Breeding Yellow Seeded Winter Oilseed Rape

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Introduction

The cultivation of oilseed rape (*Brassica napus*) has been greatly expanded during recent decades due to rapid progress in breeding and biotechnology. Today, rapeseed is one of the leading oilseed crops world-wide - with particular economic importance for Germany and other EU member states. Following oil extraction rapeseed meal contains about 40% protein with a well-balanced amino acid composition. Among other antinutritional compounds, including glucosinolates, phytate, sinapine, phenolic acid and tannins, crude fibre (and substances enclosed by it) adversely affects the usability of rapeseed meal in feed and nutrition. Regarding further improvement of winter oilseed rape, yellow seed colour is considered to have a pronounced influence on seed quality leading to a concomitant increase in digestible energy and protein content of the meal. This can be explained by the characteristically thinner seed coat of yellow as compared to black seeds (cf. FRIEDT and LÜHS 1999, BAETZEL *et al*. 1999, 2000).

Material and Methods

In the course of a winter oilseed rape breeding programme, F_1 hybrids were derived from crosses between dark-seeded, well-performing rapeseed lines and true-breeding yellow-seeded *B. napus* lines. Both inbred (F_2) and doubled-haploid (DH) mapping populations were generated. The DH population was derived from a cross between the true-breeding yellow-seeded double-low line ’T 25629’ and the dark-seeded high-erucic acid doubled-haploid line ‘DH 26-96’. The F2 inbred population 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’. In addition to visual assessment seed colour was determined and screened by using a digital optical-picture analysis system; the seed material was photographed with a digital camera and assessed for brightness values as follows: yellow (≤4.5), brown (4.5-7.5) and black (>7.5). The seed colour value of an individual sample comprises the mean value of all seeds. Crude seed composition was analysed by non-destructive near-infrared reflectance spectroscopy (NIRS) (BAETZEL *et al*. 2000).

AFLP analyses were carried out according to VOS *et al*. (1995). Linkage analyses were performed using the programme MapMaker Version 3.0b (LANDER *et al*. 1987) and a linkage map was constructed using a minimum LOD score of 3.0. The Kosambi function (KOSAMBI 1944) was used to obtain the centiMorgan (cM) values. **Results and Discussion**

Inheritance of seed colour

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 (LÜHS *et al*. 2000). The genetic segregation of the DH population 'T 25629' x 'DH 26-96' fits a 1:6:1 ratio; hence seed colour is obviously inherited in an additive manner: black seeds occur when all three loci are homozygous for the most effective ("dominant") alleles, and true yellow seediness 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. A ratio of 27 : 36 : 1 for black : brown : yellow seeds was observed in the F2 population 'Express 617' x '1012/98' corresponding to the proposed three-gene additive model (Table 1).

Table 1: Observed and expected segregation for the yellow seed trait in the DH and F2 populations, respectively, as used in this study.

Seed colour Brightness value *	DH population 'T 25629' x 'DH 26-96'		F2 population 'Express 617' x '1012/98'	
	observed	expected	observed	expected
Black > 7.5	9	11.25 (1)	574	588 (27)
Brown 4.5 – 7.5	74	67.5 (6)	788	784 (36)
Yellow ≤ 4.5	7	11.25 (1)	32	22 (1)
Total	90		1,394	
χ^2	2.68		5.15	
DF	2		2	
P	0.26		0.08	

*) Brightness values as determined by a digital-optical image analysis.

Molecular markers

The development of molecular markers linked to gene loci controlling seed colour in *B. napus* has gained importance because selection for yellow seediness is hindered due to pronounced environmental effects, e.g. temperature during seed ripening (cf. VAN DEYNZE *et al*. 1993, 1995).

In the course of the present study, marker analyses were performed in the F2 and DH populations as described above. AFLP markers were generated in both populations using 20 primer combinations, and markers from 13 SSR primer pairs have been mapped in the F2 population using an assay based on M13-tailed primers. A total of 290 SSR markers was screened for polymorphism between the parents of the two crosses; 62 and 73 SSRs proved to

be polymorphic in the crosses ‘T 25629’ x ‘DH 26-96’ and ‘Express 617’ x ‘1012/98’, respectively (Fig. 1).

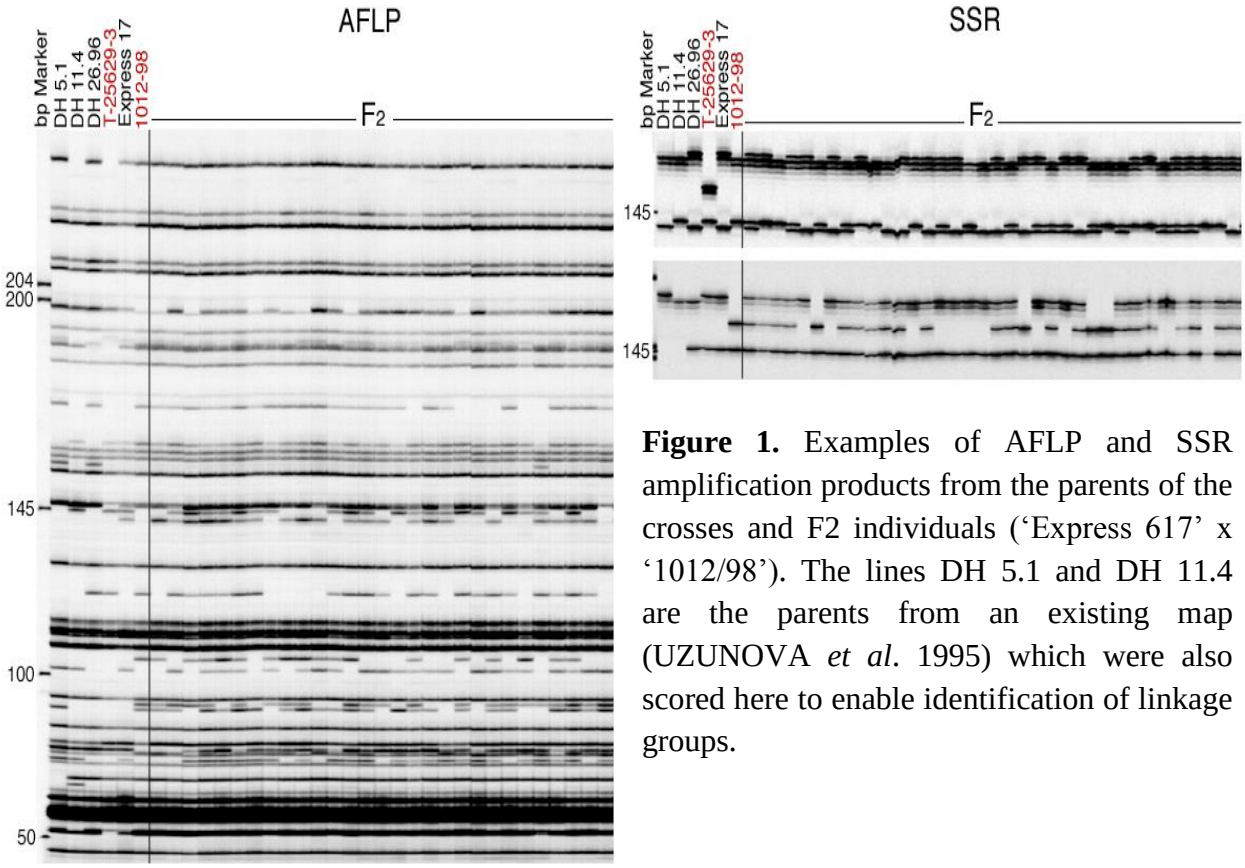

Figure 1. Examples of AFLP and SSR amplification products from the parents of the crosses and F2 individuals (‘Express 617’ x ‘1012/98’). The lines DH 5.1 and DH 11.4 are the parents from an existing map (UZUNOVA *et al*. 1995) which were also scored here to enable identification of linkage groups.

QTL analysis of seed colour

Linkage analyses were performed using MapMaker 3.0b and a linkage map was constructed based on a LOD 3.0. Centimorgan values were obtained using the Kosambi function. To enable identification of the resulting linkage groups, the parental lines from the existing RFLP rapeseed map ‘Mansholt’s Hamburger Raps’ x ‘DH11-4 Samourai’ (UZUNOVA *et al*. 1995) were also scored (Fig. 1). Based on the DH-population a genome map was generated comprising 173 AFLP markers in 19 linkage groups covering 802.6 cM. A skeleton map of 74 markers developed from this map was used for QTL analysis of the yellow seed colour trait. Three QTLs, one each on groups 1, 8 and 11 could be detected which explain 83% of the total phenotypic variance (Fig. 2). For the F2 population ‘Express 617’ x ‘1012/98’ a second AFLP map including also 7 microsatellites was developed comprising 137 markers in 19 linkage groups covering 1,419.8 cM (data not shown).

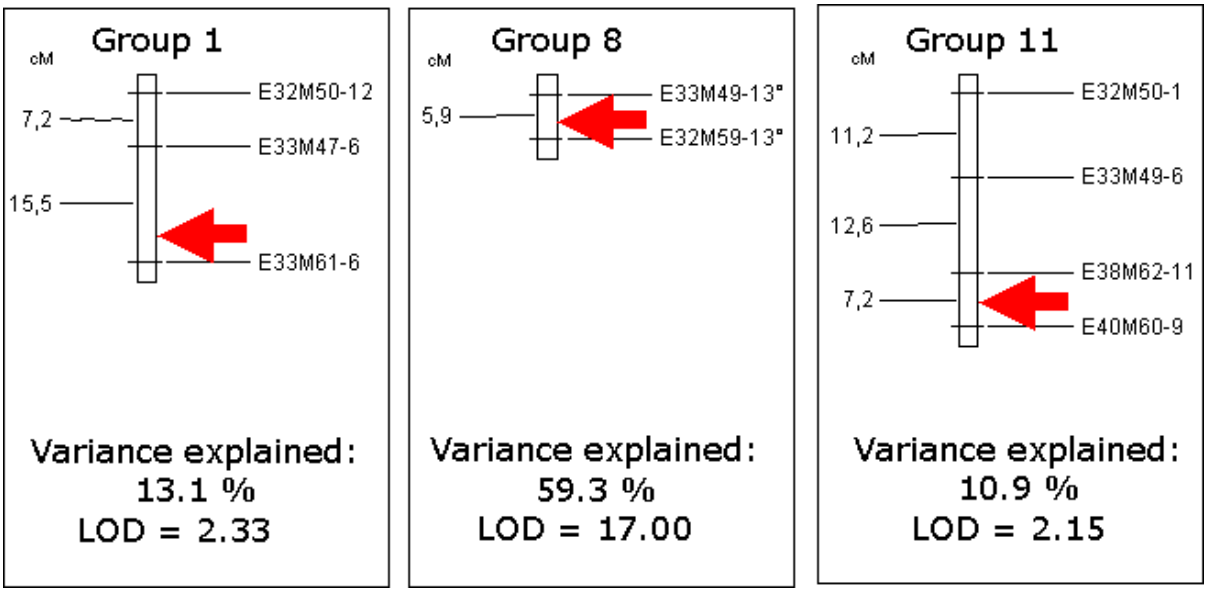

Figure 2. Localisation and characterisation of three QTLs for yellow seed colour in a skeleton map of the DH population 'T 25629' x 'DH 26-96'.

Summary and Conclusions

In this study, AFLP and SSR markers were employed for genetic mapping of the yellow seed trait after phenotypic evaluation by digital-optical image analysis and non-destructive near-infrared reflectance spectroscopy (NIRS). Three QTLs for yellow seed colour could be mapped in the population 'T 25629' x 'DH 26-96' investigated first, and valuable markers for marker-assisted selection are now available that allow more reliable selection for a trait which is strongly influenced by environmental effects. Integration of additional SSR markers will improve the accuracy of the maps and enable the reliable identification of the linkage groups carrying the QTLs. Moreover, as biochemical pathways leading to lignin and seed testa pigmentation are considered to coincide along the phenylpropanoid pathway (cf. DIXON *et al*. 2001, PEER *et al*. 2001, WINKEL-SHIRLEY 1998, 2001), exploiting the knowledge from *Arabidopsis* genomics is expected to have a strong impact on improving yellow-seeded *B. napus* and breeding of winter oilseed rape.

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