

# Development of segregating populations in the breeding of yellow-seeded winter rapeseed (*Brassica napus* L.)

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## ABSTRACT

The combination of genetic and environmental factors gives oilseed rape (*Brassica napus* L.) a range of seed colours. The introgression of genes encoding seed pigmentation, e.g. from related *Brassica* species, and subsequent expression of yellow seed colour in *B. napus* can be complex due to polyploidy ( $2n=4x=38$ ), multiple gene control and predominantly maternal determination. Transparent and thin seed testa is thought to coincide with low fibre content because the biosynthetic pathways leading to pigment and lignin synthesis have common precursors. Therefore, yellow seededness would address another seed trait in rapeseed, which is to decrease the crude fibre content of the meal in order to improve its digestibility and animal feed value, relative to soybean meal. As crude fibre is a biochemically inhomogeneous seed fraction and difficult to determine analytically, selection for light seed colour would facilitate the improvement of the meal. In the course of a quality breeding programme two different sources for yellow seededness were used to develop segregating winter rapeseed populations. Regarding the first population, derived from a cross between the dark-seeded high-erucic acid pure line ('DH26-96') and the true-breeding yellow-seeded double-low line 'T25629', doubled-haploid (DH) lines were produced. Whereby in another case, i.e. a cross between a dark-seeded double-low line ('Express 617') and the true breeding yellow-seeded double-low line '1012-98', both inbred and DH lines were generated. As temperature can modify the seed coat colour in rapeseed, the populations were grown in the field and glasshouse in order to record environmental effects. In all cases the seed colour distributions in the populations fit a trigenic ratio with black seed colour being dominant over yellow seed colour. True yellow seededness is only manifested when three homozygous recessive alleles are present, whereas all other genetic situations result in a more or less brown or black seeds.

**Key words:** *Brassica napus* - seed colour - segregating populations - inheritance - environment

## INTRODUCTION

Oil crops are important sources of energy and protein, both for human consumption and feeding livestock. *Brassica* seeds possessing transparent testa are characterised by thinner seed coats than black seeds, associated with a reduction of crude fibre content and a concomitant increase of digestible energy and protein content in the meal. Therefore, improving the nutritional value of rapeseed through the development of yellow-seeded *B. napus* varieties would have a strong impact on the relations of the feed meal market and especially on the production of oilseed rape in Europe and would be beneficial for the competitiveness with soybean meal (Friedt et al. 2002a, 2002b). In the course of a breeding programme inbred and doubled-haploid (DH) lines were generated derived from crosses between different dark-seeded rapeseed lines and different forms of yellow-seeded *B. napus* showing double-low quality. Depending on the source of yellow seededness used in the genetic studies, in most cases a trigenic inheritance has been proposed (cf. Baetzel et al. 1999, Lühs et al. 2000).

## MATERIAL AND METHODS

A set of widely divergent *Brassica* materials including *B. napus* were evaluated as sources for yellow seededness and true-breeding winter rapeseed lines were used in crosses in order to develop segregating populations. The doubled haploid (DH) population YE1 was derived from a cross between the yellow-seeded double-low line 'T 25629' and the dark-seeded high-erucic acid doubled-haploid line 'DH 26-96' as described earlier (Baetzel et al. 1999, Lühs et al. 2000).

The DH individuals were evaluated in 2001-2002 in the greenhouse (day/night: 24/20 °C, 16 hours day-light) and the field at the Field Research Station Rauischholzhausen (near Marburg/Lahn, Germany) for collecting phenotypic and quality data. A core set of 110 DH lines were used for the subsequent genetic and QTL mapping (Badani et al. 2003). At second, a F<sub>2</sub> inbred population (YE2) was derived from a cross between a dark-seeded double-low line ('Express 617') and the true-breeding yellow-seeded double-low line '1012-98', which is of different origin compared to YE1. The evaluation of F<sub>2</sub> individuals was conducted in 1999-2000 in field nurseries at Einbeck and Thüle, Germany (Friedt et al. 2002a). In addition to visual assessment seed colour was determined and screened by using a digital optical-picture analysis system (GTA Sensorik, Neubrandenburg, Germany) with brightness values as follows: yellow ( $\geq 140$ ), brown (70-139) and black ( $\leq 69$ ). Both crude seed composition and seed colour were determined by near-infrared reflectance spectroscopy as described by Baetzel et al. (2003). Chi-square goodness-of-fit tests were used to compare the observed distribution in the segregating populations to those predicted by different models for seed colour inheritance (Shirzadegan 1986, Henderson and Pauls 1992, Van Deynze and Pauls 1994). The data for seed colour were pooled, tested for heterogeneity and fitted to appropriate genetic models (cf. Mudra 1958).

## RESULTS AND DISCUSSION

The combination of genetic and environmental factors gives oilseed rape a range of seed colours. However, the introgression of genes encoding seed pigmentation, e.g. from related *Brassica* species, and subsequent expression of seed colour in *B. napus* can be complex due to allotetraploidy ( $2n=4x=38$ ), multiple gene control and predominantly maternal determination (Van Deynze et al. 1993, Lühs et al. 2000). For both environments the observed genetic segregation of the DH population 'T 25629' x 'DH 26-96' fits a 1:6:1 ratio (Table 1); hence seed colour in this material is obviously inherited in an additive manner: three dominant complementary genes simultaneously are governing the expression of black seeds and true yellow seededness is only manifested in the case of three homozygous recessive (non-functional) alleles, whereas all other genetic situations result in a more or less brown seed colour. The heterogeneity was tested according to Mudra (1958) indicating that pooled data for both environments (field, greenhouse) and the data for each single environment fit the predicted segregation. This result provides evidence that the present DH population does not exhibit inheritance with epistatic gene action, as assumed by Shirzadegan (1986) for inbred populations based on a similar source of yellow seededness. In the F<sub>2</sub> population 'Express 617' x '1012/98' (other origin than YE1) corresponding to the proposed trigenic additive gene model a ratio of 27:36:1 for black : brown : yellow seeds was observed (Table 2).

Table 1. Observed and expected frequencies for DH population YE1 segregating for seed colour cultivated in two different environments (expected frequencies in brackets)

Seed colour <sup>1</sup>	Genotype	Predicted Ratio	YE1 Field	YE1 GH <sup>2</sup>	Pooled	Total	Heterogeneity
Black $\leq 69$	Bl <sub>1</sub> Bl <sub>1</sub> Bl <sub>2</sub> Bl <sub>2</sub> Bl <sub>3</sub> Bl <sub>3</sub>	1	23 (15.4)	13 (17.5)	36 (32.9)		
Brown 70-139	bl <sub>1</sub> bl <sub>1</sub> Bl <sub>2</sub> Bl <sub>2</sub> Bl <sub>3</sub> Bl <sub>3</sub> bl <sub>1</sub> bl <sub>1</sub> Bl <sub>2</sub> Bl <sub>2</sub> bl <sub>3</sub> bl <sub>3</sub> Bl <sub>1</sub> Bl <sub>1</sub> bl <sub>2</sub> bl <sub>2</sub> Bl <sub>3</sub> Bl <sub>3</sub> Bl <sub>1</sub> Bl <sub>1</sub> bl <sub>2</sub> bl <sub>2</sub> Bl <sub>3</sub> Bl <sub>3</sub> Bl <sub>1</sub> Bl <sub>1</sub> bl <sub>2</sub> bl <sub>2</sub> bl <sub>3</sub> bl <sub>3</sub>	6	86 (92.2)	108 (105)	194 (197.2)		
Yellow $\geq 140$	bl <sub>1</sub> bl <sub>1</sub> bl <sub>2</sub> bl <sub>2</sub> bl <sub>3</sub> bl <sub>3</sub>	1	14 (15.4)	19 (17.5)	33 (32.9)		
Total			123	140	263		
$\chi^2$			4.32	1.37	0.35	5.69	5.34
DF			2	2	2	4	2
P %			10-20	50-90	50-90	20-50	5-10

<sup>1</sup> seed colour assessed by digital optical-picture system, reference dimensions = brightness values; <sup>2</sup> GH = greenhouse

Table 2. Observed and expected frequencies for F2 inbred population *YE2* segregating for seed colour based on different genetic models of trigenic control (expected frequencies in brackets)<sup>1</sup>

Seed Colour <sup>1</sup>	Predicted Ratio	Inbred lines	Predicted Ratio	Inbred lines	Predicted Ratio	Inbred lines
Black ≤ 69	27	574 (588)	16	574 (349)	12	574 (261)
Brown 70-139	36	788 (784)	47	788 (1,024)	51	788 (1,111)
Yellow ≥ 140	1	32 (22)	1	32 (22)	1	32 (22)
Total		1,394		1,394		1,394
$\chi^2$		5.15		204.98		472.54
DF		2		2		2
P %		5-10				

<sup>1</sup> seed colour assessed by digital optical-picture system, reference dimensions = brightness values

Besides seed size the colour of the seed testa has a well-known effect on the composition of storage products, such as oil and protein along with crude fibre content (cf. Morgan et. al 1998, Baetzel et al. 1999). The box-and-whisker plots in Figure 1 display the variability found in the F<sub>2</sub> inbred lines belonging to *YE2* in comparison to the parental lines. Due to an increase of oil content the novel rapeseed material segregating for seed colour possesses a higher sum of both protein and oil.

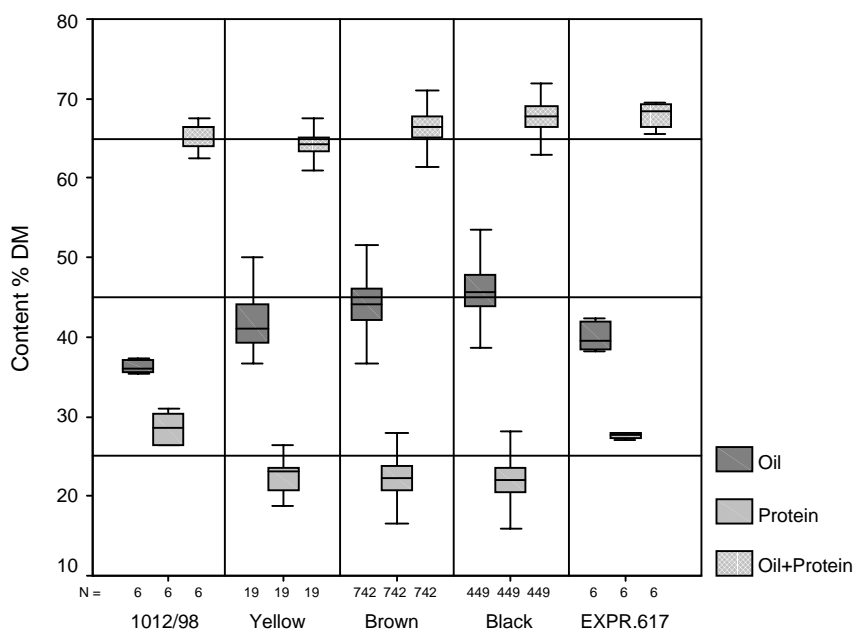


Figure 1. Oil and protein content (% of dry matter, NIRS) of F3 seeds (*YE2*) and the parental lines '1012/98' and 'Express 617' (N= number of samples)

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