GENETIC DISSECTION OF NATURAL VARIATION FOR FLOWERING TIME IN RAPESEED

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Background

Knowledge of flower development and maturity particularly in drought periods is critical for canola (*Brassica napus L.*) growers. Qualitative and quantitative trait loci (QTL) controlling flowering time have been identified in several overseas populations of *Brassica* species (Osborn et al. 1997; Zhao et al. 2010). Identification of such loci in Australian canola populations is vital for developing new canola germplasm having an optimal time of flowering to maximise yield. Flowering time is regulated by several genes and environmental cues. In Arabidopsis, five partially independent pathways capable of inducing flowering: photoperiod, autonomous/vernalisation, gibberellic acid, integrator and floral meristem identity, and repressors have been revealed (Bernier and Perilleux 2005; Dennis and Peacock 2007; Michaels 2009). Signals originating from these regulatory pathways are integrated at different levels by the set of integrator genes (Ausin et al. 2005). Other factors such as plant density, nutrient supply, drought stress and pathogen pressure can also influence flowering time. In order to dissect such complexity, understanding of genetic control of flowering is important.

In this study, we characterised 188 accessions of Brassica, collected from different parts of the world, and identified (i) genetic variation in flowering time, (ii) genomic regions associated with flowering time, and (iii) aligned these genomic regions associated with various components of flowering time with next generation sequence data of cultivars Skipton and Ag-Spectrum.

Materials and Methods

One hundred and eighty one accessions of *Brassica napus* L, one of *B. rapa*, three of *B. juncea* and two of *B. carinata*, collected from different parts of the world, were characterised for various components of flowering time [days to first flower, days to last flower, duration of flowering, response to vernalisation (no vernalisation vs. 8 wk vernalisation at 4-6°C) and photoperiod (16 hr vs. 8 hr photoperiod]. All genotypes were evaluated under controlled environment growth cabinets, glasshouse and field conditions. In order to identify loci controlling flowering time, a DH population derived from Skipton and Ag-Spectrum was also phenotyped under glasshouse and field conditions, and genotyped using molecular markers.

DNA was isolated from approx. 10 week-old glasshouse-grown seedlings using a standard phenol-chloroform method and further analysed for polymorphisms using markers based upon Diversity Array Technology (DArT P/L, Canberra, Australia), simple sequence repeat (SSR), sequence-related amplified polymorphism, sequence characterised amplified region and candidate genes. The genetic linkage map was produced using Map Manager version QTL20b (Manly *et al.* 2001) using the Kosambi mapping function, as described previously (Raman *et al.* 2009). Accuracy of the marker order within linkage groups was checked using the R/qtl statistical analysis package (Broman *et al.* 2003), RECORD computer package (van Os *et al.* 2005), and compared with previously published maps (Choi *et al.* 2007; Lowe *et al.* 2004; Piquemal *et al.* 2005; Suwabe *et al.* 2008; Suwabe *et al.* 2006). An integrated map consisting of 671 markers was subsequently employed for identifying QTL associated with flowering time using the whole genome average interval mapping approach (Verbyla

et al. 2006). All QTL analyses were conducted using the ASREML-R package (Butler *et al.* 2007). Association mapping was carried-out as described previously (Raman et al. 2010).

Results and Discussion

Natural variation in days to first flower, days to last flower, duration of flowering, and response to vernalisation was observed in the 188 genotypes grown under controlled and field conditions. The flowering time ranged from 31 to 153 days under unvernalised conditions (Fig 1) as compared to 31 to 87 days under vernalised conditions.

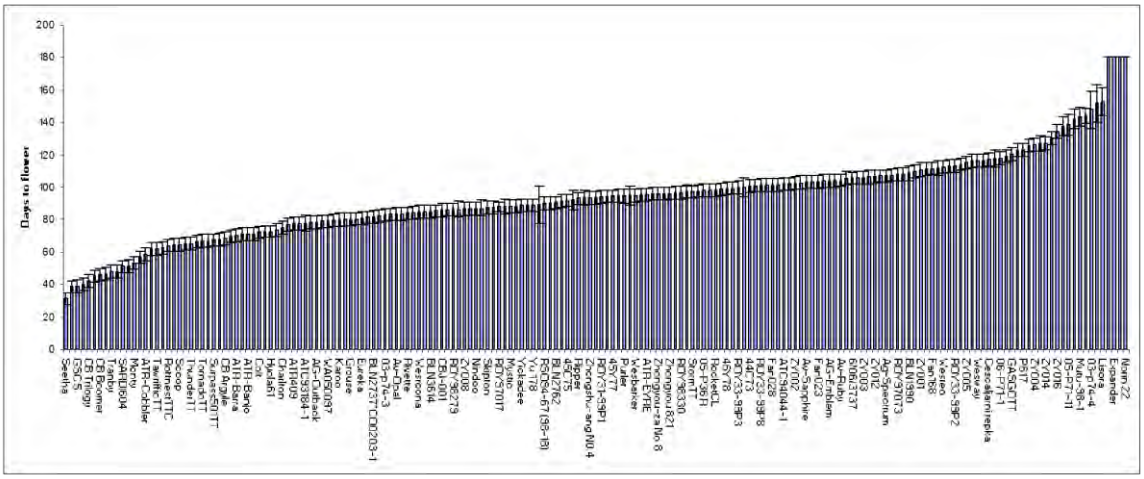

Fig 1: Phenogram showing natural variation in flowering time in 188 genotypes of Brassica.

This strongly suggested that vernalisation regulates flowering time in Brassica genotypes investigated in this study. Some of the Australian canola varieties such as CB Triology, CB Trigold, CB Tanami, and CB Pilbara didn't significantly respond to vernalisation. In order to determine location, size and effect of QTLs controlling flowering time, we performed QTL analysis utilising a framework molecular map developed in the DH population from a cross between the Australian cultivars Skipton/Ag-Spectrum. QTL analysis indicated that flowering time is a complex trait and is controlled by at least 38 loci with LOD ≥ 2.0, localized on chromosomes A1, A2, A3, A4, A5, A6, A7, A10, C1, C2, C3, C4, C5, and C6. These loci accounted for between 2% and 56% of the total genotypic variation. Flowering traits exhibited low to high heritability values ranging from 36% (duration of flowering under vernalised conditions) to 89% (days to first flower). Some of QTLs identified are shown in Table 1.

Table 1: Localisation of QTLs associated with days to flower under unvernalised conditions in a DH population from Skipton/Ag-Spectrum

Chr	Left Marker	Right Marker	LOD Score	%Gen Var (r^2)	Additive Effect
A1	*XbrPb-808330*	*Xra3-e05b*	2.4	6.0	Skipton
A1	*Xol12-F11*	*XbrPb-808614*	2.8	3.4	Ag-Spectrum
A3	*XbrPb-839739*	*XbrPb-658284*	12.0	21.4	Ag-Spectrum
A6	*Xcb10006*	*Xbrms227*	4.2	3.8	Ag-Spectrum
A7	*Xbrms186*	*Xna12-e09*	5.8	6.5	Skipton
C2	*XbrPb-660999*	*XbrPb-661396*	10.5	15.8	Skipton
C3	*Xcb10079a*	*XbrPb-661557*	9.9	10.2	Ag-Spectrum
C7	*XbrPb-660971*	*XbrPb-660868*	4.7	5.9	Skipton

We verified some of our QTL analysis results using the association mapping (AM) approach. All the 188 accessions were genotyped using 1,426 markers based upon DArT and SSR markers and further utilised for AM analysis. Some of the major genomic regions conditioning flowering time were similar. Aligning genetic regions that showed significant association with flowering time in the doubled haploid population with the genome of *B. rapa,* allowed us to co-localise associated markers with published candidate genes that control flowering time in Arabidopsis and Brassica species. In order to identify SNPs underpinning phenotypic variation for flowering time, we also analysed the whole genome GAIIx sequence data generated from parental lines Skipton and Ag-Spectrum used for mapping flowering time QTLs. Results on such SNP variants will be presented.

Conclusion

Our results indicated that both the QTL and AM approaches are suitable for associating phenotypic variation for flowering time in Brassicas with markers, and with candidate genes causing such variation. This knowledge has enhanced our understanding of the molecular mechanism controlling flowering time in the adapted germplasm available to Australian commercial canola breeding programs.

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