Winter Oilseed Rape's Meal Quality

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Rapeseed (Brassica napus L.) meal is an important source of protein, but the presence of anti-nutritional compounds, such as fibre and glucosinolates, still limits its use as a livestock feed. Understanding the genetic basis of seed fibre biosynthesis would help to manipulate its content in seeds of oilseed rape. Here, we applied high-resolution skim genotyping by sequencing (SkimGBS) and characterised 187,835 single-nucleotide polymorphism (SNP) markers across a mapping population subsequently used for a genetic mapping study (R/qtl).

QTL mapping · candidate genes · genetic markers · rapeseed meal quality · fibre · glucosinolates · seed colour

1. Introduction

Brassica napus L. (rapeseed, canola, oilseed rape, OSR) is grown worldwide for the production of vegetable oil, biodiesel, and protein-rich rapeseed meal (RSM) after oil extraction. A current major breeding aim in oilseed rape is to improve the quality of RSM by enhancing protein quality and quantity and reducing levels of anti-nutritional compounds, such as seed fibre and glucosinolates. The indigestible fibre present in the seed coat, including lignin, cellulose, and hemicellulose components, is the main anti-nutritional compound in black-seeded oilseed rape, which affects the taste and appearance of RSM and limits its use as a poultry feed [1]. The fibre also has a negative effect on seed oil and protein content in *B. napus*, as the synthesis of cellulose and hemicellulose redirects photosynthetic assimilates from oil and protein into sugar biosynthesis, which may result in reduced content of these two compounds [2][3].

Much effort has been put into the research of yellow and light-colour seeded genotypes of OSR since such genotypes have thinner seed coats, less fibre, and higher seed oil and protein content. However, these yellow-seeded genotypes have not been successfully introduced into the market due to other unfavourable agronomic traits. Seed coat colour is a difficult morphological marker for selection due to its low heritability, multiple gene inheritance, and maternal and environmental effects (light, temperature) regulating the trait [4]. Fibre, when compared to seed colour, is a more stable trait for selection [5]; therefore, understanding the genetic mechanism regulating seed fibre content is essential in improving seed oil content and meal value of *B. napus*.

Numerous studies have been performed to identify QTL for seed colour and fibre content with different effects in different genetic backgrounds. However, many of these studies revealed one major locus on chromosome A09 that explained most of the trait variation [6][7][8][9][10][11]. The QTL for fibre and seed colour are linked, as the biochemical pathways leading to the synthesis of these two economically important traits have common precursors, such as *p*-coumarate [6][12]. Although the genetic mechanism of seed fibre and colour biosynthesis have been extensively studied in Arabidopsis, the identification of their functional orthologs and understanding their mode of action in *B. napus* remains challenging. The complexity of the *B. napus* genome, which is characterised by multi-gene families, homeologous exchanges [13], and gene presence/absence variation (PAV) [14][15], further complicates the identification of key genes regulating important agronomic traits across different environments and cultivars of oilseed rape. However, whilst the latest genotyping technologies and bioinformatics allowed the identification of some candidate genes regulating fibre content and seed colour in *B. napus* [5][7][8][16], further investigations are required.

Here we performed QTL mapping analysis to unravel the genetic basis of traits affecting RSM quality, including seed oil, protein, fibre, glucosinolate, and seed coat colour using a doubled haploid (DH) mapping population derived from a cross between lines with high variation for fibre traits: yellow-seeded ‘Z114’ and black-seeded ‘M305’. The aim of the study was to identify SNPs significantly associated with the traits of interest in order to establish candidate genes and develop genetic markers that could be used in breeding programmes aimed to improve RSM.

2. Discussion

Genotyping by sequencing allows analysis of genome-wide sequence variation among individuals, which enables accurate and efficient identification of genes controlling important agronomic traits [17].

We observed a large variation in SNP coverage between the chromosomes of the parental lines, from 574 on chromosome A08 to 15,152 on chromosome C07. Whilst A08 has the lowest number of SNPs, the short length of this chromosome makes it appears more extreme. The low marker density on chromosome A08 has already been observed in our previous study [18], and it might be due to the missing read coverage in both of the parental lines. Missing read coverage could be caused by differences between the genome of the parental lines and the Darmor reference that we used to align the reads or due to the genomic differences between those lines. The low SNP density on chromosome A08 may also be a result of a relatively low level of genetic diversity between the parental lines as they both represent low erucic acid and low glucosinolates (double zero) winter-type oilseed rape [19]. Intensive breeding of double zero oilseed rape led to a restricted gene pool, which reduces its genetic variation. Chromosome A08 could also represent a genomic region of identity-by-descent that was not efficiently disrupted by recombination during selection [20].

Seed fibre and glucosinolates considerably reduce the value of *B. napus* meal, especially for poultry; therefore, the identification of functional candidate genes related to these traits is of importance [21][22][23][24]. In the studied population, derived from the yellow-seeded ‘Z114’ and black-seeded ‘M305’ DH lines, the highest phenotypic variation was found for fibre (ADF/NDF) and SCC. These traits were correlated with glucosinolates and protein content in previous studies [25]. Decreased amounts of cell wall polysaccharides in seeds containing less fibre can cause increased carbon availability for protein deposition [26].

The correlation found between ADF/NDF/SCC/GLS/SPC can be reflected in the identification of common QTL for the studied traits (e.g., qSCC/ADF/NDF/GLS) and many interesting candidate genes related to plant cell wall, lignin biosynthesis underlying SPC and GLS-QTLs. The QTL for these traits was found on chromosomes A02, A04, A06, C02, C06, C07, and C08, with PVE ranging between 21.52% and 31.65%. The regions of A02, A04, A06, C02, C06, and C08 were also found to be correlated with seed fibre by Miao [11]. It is difficult to compare the positions of the identified QTL regions due to different reference genomes used by Miao (ZS11) and here in this study (Darmor). QTL for ADF and GLS were found previously on chromosome C02 [10], whereas A06, C08, and A09 regions were repeatedly detected for SCC in a GWAS study performed by Wang 4. Here we found a similar region of A06 (20.5 Mbp) and C08 (distal end) to that found in the Wang GWAS study. Interestingly, the distal region of chromosome C08 correlated with SCC, NDF, and GLS in our study showed high homology with a region of chromosome A09 [4][13], detected as a major QTL for seed fibre and seed colour in various genetic backgrounds [2][5][6][7][8][9][27]. Major QTL for seed colour were also detected on A09 or C08 chromosomes, depending on the genetic background [28], which also indicates that different black-seeded forms may possess different seed colour genes. Previous studies also showed a correlation with seed fibre on chromosomes A05 and C05; however, here, we did not detect any QTL on these chromosomes [3][10].

A number of candidate genes associated with seed fibre deposition, seed coat development, flavonoid, and anthocyanin biosynthesis were identified in previous studies [1][4][16]. Strong candidates include cinnamoyl-CoA reductase 1 (*CCR1*) and cinnamyl alcohol dehydrogenase (*CAD2/CAD3*), *SEC8*, *PAL4*, *CESA3*, and *GPAT5* [2][3][5][7][8]. Some of the candidate genes identified in this study belong to the same gene family but are located on a different chromosome. The most interesting candidate genes *BnaC02g38340D* and *BnaC02g38710D* were identified here for seed fibre, and SCC was located on chromosome C02 and encoded transparent testa 10/laccase-like 15 *(TT10/LAC15)* and transparent testa 4 (*TT4*), respectively. In other studies, *TT4* was found to be associated with ADL (acid detergent lignin) on chromosome C09 (*BnaC09g43250*) [3], and *TT10* was detected as a major gene for SCC and fibre on A09 [5][9]. Transparent testa (*TT*) are key enzymes in proanthocyanidins and lignin biosynthesis pathways [9][29][30][31].

Since the highest phenotypic variation in the Z114 × M305 mapping population was found for ADF/NDF and SCC, the strongest emphasis on the identification of candidate genes was made for genes known to be involved in carbohydrate metabolism and flavonoid biosynthesis. In the *qGLS-A02* region, one of the identified genes, Korrigan2 (*KOR2*), encodes endo-1,4-β-d-glucanase, known to be involved in cellulose synthesis [32][33]. Interestingly, the same region of chromosome A02 was found to be correlated with seed fibre in a study performed by Miao [11]. The interesting candidate genes underlying QTL regions on chromosome A04 include pectin methylesterase (*BnaA04g27070D*) and pectin lyase-like protein (*BnaA04g25420D*). The presence of SNP variation in these genes and their relatively close physical locations from the QTL (100–500 kbp) indicates that they are very likely regulators of fibre composition in *B. napus* seeds. Another gene, *BnaA04g03060D,* located 10 kbp from *qGLS-A04*, encodes β-1,3-glucanase 3, glycoside hydrolase, which functions in cell wall degradation [34]. Other interesting genes underlying QTL on chromosome C08 include cellulase, glycosyl hydrolase family 5 *GH5,* and UDP-glycosyl transferase *UGT73C7,* which are known to be involved in carbohydrate metabolic process and cell wall lignification [35][36]. Another strong candidate is peroxidase 64 (*PRX64*) (*BnaC07g05860D*), located 173 kbp from the *qSCC/ADF-C07,* the major oxidase enzyme known to play a role in proanthocyanidins and lignin biosynthesis [9][29][30][33]. RING-type E3 ubiquitin transferase (*CMPG1*) (*BnaC07g05860D*) with two SNPs located 16,6 kbp from the QTL. These genes are known to play a role in lignin biosynthesis and response to chitin [37][38][39]. A key gene found to be correlated with seed colour in previous studies, namely transparent testa 12 (*TT12*), was not identified in this study. *TT12* encodes a multidrug and toxic compound extrusion (MATE) secondary transporter that is specifically expressed in the developing seed coat and is involved in the transportation of proanthocyanidin precursors into the vacuole [40]. It was found that the *BnaC06g17050D* gene, which is orthologous to Arabidopsis *TT12*, is associated with seed coat colour in oilseed rape [4]. However, we could not find any association between this gene and SCC in the present study.

3. Conclusions

In conclusion, a QTL genetic mapping study using an NGS SkimGBS approach allowed us to identify several promising genes, including PE, PLL, TT10/LAC15, SUS2, and GH5, which provides insight into the complex genetic architecture of seed fibre and colour biosynthesis in B. napus. Understanding the mechanism of action and causal polymorphisms of these genes will provide a better understanding of the role of those genes in the regulation of complex traits affecting RSM quality.

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