Rapeseed Meal Quality Breeding – The European Situation

Wolfgang Friedt, Ana-Gloria Badani, Benjamin Wittkop, Wilfried Lühs and Rod Snowdon

Department of Plant Breeding, IFZ Research Center for BioSystems, Land Resources and Nutrition, Justus-Liebig-University, Heinrich-Buff-Ring 26-32, D-35392 Giessen, Germany

Phone: +49 641 99-37420, Fax: +49 641 99-37429, e-mail: wolfgang.friedt@agrar.uni-giessen.de

Rapeseed (*Brassica napus* ssp. *napus*, 2n = 38, genome AACC) is the leading oilseed crop in Europe and the second most important worldwide after soybean. In Germany, the current acreage is approx. 1.3 Million ha with an average seed yield of 4 t/ha (2004). Rapeseed oil is predominantly used as vegetable oil, however the use for biodiesel and as a lubricant substrate for biodegradable polymers is also significant. Rapeseed cake or meal as a residue of oil extraction is used as a protein source in animal nutrition, where the favourable composition of essential amino acids, especially high contents of sulphuric amino acids such as methionin and cystein (Downey & Bell 1990), are particularly noteworthy. The protein content in rapeseed meal after oil extraction varies from 40-50% (% dry matter). In addition the meal is rich in minerals (*Ca, Mg and P*) and vitamins (*B4 and E*) (Thies 1994). However, due to its somewhat bitter taste, adstringency and contents of antinutritive components (sinapate esters, phytate, tannins, crude fibre) the value of rapeseed meal-protein is comparatively low in comparison to soybean meal-protein. Therefore, a reduction of crude fibre and antinutritive components in rapeseed is an important breeding aim in order to increase the meal quality and allow an increase in the proportion of rapeseed meal that can be implemented in animal feed mixtures.

Yellow or light seeds are therefore of particular interest for breeding of winter oilseed rape in Europe because of their association with a thinner seed coat that results in reduced crude fibre content. This considerably improves the feed quality of rapeseed meal (Shirzagedan & Röbbelen 1985, Slominsky et al. 1994, 1999). Crude fibre, a heterogeneous fraction including cellulose, hemicellulose and lignin as indigestible components, is a major component influencing the nutritional value of seed meal. Light seed colour and low fibre content are considered to coincide because the biochemical pathways leading to lignin and pigment synthesis have

common precursors, such as p-cumarate (Theander et al. 1977, Whetten et al. 1998). Furthermore, the reduction in testa thickness in yellow-seeded oilseed rape has also been found to be associated with increased seed oil and/or protein content per dry weight (Xiao and Liu 1982, Piotrowska et al. 2003).

True breeding commercial light-seeded winter oilseed rape varieties are to date not available on the European market. to the main reason for the difficulties in breeding of yellow-seeded winter rapeseed is the strong environmental modification of the trait, particularly caused by temperature (cf. Van Deynze et al. 1993), which makes selection of stable yellow-seeded phenotypes difficult. The introduction of novel yellow-seeded germplasm with reduced crude fibre and the development of tightly-linked molecular markers for marker-assisted selection of these traits are two important current breeding and research aims in Germany and France, two of the largest European rapeseed producers. Novel genetic variation for seed colour originates mainly from interspecific crosses with light-seeded *B. rapa*, *B. oleracea*, *B. juncea* or *B. carinata* genotypes.

Different mechanisms have been proposed for the inheritance of yellow seed colour in crop brassicas. According to Shirzadegan (1986), Henderson and Pauls (1992) and Van Deynze et al. (1995) three genes are involved in the genetic control of testa colour in *B. napus*, and only lines that are homozygous for recessive alleles at all three loci will breed true for yellow seed-colour. Heneen and Brismar (2001) suggested a combination of maternal and embryonal control of seed colour in the diploid species *B. alboglabra* (2n = 18) with loci on three different C-genome chromosomes, whereas Lionneton et al. (2004) identified two Mendelian loci responsible for inheritance of yellow seeds in *B. juncea*. On the other hand, Somers et al. (2001) localised a single major gene explaining 72% of the variance for seed colour in a cross between a yellow-seeded and a black-seeded *B. napus*, and suggested that this gene interacts in a dominant manner with two additional epistatic loci that each explain around 10% of the variance in seed colour. This finding was supported by Liu et al. (2005), who analysed segregation ratios in crosses between black-seeded and resynthetic yellow-seeded rape. Their results show that a partially dominant gene for yellow seed colour exhibits epistatic effects on two dominant genes for black seed colour.

In order to identify gene loci contributing to yellow seed colour in winter oilseed rape, Badani et al. (2005a) performed segregation analyses and mapped quantitative trait loci (QTL) for seed colour in segregating populations derived from two different yellow-seeded rapeseed sources (Baetzel et al. 2003). In both crosses a major, partially dominant QTL contributing

more than 60% of the variation in seed colour was found at the same map position in both crosses. Either one or two further loci were found to also contribute to the seed colour in an epistatic manner. These results correspond to the QTL analysis by Somers et al. (2001) and the segregation patterns described by Liu et al. (2005) for seed colour in *B. napus*. They also support the suggestion of these authors that a partially dominant gene for yellow seed colour interacts epistatically with other loci to determine seed colour in rapeseed.

The colour of the seeds is however not the deciding factor in terms of meal quality, but rather the content of dietary fibre. Hence, Badani et al. (2005a) also analysed acid detergent fibre (ADF), the fraction of seed meal comprising the indigestible cell wall compounds cellulose and lignin, and confirmed that ADF content is closely correlated with seed colour (Fig. 1). The ADF contents of intact seed samples varying in seed colour were also estimated by near-infrared spectroscopy (NIRS), and Font et al. (2003, 2005) developed an NIRS calibration for ADF in *B. napus* from measurements of absolute ADF content. Based on corresponding NIRS measurements a large QTL for ADF was found to co-localise with the major QTL for yellow seed colour (Badani et al. 2005a), hence molecular markers for this locus could be used for targeted reduction of antinutritive fibre in rapeseed meal.

Another major anti-nutritive component in seeds of most members of the Brassicaceae family are the sinapate esters, and reduction of sinapine (sinapoylcholine) in the seed is a further important breeding aim in European winter rapeseed with regard to an overall improvement of the meal quality. Sinapine is formed via a two-step biosynthesis, whereby sinapate supplied by the phenylalanine/hydroxycinnamate pathway is converted to 1-*O*-sinapoylglucose by the enzyme UDP-glucose:sinapate glucosyltransferase (SGT). The energy-rich β-acetal ester 1-*O*-sinapoylglucose is subjected to a transacylation reaction catalyzed by 1-*O*-sinapoylglucose:choline sinapoyltransferase (SCT), which transfers the sinapoyl moiety from 1-*O*-sinapoylglucose to choline, resulting in the accumulation of sinapine. Mutant analysis in Arabidopsis and homology-based cloning strategies led to the identification of genes encoding SGT and SCT in *B. napus* (Milkowski et al. 2000, 2004). The recently introduced method of double-stranded RNA interference (dsRNA*i*) has proven to be an effective tool for gene silencing and could be used to down-regulate these key sinapine genes in oilseed rape. Since the suppression effect is dominantly inherited, the dsRNA*i* approach is attractive for manipulation of polyploid crop plants and for hybrid breeding. By use of regulated promoters, the silencing effect can be triggered during specific stages of plant development, thus avoiding detrimental effects that could be caused by constitutive silencing.

Another modern breeding technique which is likely to play an increasingly important role in European rapeseed breeding in coming decades is TILLING (Targeting Induced local lesions in genomes; McCallum et al. 2000), which allows the generation and targeted utilisation of allelic mutations in genes of interest. This is particularly relevant for the generation of novel germplasm with mutations in key genes of important biochemical pathways. The principle of TILLING is based on DNA amplification by PCR of target genes of individuals from a mutant population, followed by mixture, de- and re-naturation of the PCR products and the treatment of these double-stranded DNA fragments with the *Cel1* endonuclease. The advantages of the TILLING technology are: (1) The chemical mutagenesis can be applied to any organism; (2) A very broad spectrum of alleles can be obtained representing silent, partial or complete loss-of-function, and gain- or change-of-function mutations; (3) Mutant alleles created in crop species genes with beneficial trait expression can be directly integrated in traditional breeding programs without involvement of transgenic approaches; (4) The TILLING technique is useful for the provision of allelic series, the efficient identification of mutations in (candidate) genes of interest and the low cost scanning and characterization of the gene pool for allelic or haplotype variation in genes of interest. The TILLING strategy relies on large populations of mutagenised plants that are investigated for point mutations within a given sequence. New European TILLING resources currently being developed for oilseed rape are likely to play a central role in future breeding for new and improved seed and meal quality traits.

RNAi and TILLING can potentially be used to target antinutritive components like sinapine and phytate in yellow-seeded oilseed rape genotypes, raising the possibility of novel varieties with higher protein yield and quality combined with an improved overall meal digestibility. Such varieties could also open the market for the use of rapeseed protein as an alternative to soybean for human nutrition, a development that would considerably increase the competitiveness of European rapeseed meal in comparison with imported soybean meal.

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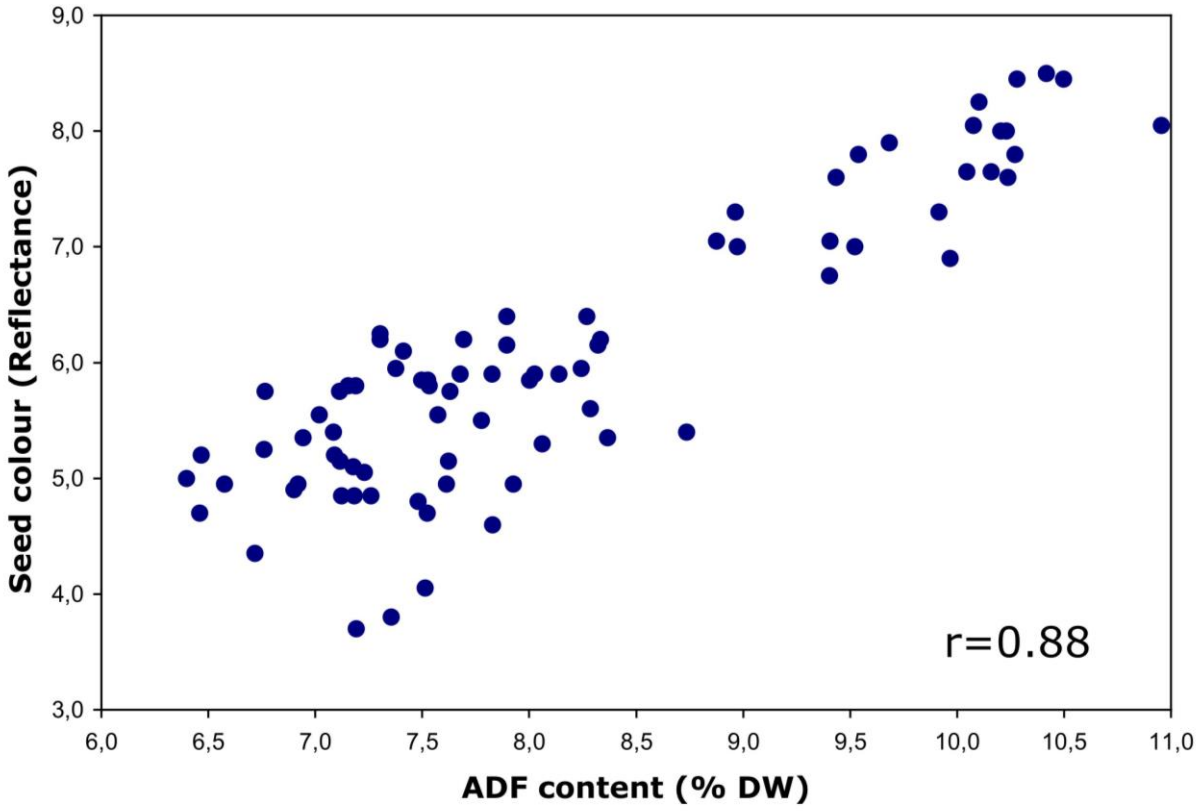

Figure 1. Correlation between seed colour (absorbance) values and content of acid detergent fibre (ADF) in a segregating doubled haploid population derived from a cross between a yellow-seeded 00-quality winter oilseed rape line and a black-seeded ++-quality line. The ADF content (as percentage of total seed dry weight) was strongly correlated to seed colour, with light-seeded genotypes (lower absorbance values) being associated with a significant reduction in ADF. For more details see Badani et al. (2005b).