

Improving rapeseed meal quality by reduction of condensed tannins

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Abstract

Condensed tannins can potentially have a major impact on animal nutrition, particularly because of their ability to form indigestible, astringent or bitter-tasting complexes with proteins. One option to overcome this problem is the breeding of yellow-seeded rapeseed with reduced condensed tannins in the seed coat. This might be achievable via selection of genotypes with smaller endothelium cells and consequently a spatial reduction in condensed tannin accumulation (seed coat structural cell mutants), or alternatively by selection of genotypes with reduced biosynthesis of condensed tannins (flavonoid biosynthesis mutants). Both types of *transparent testa* (*tt*) mutants are well-characterised in *Arabidopsis*, however the genetic basis of the yellow-seed trait in the polyploid genome of rapeseed is still not completely understood. As part of ongoing research into breeding of yellow-seeded rapeseed, the aim of this work is the development of analytical and screening procedures for condensed tannins in oilseed rape seeds, with a view towards isolation and characterisation of the responsible genes in yellow-seeded mutants.

Introduction

Plant tannins make up a distinctive group of high molecular weight phenolic compounds that have the ability to complex strongly with proteins, starch, cellulose and minerals. Chemically three groups of tannins are distinguishable: phlorotannins, hydrolysable and condensed tannins (*syn.* proanthocyanidins). The phlorotannins have been isolated from species in several genera of brown algae, whereas the hydrolysable and condensed tannins are widely distributed throughout the plant kingdom. Plant tissues containing tannins include bark, wood, fruit, seeds, leaves, roots and plant galls. Different groups of tannins have been associated with the maintenance of seed dormancy, while others have allelopathic and bactericidal properties. In rapeseed (*Brassica napus* L.) condensed tannins are largely responsible for the dark colour of the seed coat, where they accumulate predominantly in the endothelium cell layer between the outer integument and the aleurone layer. Whereas the proportion of condensed tannins in the cotyledons of *B. napus* seeds is comparatively low (only 0.1-0.5% of dry weight), condensed tannins in dark-seeded *B. napus* can comprise up to 6% of the seed coat. This means that they contribute significantly to rapeseed meal, with a total content of up to 800 mg/100g after oil extraction.

By localising quantitative trait loci (QTL) for condensed tannin content in *B. napus* seeds and comparing these to the positions of promising candidate *tt*-genes, we hope to develop closely-linked molecular markers for selection regarding important genes involved in the accumulation of antinutritive tannins in rapeseed meal.

Methods and Materials

A dense genetic map was generated from a population of 166 doubled-haploid lines derived from a cross between an inbred line of the black-seeded German winter oilseed rape cultivar 'Express' and the true-breeding, yellow-seeded line '1012/98', both with 00-seed quality. Significant QTL involved in seed colour, total flavonoids and in the contents of important flavonoid compounds were mapped using the software PLAB-QTL based on analyses of seeds grown in field trials in Einbeck and Gross Gerau, Germany from 2003-2005. Seed colour was measured quantitatively based on digital reflectance values. Total condensed tannins were estimated by spectrometry based on the vanillin assay, while individual flavonoid compounds were quantified via HPLC using standards to identify peaks.

Results

Large differences were found between the black- and yellow-seeded mapping parents in total condensed tannin content and in different flavonoid compounds measured by HPLC (Figure 1). In particular the yellow-seeded line showed an absence of epicatechin, which in *Arabidopsis* is thought to be the major precursor of condensed tannins in the seed coat (Lepiniec et al. 2006). However the correlation of total flavonoid content with seed colour was comparatively low (Figure 2). This suggests that a significant proportion of the total seed flavonoids are non-coloured flavonoids that do not influence the seed colour but may still have a nutritional effect by forming indigestible compounds with proteins, for example.

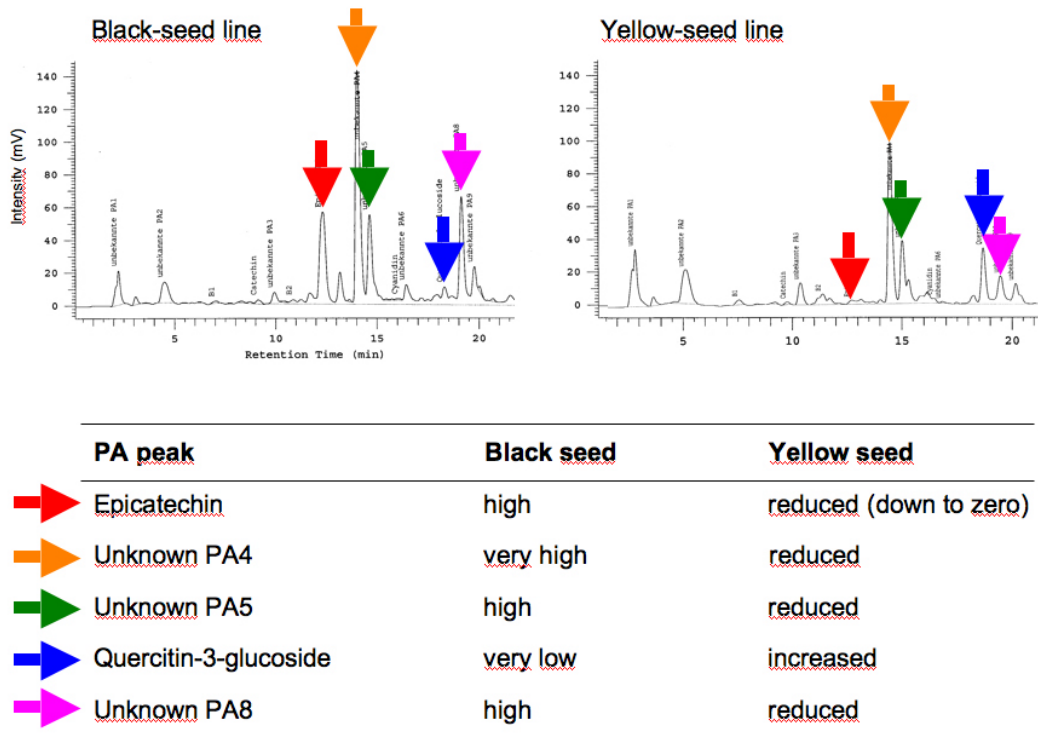


Figure 1: HPLC measurements of flavonoid compounds in black and yellow seeded lines allowed the identification of components with large differences in levels associated with yellow seed colour. In particular epicatechin, a precursor of condensed tannins, was absent in the parental yellow-seeded line, while quercitin-3-glucoside was significantly increased compared to the black-seeded parent. A number of unidentifiable proanthocyanidins, presumably including condensed tannins, also showed significant reductions in yellow-seed lines.

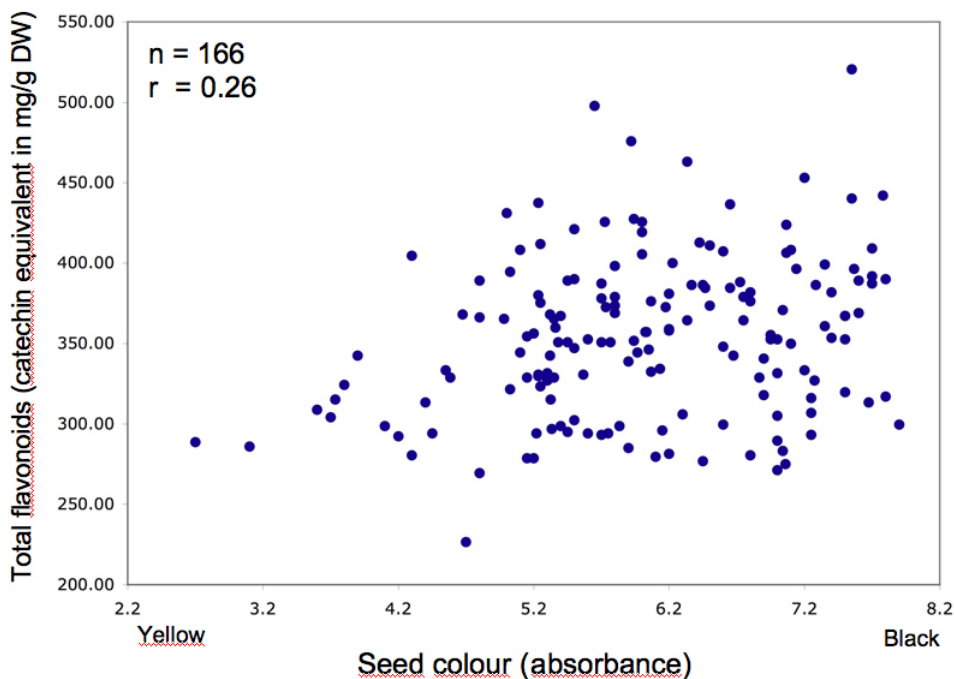


Figure 2: Correlation between seed colour and total seed flavonoid content in 166 DH lines from the cross Express 617 (black seed) x 1012-98 (yellow seed). The correlation is very weak, suggesting that a significant proportion of the total flavonoids are composed of colourless compounds that have no effect on seed colour.

Preliminary localisation of significant QTL for total flavonoids and individual flavonoid compounds indicated that some loci co-localise with major QTL for seed colour that were described previously by Badani et al. (2006). On the other hand numerous QTL were detected that do not appear to have a significant effect on seed colour but may represent epistatic gene loci that contribute to the high environmental variation seen in yellow- and brown-seeded *B. napus* lines. By comparing the levels of flavonoid compounds with QTL it may be possible to identify genes within the flavonoid biosynthesis pathway

(Routaboul et al. 2006, Lepiniec et al. 2006; Figure 3) that are responsible for minor differences in seed colour in *B. napus*.

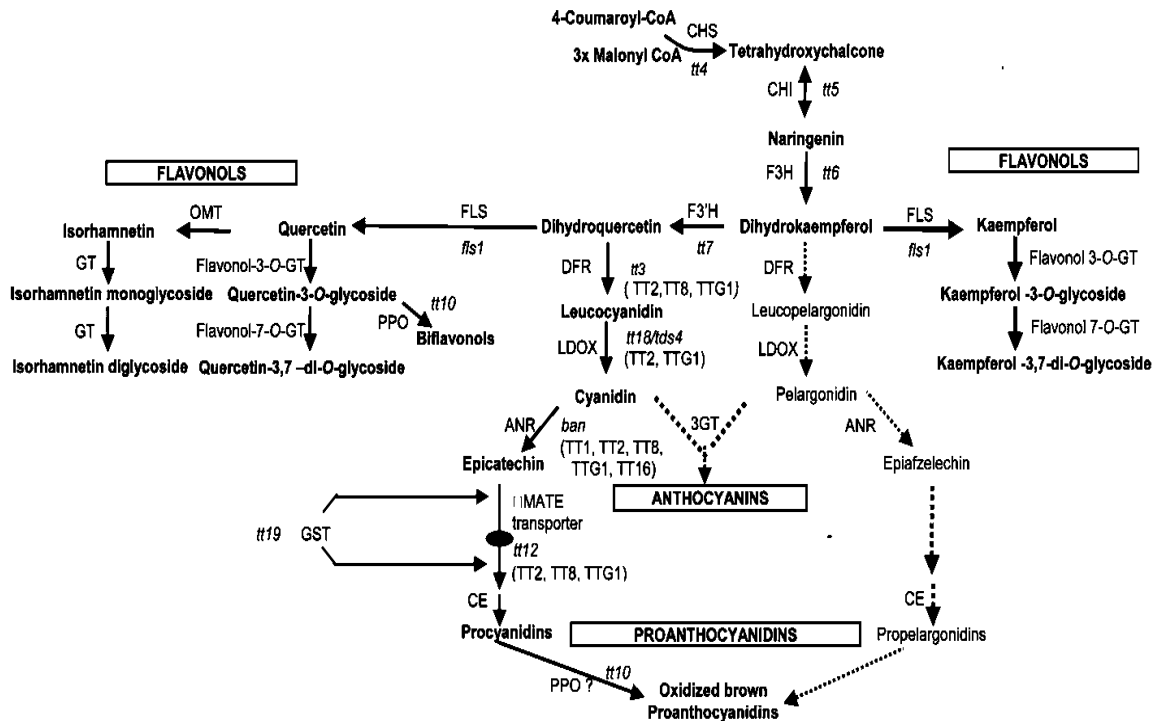


Figure 3: The flavonoid biosynthesis pathway of Arabidopsis, including key structural and regulatory *transparent testa* (TT) genes. Detailed knowledge of flavonoid biosynthesis can help to identify genes involved in differences observed in flavonoid composition between yellow- and black-seeded oilseed rape lines. In particular, the lack of epicatechin in yellow-seeded lines may point to mutations in structural or regulatory genes directly involved in epicatechin accumulation. Diagram from Routaboul et al. (2006).

Discussion

Mapping of candidate genes for flavonoid biosynthesis and comparison to QTL for major flavonoid compounds will enable us to identify genes involved in the expression and control of seed colour in yellow-seeded oilseed rape. Mapping of orthologous copies for relevant Arabidopsis *transparent testa* genes, with the help of synteny-based and map-based cloning techniques, is currently in progress to help achieve this aim.

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