QTL mapping for yellow seed colour in oilseed rape (*Brassica napus*)

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ABSTRACT

Elucidating the genetic control of yellow seed colour is one primary aim of the collaborative project "Genome analysis in rapeseed (GARS)" within the German genomics initiative "GABI". In order to identify gene loci contributing to this trait, segregating mapping populations have been developed from two distinct sources of yellow seed colour. In each case the segregation ratios for the trait suggest a trigenic inheritance with expression of three homozygous recessive alleles necessary for manifestation of the yellow-seed trait. For each population genome maps constructed using AFLP and SSR markers are being used to identify molecular markers and QTL associated with seed colour.

Key words: Rapeseed – seed colour – QTL mapping – AFLP – SSR

INTRODUCTION

The yellow seed colour trait is of particular interest for oilseed rape breeding not only due to its association with increased oil and protein content, but also because a reduced crude fibre content after oil extraction improves the feed quality of rapeseed meal (see Baetzel et al. 2003). Depending on the source of yellow seediness, two to three genes are thought to be involved in the genetic control of testa colour in *Brassica napus*, whereby the expression of homozygous recessive alleles at all loci is necessary for manifestation of yellow seed colour. For *Arabidopsis thaliana* a large number of different mutants have been described which show the transparent testa (*tt*) phenotype and the corresponding genes have been identified and in some cases cloned (reviewed by Winkel-Shirley 2001). This project aims to exploit comparative genetic and physical mapping to identify and characterise the *B. napus* loci for seed colour. We are particularly interested to investigate if any of the known Arabidopsis *tt* mutants correspond to *B. napus* loci for yellow seediness.

MATERIALS AND METHODS

In order to identify gene loci contributing to this trait, segregating mapping populations have been developed from two distinct sources of yellow seed colour. Pure yellow-seeded lines were developed by microspore culture. The first population (*YE1*) derived from a cross between a yellow-seeded *B. napus* wintertype '25629-3' and the black-seeded DH line 'K26-96'. 110 doubled haploid (DH) lines produced by microspore cultivation were used for the subsequent genetic and QTL mapping. Additionally, a second population (*YE2*) comprising 179 F_2 individuals was derived from a cross between the black-seeded oilseed rape variety 'Express 617' and the yellow-seeded line '1012/98'. Assessment of seed colour for localisation of QTL associated with seed colour was performed by digital image analysis on F_3 seed or DH lines after propogation of the populations under field and/or controlled greenhouse conditions. Genetic maps were generated using 28 AFLP primer combinations. In order to align the resulting maps they are being expanded using SSR markers from public and commercial sources, preferentially selected as polymorphic in both crosses.

RESULTS AND DISCUSSION

Genome maps (Fig 1) were produced for the DH population from *YE1* and the F_2 population from *YE2*. In each population one large-effect QTL and two small-effect QTL for seed colour were detected (Fig 2). To date seven linkage groups could be aligned using markers common to both maps. The linkage groups bearing the second-largest seed colour QTL contain four common markers, suggesting that these QTL correspond to the same locus in the two yellow-seeded sources. On the other hand, reference markers suggest that the large-effect QTL are possibly on different linkage groups in the two populations and therefore may represent different genetic loci. To study in depth the genetic mechanisms of yellow-

seediness in *B. napus*, potential associations between the seed colour QTL and *Arabidopsis thaliana* transparent testa (*tt*) genes are to be examined by localising the respective *B. napus* orthologues for selected *tt* loci in the existing maps.

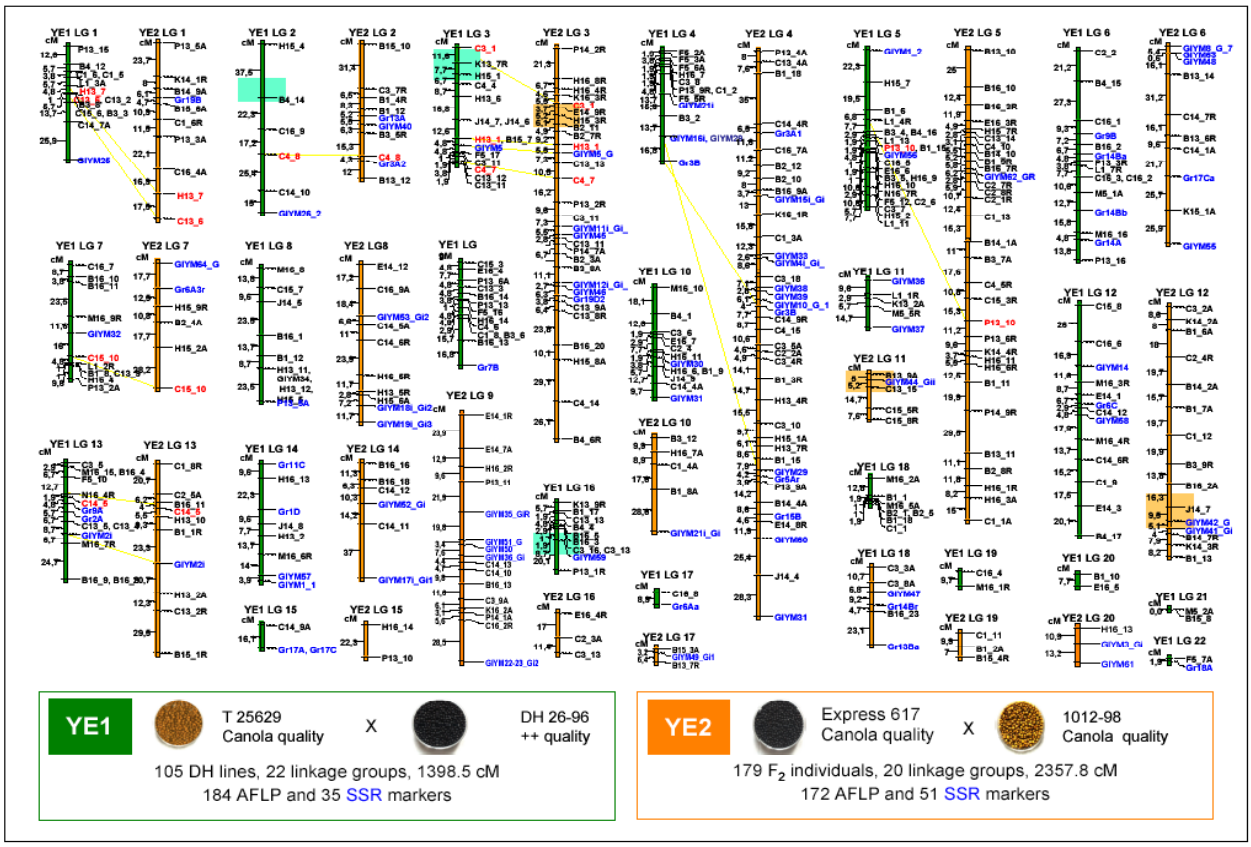

Fig. 1 Genetic maps describing linkage of AFLP and SSR markers for a DH population from *YE1* (green) and an F1 population from *YE2* (orange), two different segregating crosses between distinct yellow and black seeded oilseed rape genotypes. To date seven linkage groups could be positively aligned between the two maps based on common markers (yellow lines). Alignment of the remaining linkage groups is being achieved with reference markers from two other maps. In each map seed colour QTL (coloured blocks) were found on three linkage groups, one with a very large effect and two with smaller effects.

The genetic maps were constructed primarily using AFLP markers. Using 27 AFLP primer combinations, 211 polymorphic loci could be identified in the DH population originating from *YE1*, and 245 in the F_2 population from *YE2*. The high quality of the AFLP patterns allowed around 80 % of the markers to be codominantly scored in the F_2 population. To assist in map alignment the marker analyses included all mapping parents from both populations, together with the parents from another oilseed rape genome map from the University of Göttingen (Uzunova et al. 1995). To increase the marker density and assist in map alignment, the AFLP maps have been supplemented with SSR markers. To date 35 polymorphic SSR loci have been mapped in *YE1* and 51 in *YE2*. Because the two mapping populations originate from quite distinct crosses, only a relatively low number of common markers could be localised in both maps. To assist in alignment of those linkage groups where no common markers could be identified, further reference SSR markers with known positions in other *B. napus* maps are being added to the two maps.

Seed colour QTL (Fig. 2) were detected based on data from plants grown under controlled conditions in the glasshouse (YE1) and in the field (both populations) using a digital image analysis system to score colour values for seed lots. In both cases one large QTL accounted for more than 50 % of the total phenotypic variation for the trait. Linkage group alignment identified four common consensus markers in the

linkage groups containing the second largest QTL, suggesting that this QTL represents the same genetic locus in each of the two yellow-seeded rapeseed sources. Supplementation of the linkage groups containing the remaining QTL with further consensus SSR markers is progressing to enable us to determine whether the same major loci control seed colour in the two crosses. Information on the genetic basis of the respective QTL will be obtained through candidate gene and microsynteny analyses. In particular we hope to map homoeologous loci to *Arabidopsis tt*-genes (Lotz et al. 2003) in *B. napus* and compare whether any of these candidate genes involved in flavonoid biosynthesis are located in genome regions associated with seed colour QTL.

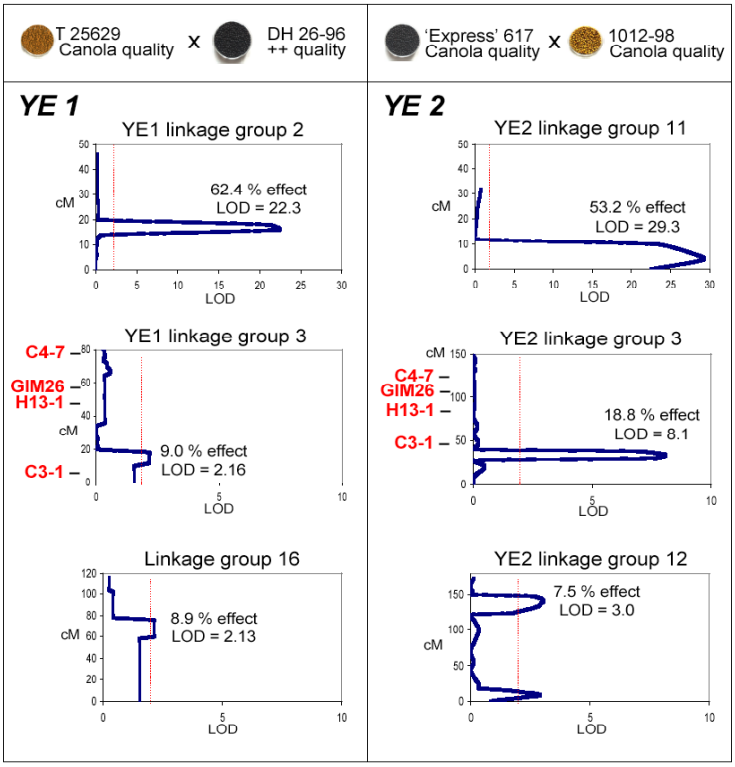

Fig 2. For each of the two yellow-seed sources, three seed colour QTL were detected. In both cases one large QTL accounted for more than 50 % of the total phenotypic variation for the trait and the other QTL showed smaller effects for which the significance is still under investigation. The linkage group with the largest QTL in the population *YE1* contains one AFLP marker in common with a linkage group that shows no QTL in population *YE2*. This may indicate that different major loci are responsible for expression of seed colour in the two yellow seeded sources. The second-largest QTL in the respective populations appear to represent the same locus, as evidenced by the common AFLP marker C3-1 adjacent to the QTL and three further aligned markers on the respective linkage groups in the two maps.

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