Plant Biotechnology Journal

Plant Biotechnology Journal (2014), pp. 1–10 doi: 10.1111/pbi.12186

SNP markers-based map construction and genome-wide linkage analysis in *Brassica napus*

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Received 29 July 2013;
revised 29 January 2014;
accepted 21 February 2014.
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Keywords: genetic linkage mapping, single nucleotide polymorphism, quantitative trait loci, blackleg resistance, flowering time.

Summary

An Illumina Infinium array comprising 5306 single nucleotide polymorphism (SNP) markers was used to genotype 175 individuals of a doubled haploid population derived from a cross between Skipton and Ag-Spectrum, two Australian cultivars of rapeseed (*Brassica napus* L.). A genetic linkage map based on 613 SNP and 228 non-SNP (DArT, SSR, SRAP and candidate gene markers) covering 2514.8 cM was constructed and further utilized to identify loci associated with flowering time and resistance to blackleg, a disease caused by the fungus *Leptosphaeria maculans*. Comparison between genetic map positions of SNP markers and the sequenced *Brassica rapa* (A) and *Brassica oleracea* (C) genome scaffolds showed several genomic rearrangements in the *B. napus* genome. A major locus controlling resistance to *L. maculans* was identified at both seedling and adult plant stages on chromosome A07. QTL analyses revealed that up to 40.2% of genetic variation for flowering time was accounted for by loci having quantitative effects. Comparative mapping showed *Arabidopsis* and *Brassica* flowering genes such as *Phytochrome* A/D, *Flowering Locus C* and agamous-Like MADS box gene *AGL1* map within marker intervals associated with flowering time in a DH population from Skipton/Ag-Spectrum. Genomic regions associated with flowering time and resistance to *L. maculans* had several SNP markers mapped within 10 cM. Our results suggest that SNP markers will be suitable for various applications such as trait introgression, comparative mapping and high-resolution mapping of loci in *B. napus*.

Introduction

Rapeseed, *Brassica napus* L., (2n = 4× = 36, genome AACC) is grown mainly for the production of edible oil for human consumption and is the most important member of the Brassicaceae family, which consists of more than 3350 species. It originated as a result of interspecific hybridization between *Brassica rapa* (AA genome, 2n = 2× = 20) and *Brassica oleracea* (CC genome, 2n = 2× = 18). Recently, the genomes of *B. rapa* and *B. oleracea* have been sequenced and annotated (Wang *et al.*, 2011b, http://www.ocri-genomics.org/bolbase). Recent innovations in genome sequencing technologies and bioinformatics have made it possible to exploit molecular markers based on the variation in gene sequences. As a result, single nucleotide polymorphism (SNP) and insertion/deletion (InDEL) markers have been developed in various organisms, including *B. napus* (Ganal *et al.*, 2009; Hu *et al.*, 2012; Trick *et al.*, 2009). SNPs are currently being recognized as the markers of choice for genetic diversity analysis, genome-wide association studies and genomic selection due to their abundance in the genome (every 44–75 bp), high rate of polymorphism and their suitability in assaying several thousand markers in parallel on automated platforms (Ganal *et al.*, 2012; Gore *et al.*, 2009). Second-generation, high-throughput sequencing technologies, which are available on platforms provided by a number of companies such as Illumina, have been employed for genome sequencing in various crops including Brassicas (Bancroft *et al.*, 2011; Michael and Alba, 2012; Trick *et al.*, 2009; Wang *et al.*, 2011b) and can be employed in the discovery of SNPs. Recently, several SNP markers have been identified in *B. napus* (Bus *et al.*, 2013; Delourme *et al.*, 2013; Durstewitz *et al.*, 2010; Tollenaere *et al.*, 2012; Trick *et al.*, 2009) and further assayed for polymorphisms on various high-throughput platforms such as GoldenGate, Infinium and second-generation sequencing, permitting rapid scoring of several thousand markers in parallel (Ganal *et al.*, 2012). Dalton-Morgan *et al.* (2014) developed an Infinium array comprising 5306 SNP markers (designated as the 6K_Evie *B. napus* chip) from *B. napus* short read sequence data. However, its utility in high-density map construction and identification of qualitative and quantitative trait loci in *B. napus* using genome-wide linkage analysis has yet to be explored.

Two Australian *B. napus* cultivars, Skipton (S) and Ag-Spectrum (AS), exhibit genetic variation for flowering time,

Please cite this article as: Raman, H., Dalton-Morgan, J., Diffey, S., Raman, R., Alamery, S., Edwards, D. and Batley, J. (2014) SNP markers-based map construction and genome-wide linkage analysis in *Brassica napus*. *Plant Biotechnol. J.*, doi: 10.1111/pbi.12186

