explanation could be deteriorated plant resistance to pathogens and cold. However, the variability coefficient of negative traits (especially the weight of 1000 seeds) has given the possibility of yield improvement (Piotrowska *et al.* 2003).

After several generations of selection a collection of stable yellow-seeded lines has been created. The agronomical value of selected lines was evaluated in field trials in completely randomized block design in 4 replications, in seasons 2008/2009 and 2009/20010. Single lines reached yield near the level of standard black seeded cultivars. Average seed oil content of investigated lines was about 47%, protein – 19.5%; fiber ADF – 85% and fiber NDF – 90% in relation to the black-seeded lines. Yellow seeded lines

were evaluated considering their resistance to diseases. Lines with increased resistance to *Leptosphaeria* spp. have been discovered, but no lines with increased resistance to *S. sclerotiorum*.

For the development of hybrid varieties yellow-seeded CMS *ogura* lines and *Rfo* – restorer lines displaying genetic distance have been selected.

A few-year long experience gives evidence to quite important weather impact on the yellowseedness trait, cold and rainy period during seeds maturation contributed to the development of darker seeds.

Seed colour and fibre content inheritance

The determination of yellow seed colour was investigated in F_1–F_3 generations obtained by crosses in complete diallelic design of 4 yellow-seeded doubled haploid lines (DH) and 3 black-seeded DH lines. Initial yellow-seeded lines were characterized by seeds colour at 4–5 level in five degree colour scale (1 – dark, 5 – yellow colour).

Yellowseedness is determined by the maternal plant genotype (the same colour of F_1 seeds as maternal lines seeds). The pollinator genotype effect is negligible because the external layers of seed coat derive from maternal tissues. Intermediate coloration of F_2 seeds (2 or 3 in adopted scale) and the ratio of 1 : 15 – yellow to the rest – in seed generation F_3, allow to state (on the basis of χ^2 calculation) the determination by two pairs of alleles with probable occurance of epistasis phenomenon (Table 1).

Table 1. Segregations of seed colour in F_3 progeny of hybrids between yellow and black-seeded DH-lines. Testing accordance of seed colour segregation at the ratio 1 : 15.

Item	Combinations	Seed colour						Total	$\chi^2_{cal.}$
		5	4	3	2	1	b		
1	DHy 38 × DHb	3	8	7	21	9	6	54	0,0049
2	DHy114 × 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
1 – 4 total		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
5-7 total		14	21	27	44	15	68	189	0,2571
1-7 total		23	33	48	102	42	120	368	0,0116

1 – brown

2 – brown with yellow overcolouring

3 – yellow and brown

4 – yellow with brown overcolouring

5 – yellow

DHy – all yellow seeded lines

DHb – all black seeded lines

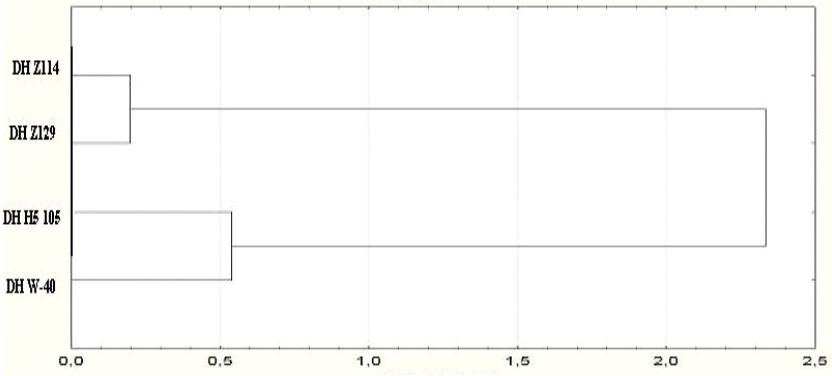
$\chi^2_{0,05;1}$= 3,841 – critical value

Similar examinations were carried out to get insight into the inheritance of fibre content. Both cumulative genes and partial domination, analyzed by Hayman method (1954), have significant impact on fibre content. Although not large difference in fibre content high inheritance of this trait (H_{ss} = 0.82 and H_{SL} = 0.95) was observed.

Characteristic of mapping population

In order to identify major QTL contributing to reduced seed coat and seed colour as well as to develop markers for effective breeding of yellow seeded varieties two mapping populations were created. Both consisted of doubled haploid lines developed from F_1 hybrids of two yellow-seeded and two black-seeded DH lines (Figure 1). They are currently phenotypically analyzed and mapped by molecular markers.

Dendrogram of investigated DH lines



I mapping population:
DHz 114 × DH H_5 105 = 71 lines
DH H_5 105 × DHz 114 = 21 lines
II mapping population:
DH W-40 × DHz 129I = 32 lines
DHz 129I × DH W-40 = 70 lines

Fig. 1. Genetic distance of parental lines calculated by the presence of 101 polymorphic RAPD amplification products.

Until now investigations carried out with 32 RAPD primers have shown in most cases polymorphism of DNA amplification products in both populations. This was the case with nine of eleven tested specific primers, that should generate markers linked with yellowseedness. Their usefulness was confirmed in earlier publications (Somers *et al.* 2001; Li *et al*. 2003; Liu *et al.* 2006 – modified ; Yan *et al*. 2007) and with all of twenty-one tested standard primers. Not all proposed primers could generate markers linkage with our source of yellowseednes, what shows table 3.

Table 3. Specific RAPD primers (11/9) UBC –Somers 2001; LC – Li 2003; YL – Yan 2007; S11nn – Liu 2006 – modified. AP – amplification product; PAP – polymorphic amplification product. I and II – Populations.

No.	Primer	Sequence	colour	AP I/II	PAP I/II	Potential linkage
I	UBC282	GGGAAAGCAG	yellow	14	0	_____
II	UBC335	TGGACCACCC	yellow	13/14	5/9	YES
III	UBC555	GTGAACAGCA	yellow	11/11	4/4	YES
IV	UBC88	CGGGGGATGG	black	23/22	5/7	rather NO
V	UBC486	CCAGCATCAG	yellow	17/19	6/8	NO!
VI	UBC365	TAGACAGAGG	black	17/20	3/7	NO!
VII	UBC89a	GGGGGCTTGG	yellow	17/19	6/5	rather NO
VIII	LC	ATTCGGTAGG	yellow	18/21	7/13	YES
IX	YL1078	ACCCGGAAAC	black	15/17	6/6	YES
X	S1129	GGGGGAGATG	yellow	8	0	_____
XI	S1130	CTGTGTGCTC	yellow	17	4/5	YES

Nowadays AFLP markers are used, in it 12 described in the literature as linked with the colour of seeds (Negi *at al.* 2000; Sabharwal *et al*. 2004; Yan *et al.* 2007). In addition, the genome map will be also complemented by an analysis using microsatellite markers.

Conclussion

Yellow colour of seeds in rapeseed is conditioned by many genetic and physiological agents, also has significant impact on many features. Some of these changes are very variable. Therefore it is not possible to use them as a morpfological marker of seed quality, especially fibre content. However the localization of major QTLs with important contribution to seed colour and fibre content as well as development of molecular markers linked to genes responsible for these traits should make breeding of yellow seeded cultivars possible.

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