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Identification of quantitative trait loci controlling developmental characteristics of *Brassica oleracea* L.

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Abstract A segregating population of F_1-derived doubled haploid (DH) lines of *Brassica oleracea* was used to detect and locate QTLs controlling 27 morphological and developmental traits, including leaf, flowering, axillary bud and stem characters. The population resulted from a cross between two very different *B. oleracea* crop types, an annual cauliflower and a biennial Brussels sprout. A principal component analysis (PCA), based on line means, allowed all the traits to be grouped into distinct categories according to the first five Principal Components. These were: leaf traits (PC1), flowering traits (PC2), axillary bud traits (PC3 and 5) and stem traits (PC4). Between zero and four putative QTL were located per trait, which individually explained between 6% and 43% of the additive genetic variation, using the multiple-marker regression approach to QTL mapping. For lamina width, bare petiole length and stem length two QTL with opposite effects were detected on the same linkage groups. Intra- and inter-specific comparative mapping using RFLP markers identified a QTL on linkage group O8 accounting for variation in vernalisation, which is probably synonymous with a QTL detected on linkage group N19 of *Brassica napus*. In addition, a QTL for petiole length detected on O3 of this study appeared to be homologous to a QTL detected on another *B. oleracea* genetic map (Camargo et al. 1995).

Keywords *Brassica* vegetables · QTL · Developmental traits · Genetic analysis · Linkage mapping

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Introduction

The genus *Brassica* is of agronomic and dietary importance. Its component species, including *Brassica napus*, *Brassica rapa* and *Brassica oleracea*, provide an array of vegetable, salad, oil, mustard and fodder crops. The vegetable brassicas (*B. oleracea*) form the most-diverse morphological group including cabbage, kale, cauliflower, broccoli, Brussels sprouts, collard and kohlrabi. Until recently, most analysis of quantitative trait loci (QTLs) in *Brassica* has focused on locating QTLs for disease resistance (Camargo et al. 1995; Pilet et al. 1998; Voorrips et al. 1998), oil quality (Toroser et al. 1995; Uzunova et al. 1995; Thormann et al. 1996) and flowering time (Ferreira et al. 1995; Osborn et al. 1997; Bohuon et al. 1998; Rae et al. 1999). Early inheritance studies focused on morphological traits, although complex inheritance was often observed, suggesting that the traits were controlled by many genes. Kennard et al. (1994) attempted to resolve these complex inheritance patterns for a range of morphological traits using single locus ANOVA on an F_2 population of a cabbage×broccoli *B. oleracea* cross. More recently, Lan and Paterson (2000) used three F_2 populations to resolve a series of QTLs associated with cauliflower curd traits. Identification of QTLs for a particular trait can contribute to crop-improvement strategies through marker-assisted selection (MAS) (Mohan et al. 1997), especially where the traits are of high value.

Several analytical approaches to QTL analysis are available. These include single-locus ANOVA, flanking-marker methods either by interval mapping (Lander and Botstein 1989; Jansen 1993) or by multiple regression (Haley and Knott 1992), and the multiple-marker regression approach (Kearsey and Hyne 1994). The latter method is appropriate for use with populations derived from the F_1. The ability to detect accurately the position of QTLs is often limited by low heritabilities and small population sizes (Darvasi et al. 1993; Hyne et al. 1995), which results in large confidence intervals. Therefore, the ability to compare results with other QTL experi-

ments is useful, but is often restricted by the lack of common markers or the existence of marker synonyms.

We describe the detection and location of QTLs controlling morphological and developmental traits, using the multiple-marker regression approach (Kearsey and Hyne 1994), in a cross between two very different *B. oleracea* varieties, an annual cauliflower and a biennial Brussels sprout. The resulting F_1-derived doubled-haploid (DH) mapping population had previously been used to construct a detailed linkage map of the *B. oleracea* genome based on RFLP, AFLP and simple sequence repeat (SSR) markers (Sebastian et al. 1999). Where possible, clarification of the QTL positions was made through comparative mapping.

Materials and methods

Plant material

A population of 97 DH lines was generated at HRI, Wellesbourne. These lines were developed through anther culture of the F_1 produced from a cross between two DH parents, a cauliflower line derived from the F_1 'Nedcha', *B. oleracea* var. *botrytis* (CA25), and a Brussels sprout line derived from the F_1 'Gower', *B. oleracea* var. *gemmifera* (AC498). A linkage map for this mapping population had previously been developed, based on RFLP, AFLP and SSR markers (Sebastian et al. 1999). A subset of 86 DH lines was used in the field trials.

Trial design

Preliminary field trials were held during 1992 and 1993 at HRI, Wellesbourne, where a subset of the lines from the DH mapping population were assessed for a limited number of traits (Table 1). In each of these years, for each of two replicate field plots ten plants per line were grown in rows, with 0.4 m between each plant, in an independently randomised design, with guard plants at the end of each row.

The main trial was conducted in 1998 at the University of Birmingham and was based on two adjacent, independently and completely randomised, blocks. Each block contained five replicate plants of each DH line together with 15 replicates each of the Nedcha and Gower DH parental lines. Seeds were sown on April 21st in pots in their randomised positions in an unheated glasshouse. After 9 days they were moved into an open polytunnel to harden off, and were transplanted into the field at the 4–5 leaf stage, arranged with a spacing of 0.5 m between plants in a row and 0.75 m between rows. Guard plants were grown around the

Table 1 Summary of trait designations and their associated descriptions. Traits are grouped according to their Principal Components, i.e. leaf, flowering, axillary bud and stem traits

Trait		Trait description
Leaf trait		
LL	Leaf length	Leaf length from base of petiole to tip of lamina (mm)
LW	Lamina width	Lamina width at widest point (mm)
LPL	Lamina petiole length	Petiole length encompassing lamina (mm)
BPL	Bare petiole length	Length of bare petiole (mm)
APL	Auricle petiole length	Petiole length encompassing auricle (mm)
WPL	Wing petiole length	Petiole length encompassing wings (mm)
LS	Leaf shape	Leaf shape scored as 1 (petiolate with min. expression of wings and auricles) to 6 (laminate and twisted or folded)
LAS	Leaf apex shape	Leaf apex scored as flat (1: AC 498), rounded (2) or pointed (3: CA 25)
MW	Midrib width	Midrib width at node (mm)
AW	Auricle width	Auricle width at widest point (mm)
LN	Lobe number	Number of lobes
WN	Wing number	Number of wings
PA	Presence of auricles	Auricles scored as absent (0) or present (1)
LT	Leaf type	Predominant leaf type in mature plant scored as laminate (0) or petiolate (1)
LTZ	Leaf transition zone	Transition from petiolate to laminate leaves scored as 1 (~ at base of stem), 2 (~ at middle of stem), 3 (~ at top of stem)
Flowering traits		
DF	Days to flowering	Number of days from sowing to appearance of first open flower
VN	Vernalisation	Vernalisation scored as the absence (1: AC 498) or presence (2: CA 25) of flowering or budding at day 156 from sowing)
FH	Flowering height	Apical height at flowering (mm)
Axillary bud traits		
AB	Axillary buds	Distribution of axillary buds scored as totally absent (0: CA 25) through to present at all nodes (5: AC 498)
AT	Axillary bud type	Type of axillary bud scored on mature plant as 1 (small leafy, shoot like) through to 5 (dense sprout like)
NN	Node number	Number of nodes in mature plant measured at harvest
NL	Leaf number	Number of leaves at day 45 after sowing
Stem traits		
PH	Plant height	Apical height at day 45 (mm)
MH	Maximum height	Maximum plant height at day 121 after sowing (mm)
SL	Stem length	Stem Length from cotyledon scar to top of stem in mature plant measured at harvest (mm)
IL	Internode length	Average internode length measured as SL/NN

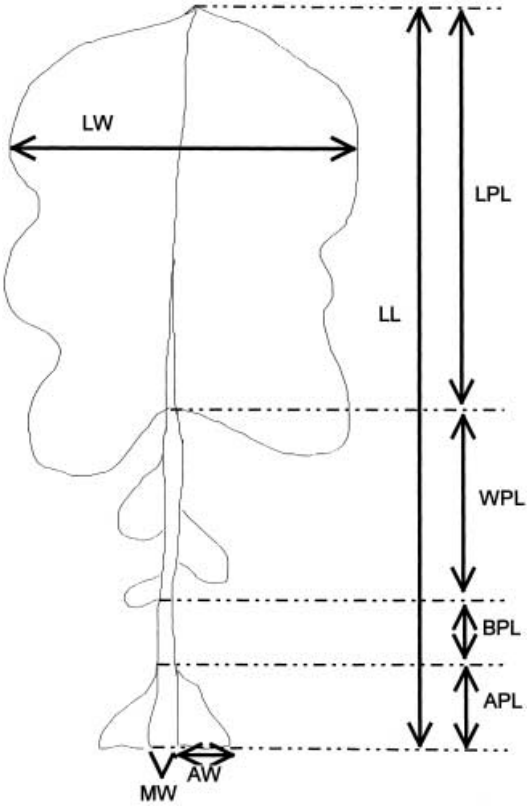

Fig. 1 Diagram of leaf traits showing the measurements taken from photocopies of fully expanded 9th leaves. See Table 5.1 for trait descriptions

perimeter of the trial to prevent edge effects. The trial was protected from bird damage by netting and was sprayed intermittently for insect control.

Traits

Measurements on 27 traits were recorded throughout the 1998 trial, descriptions of which are in Table 1. The leaf traits were scored for block-1 plants only, from photocopies of fully expanded ninth leaves taken at 78 days from sowing (Fig. 1). Axillary bud characters were scored at day 142 and traits measured on mature plants were taken at day 162. All plants were scored for any given trait on the same day, except for the flowering traits which were taken on day of the appearance of the first open flower.

Data analysis

Analyses of variance (ANOVA) were carried out separately for all traits to detect line or block effects. Data were pooled for lines across blocks for all characteristics except for the axillary bud traits (Table 1) which had significant line×block interactions, and for the leaf traits which were only measured for block 1. ANOVA using the General Linear Model was then performed on the combined data with both blocks and lines, being designated as random effects. Pearson correlation coefficients were calculated between all traits and a principal component analysis (PCA) was performed using a correlation matrix to indicate trait groupings.

A linkage map already existed for this mapping population (Sebastian et al. 1999) based on the original 97 DH lines with 223 markers mapping to nine linkage groups, giving a total map length of 831 cM (Kosambi). QTL analysis was carried out using a subset of 75 loci evenly spaced at approximately 10-cM intervals and with the most-complete genotype information. The marker-regression approach (Kearsey and Hyne 1994) for QTL detection and mapping was then performed based on line means, using the software available at the web site (http:/web.bham.ac.uk/g.g.seaton/) according to Bohuon et al. (1998). Where a single QTL model was significant, confidence intervals for the most-likely QTL position and its additive effect were obtained by performing 1000 simulations.

The presence of one or more QTLs was tested using ANOVAs. Usually a single QTL-model on a given linkage group was accepted when the residual mean square in the ANOVA was not significant ($P>0.05$) and the regression mean square was significant. Given the number of tests carried out here, only those significant to $P<0.02$ will be discussed.

Results

Variance components

Simple ANOVA showed that there were no significant line×block interactions for any character except for the axillary bud traits. Data for blocks 1 and 2 axillary bud traits were therefore analysed separately, whilst data were combined for lines across blocks for the remaining characters. ANOVA detected highly significant ($P<0.01$) differences between DH lines for all traits and allowed estimates of additive genetic variance, $V_A[=(1/2)\Sigma a^2]$, to be made (see Kearsey and Pooni 1996 for a definition of terms). Figure 2 shows examples of the phenotypic distributions of the segregating DH population for various traits based on line means. PCA based on line means for all traits showed that 82% of the observed variation could be explained by the first five principal components (PC). Examination of the trait weightings for each PC showed that the traits (Table 1) could be grouped into four main categories: leaf traits (PC1, explaining 43% of variation), flowering traits (PC2, 15%), axillary bud traits (PC3 and 5, 15%) and stem traits (PC4, 9%). This suggested that these trait groupings may largely be controlled by similar genes. The axillary bud traits for blocks 1 and 2 explained most of the variation in PC5 and 3 respectively, but are considered together for further discussion. There was significant correlation (Pearson's coefficient) between the same pairs of traits found within the trait groups defined by the PCA.

QTL analysis

Although ANOVA detected significant differences between the DH lines for all 27 traits, QTLs were detected for only 17 traits at the significance level of $P<0.02$. A total of 32 QTLs were identified for the 17 traits which spanned all nine linkage groups of the *B. oleracea* genome. A single QTL alone was detected for nine traits, while four QTLs were located for both lamina width (LW) and stem length (SL). Table 2 summarises the locations and associated additive effects of significant QTLs for all traits, together with their confidence intervals and the percentage of the additive genetic variation explained by each QTL. In addition, a summary of the

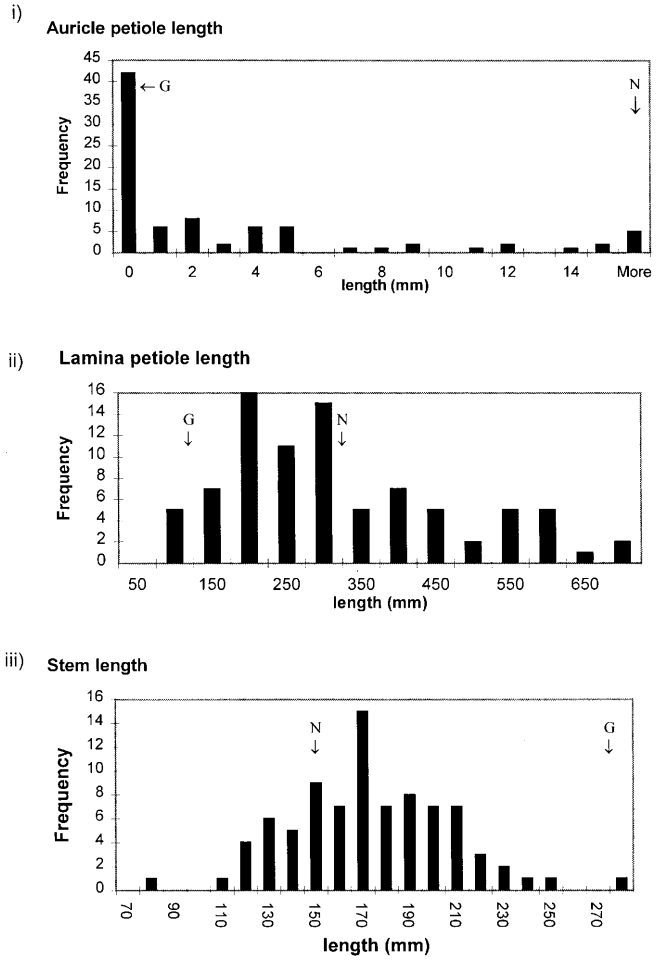

Fig. 2i–iii Examples of the phenotypic distributions of the cauliflower×Brussels sprout DH population for a range of traits: **i**) lamina petiole length, **ii**) auricle petiole length, **iii**) stem length. The positions of the mean values for the Nedcha parent line CA25 (*N*) and the Gower parent line AC498 (*G*) are shown

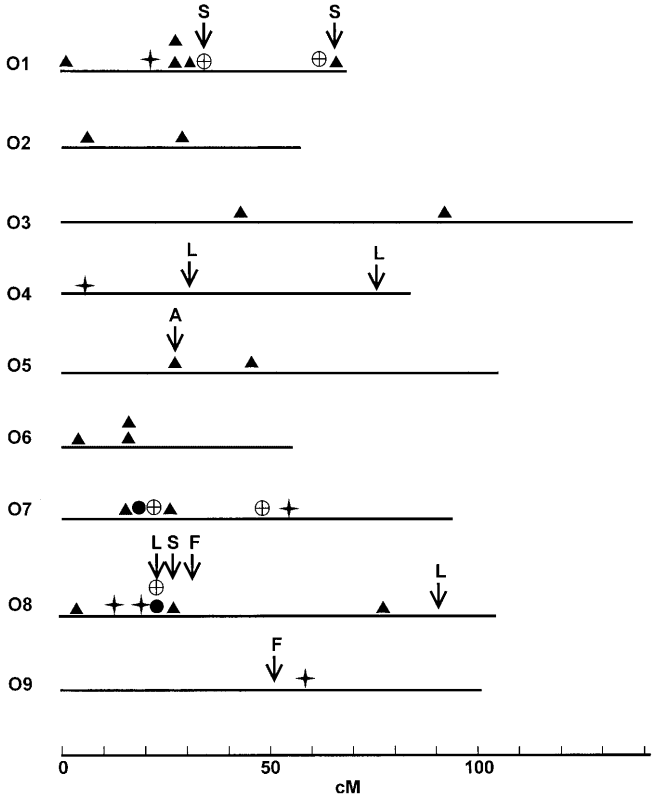

Fig. 3 Distribution of QTLs for each trait category over the nine *B. oleracea* linkage groups O1–O9: ▲ leaf traits, ● flowering traits, ❖ axillary bud traits, ⊕ stem traits. The position of QTLs, based on the PCA weighted line means, for each trait category, are also shown. *Arrows* indicate PCA QTL for leaf traits (*L*), flowering traits (*F*), axillary bud traits (*A*) and stem traits (*S*). A scale indicating genetic distance is shown in cM

marker regression ANOVAs is shown with probabilities for the regression and residual mean squares based on a one-QTL model.

Figure 3 shows the distribution of the significant QTLs for each category of traits over the nine linkage groups of the *B. oleracea* genome. Two different methods were used to identify QTLs based on data adjusted with PC weightings. Firstly, for each PC, weighted trait-component values were calculated for each plant and averaged for each DH line. QTL analysis was then carried out using these mean component values for each of the five PCs in order to identify QTLs associated with each trait category. For example, regions of the genome associated with leaf traits were identified through QTL analysis of the weighted trait values of individual plants. These were based on the trait weightings for PC1, which were then averaged across all individuals of each DH line. The positions of these significant PC-QTLs are also indicated in Fig. 3. It was observed that four out of the ten significant QTLs ($P<0.05$) were positioned where no significant PC-QTLs from the component traits were found. Very similar results to the actual data were obtained, following an alternative approach. In this case PC-QTL analysis was performed for each individual characteristic by applying the line weightings from just the first PC to the original line means. These QTL positions are shown in Table 2. Using the latter method only two additional QTLs were observed at a significance level of $P<0.02$. It was also found that PC-QTLs were not always detected where QTLs were significant ($P<0.02$) with the actual trait data.

Leaf traits

The cauliflower parent, Nedcha DH line CA25, has predominantly long broad laminate leaves whilst the Brussels sprout parent, Gower DH line AC498, has exclusively petiolate leaves. The resulting DH population displayed a range of intermediate phenotypes encompassing the transition from petiolate to laminate leaf types at varying positions up the stem of the plant leaf transition zone (LTZ). Measurements of leaf characters, apart from leaf type (LT) and (LTZ), were taken from

Table 2 Summary of analysis for traits with significant QTL effects following marker regression. For each trait the linkage group and position of each QTL are given. QTL positions are given for the individual traits and for the PCA based on line means. Marker regression ANOVAs are given with probabilities for the regression and residual mean squares based on a single QTL model. Confidence intervals for the QTL positions are given where just a single QTL per linkage group was found. The additive effects and the percentage of additive genetic variance (V_A) explained by each QTL are described, as well as the heritability associated with each trait

Trait	Linkage group	QTL Position (cM)		Confidence interval (cM)	Additive effect	%V_A	Heritability %	Regression *P*	Residual *P*
		Individual trait	PCA combined trait						
Leaf traits									
LW	6	16	20[a]	12.4	8.9	14.5	31.7	<0.01	0.02
	7	16	20	8.4	13.3	32.4		<0.01	0.24
	8	28	28		–15.6	44.5[b]		<0.01	<0.01
	8	80	94	16.2	11.0				
LPL	3	44	46	12.7	13.6	16.9	34.2	<0.01	0.18
	6	16	–	16.1	10.6	10.2		0.015	0.41
	7	26	–		10.7	10.6		0.011	0.46
BPL	1	28	–		–11.2	45.4[b]	21.4	<0.01	0.04
	1	70	–		3.8				
	2	6	–	13.8	–5.1	11.3		<0.01	0.2
APL	1	28	30	13.8	2.4	14.3	36.3	0.012	0.47
WPL	1	0	0	11.3	–6.6	25.6	17.6	<0.01	0.2
LAS	3	94	–	10.1	0.2	37.8	18.1	<0.01	0.1
MW	2	30	30	10.9	1.0	22.6	10.5	<0.01	<0.86
LN	6	4	0	11.3	0.3	26.0	16.8	0.012	0.53
WN	8	4	–	17.3	0.4	17.7	26.7	<0.01	0.13
LT	1	32	32	8.3	–0.2	24.9	35.6	<0.01	0.65
	5	28	–	16.8	0.1	11.0		0.015	0.73
LTZ	5	46	46	16.7	–0.2	11.9	24.0	0.013	0.68
Flowering traits									
VN	7	18	–	12.3	–0.2	14.1	54.9	<0.01	0.28
	8	24	24	11.8	0.2	23.0		<0.01	0.51
Axillary bud traits									
Block 1									
NN	8	22	22	7.9	–13.5	42.9	38.9	<0.01	0.23
NL	4	6	4	17.0	–0.6	12.8	37.0	0.018	0.25
	7	56	56	14.6	–0.6	14.1		<0.01	0.68
Block 2									
NN	8	14	18	14.1	–9.3	17.9	38.6	<0.01	0.13
	9	62	–	18.3	–7.3	10.9		0.018	0.13
AB	1	22	22	14.4	–0.3	10.8	29.9	0.011	0.04
Stem traits									
PH	7	22	–	16.8	0.6	9.1	51.0	0.018	0.81
SL	1	34	34		–97.8	20.6[b]	51.1	<0.01	<0.01
	1	68	68		65.7				
	7	48	48	15.7	48.8	6.6		0.015	0.54
	8	24	24	12.6	–63.6	11.3		<0.01	0.46

[a] Position of significant QTLs based on PCA weighted trait values

[b] %V_A for the two QTLs on the same linkage group allowing for linkage in repulsion. The combined additive effect of the two QTLs was calculated as, $a^2_A+a^2_B+2(1–2R)a_Aa_B$

photocopies of fully expanded 9th leaves from block-1 plants only (see Fig. 1). Since the leaves at this stage were predominantly (97%) petiolate, the laminate leaves were eliminated from the analysis.

No significant QTLs were detected for the characters leaf length (LL), leaf shape (LS), auricle width (AW) or the presence of auricles (PA) at $P<0.02$. Between one and four significant QTLs were detected for each of the remaining 11 leaf traits (Table 2). These were located across all linkage groups of the *B. oleracea* genome apart from O4 and O9. Significant regression ($P<0.01$) and residual ($P<0.05$) MS for a single-QTL model suggested that the characters lamina width (LW) and bare petiole length (BPL) have two QTLs on O8 and O1 respectively. In both cases the two QTLs are linked in repulsion.

A total of five QTLs for leaf traits were detected on O1. A decreasing allele from the AC498 Brussels sprout parent for auricle petiole length (APL) was detected at 28 cM, and an increasing allele for leaf type (LT) at

32 cM. A single QTL in this region of O1 is suggested which controls BPL, APL and LT. An increase in BPL would be expected to be accompanied by a decrease in APL, which in turn defines whether a leaf is petiolate rather than laminate.

QTLs for lamina width (LW) and lamina petiole length (LPL) were detected in similar regions of linkage groups O6 and O7. For all four QTLs the cauliflower DH parent line contributes the increasing allele, consistent with the parental values which are 1.6- and 2.7-times larger for CA25 than AC498.

PCA QTL analysis of the line means based on individually weighted trait values detected significant QTLs in the same regions as QTLs located from the actual trait data. Differences in QTL positions can be accounted for by the confidence intervals of the individual QTL. No PC-QTLs were detected for eight out of the 19 leaf-trait QTLs. Two additional PC-QTLs were significant for leaf-apex shape (LAS) at 16 cM on O1 and wing number (WN) at 0 cM on O1, for which there were no significant leaf-trait QTLs. Analysis of the first PCA weighted trait means located two QTLs on each of O4 and O8 (See Fig. 3). The two QTLs on O8 were located in both regions associated with QTLs for lamina width (LW). However, no QTLs for leaf traits were detected on O4, even though two QTLs based on the first PCA weighted trait means were located on that linkage group.

Flowering traits

Cauliflowers are annuals. Brussels sprout plants are characteristically biennial, producing axillary sprouts in the first year and then flowering in the second year after a suitable period of vernalisation. Surprisingly, 27% of the Brussels sprout DH AC498 parental plants flowered before the end of the 1998 trial period, during the first year. However, as expected, approximately 51% of the DH lines of the mapping population flowered in the same period. As a result, scores for days to flowering (DF) and flowering height (FH) were recorded for 44 lines only. Analysing data for so few lines reduces the power of QTL detection (Hyne et al. 1995) and as a result no significant QTLs for DF, AS or FH were detected.

Two QTLs for vernalisation were detected, on O7 (at 18 cM) and O8 (24 cM). Together these explained 37% of the additive genetic variance for this trait (Table 2). Vernalisation requirement (VN) was scored as 1 (the absence) or 2 (the presence) of flowering or budding by day 156 after sowing. The QTL on O8 has an additive effect of 0.2, therefore the DH parent line AC498 has the decreasing allele requiring vernalisation and explains 23% V_A. However, the QTL detected on O7 has a negative additive effect of –0.16, which explains 14% V_A. In this case the parent line AC498 contributes the increasing allele promoting flowering in the first year. It had not been expected that AC498 would provide this increasing allele. However, in the 1998 trial almost a third of AC498 parent plants flowered unexpectedly in the first year. This may indicate an interaction between a specific allele and particular environmental conditions.

Axillary bud traits

Axillary bud traits are important for Brussels sprout formation. ANOVA of the four traits in this group, axillary bud distribution (AB), axillary bud type (AT), node number (NN) and early leaf number (NL), revealed that all had a significant interaction between lines and blocks. As a result the data for each block were analysed separately. No significant QTLs were detected for axillary bud type in either block. QTL analysis involving the PCA weightings detected only a single QTL for axillary bud traits on O5. No other significant QTLs for any of the axillary bud traits were found on O5. A single QTL for AB was located on O1 in block 2, and two QTLs were detected for NL in block 1.

For both blocks, a QTL for node number (NN) was detected in similar regions of O8 at 22 cM (block 1) and 14 cM (block2), in both cases, as would be expected. The AC498 Brussels sprout parent line possessed the allele having an increasing effect on node number. The heritabilities of NN in each block were approximately equal at 39%. However, the percentage of additive genetic variation explained by the two QTLs on O8 differs by more than a factor of 2. The QTL for block 1 explains 43% V_A, while only 18% is accounted for by the block-2 QTL. An additional significant QTL ($P<0.02$) was located on O9 at 62 cM, with block-2 data, explaining a further 11% V_A.

Stem traits

There was a marked difference in height between the cauliflower and Brussels sprout parental lines. At maturity the average stem length of the cauliflower parent was 153 mm, whilst that of the Brussels sprout parent was 975 mm. Even at day 45 after sowing the difference between the parental lines was apparent, with the cauliflower parent having an average apical height of 37 mm and the Brussels sprout parent a height of 91 mm. In this analysis no significant ($P>0.02$) QTLs were detected for either maximum height (MH) or internode length (IL). Four QTLs were detected for stem length (SL). One of these, not surprisingly, was located in a similar region to the QTL for early plant height (PH) on O7 at 22 cM. This and the QTL for SL, found at 48 cM on the same linkage group, explain only 9% V_A and 7% V_A respectively, with corresponding effects. Three further QTL for SL were detected, two on O1 linked in repulsion and another on O8. Each of these trait QTLs had corresponding PC-QTLs, indicating a strong association with stem traits in these areas of the genome.

Discussion

The present study identified at least 32 QTLs, which individually explain between 6% and 43% of the additive genetic variation for a range of 17 morphological traits of *B. oleracea*. No QTLs were detected for a further ten traits under the strict significance levels ($P<0.02$) used in this study to allow for the large number of individual tests performed.

No more than four significant QTL were located per trait. This is consistent with previous QTL studies (Kearsey and Farquhar 1998), and a possible detection limit of 12 QTLs (Hyne and Kearsey 1995). For only three traits were two QTLs located on a single linkage group: lamina width (LW; O8), bare petiole length (BPL; O1) and stem length (SL; O1). In each case, the two QTLs had opposite effects. There may be other traits in this study for which two, or more, QTLs exist per linkage group. However, it is more difficult to detect two QTLs on a linkage group which have the same effects. This is due to the fact that, unless they are of unequal sizes or very far apart, they will appear as a single QTL located somewhere in the middle of the two actual QTLs (Hyne and Kearsey 1995).

Considerable variation was observed in the number of QTLs detected per linkage group. The 32 QTLs found in this study were distributed over all nine linkage groups of the *B. oleracea* genome. However, whilst five linkage groups had two or fewer QTLs, O1, O7 and O8 each had six or more QTLs spanning their lengths (See Fig. 3). Mansur et al. (1993) found that QTLs for many developmental traits in soybean were associated with just three linkage groups. This could arise from the clustering of genes or pleiotropy. In the present study, measurements of early plant height (PH) and stem length at maturity (SL) were scored. On O7, a single QTL for each of these two traits was detected within the same region, taking into account the confidence intervals associated with the QTL positions. Both the QTLs had the same gene action and explained very similar amounts of additive genetic variation, with the CA25 parental allele having an increasing effect on both traits. We would not expect the CA25 parent to contribute alleles having an increasing effect on this phenotype. This is because the mean values for PH and SL, measured at two very different stages during plant development, were much lower for the CA25 parent than the AC498 parent. However, this does support the argument that these two traits are controlled by the same putative gene affecting stem traits on O7. In a similar manner, QTLs for both lamina width (LW) and lamina petiole length (LPL) (which is effectively a measurement of lamina length) were found on O6 and O7 in close proximity to one another. Here, CA25, as expected, contributed the alleles having an increasing effect on both LW and LP. As a result, the QTLs identified here may actually be controlling the overall potential size of the lamina rather than its individual dimensions. This is supported by an earlier study into the morphological variation in *B. oleracea* using single-factor ANOVA (Kennard et al. 1994), who found significant marker-trait associations on two linkage groups for lamina width, lamina length and petiole length. Again, all traits had the same gene action, suggesting that the same putative gene may be involved in controlling all three characters. Our inability to compare the markers used in the earlier and the present study prevents direct comparisons being made to determine whether the regions of the genome affecting lamina length and width in the two studies are homologous.

For seven out of the 17 morphological traits for which significant QTLs were detected, the increasing allele did not come from the parent with the greater mean value. This is not an uncommon occurrence. A study of morphological variation in *B. rapa* (Song et al. 1995) found that, although alleles usually had the expected effect, some minor alleles had opposite effects. Similarly, Bohuon et al. (1998) found that the earlier-flowering *B. oleracea* var. *alboglabra* parent line, A12DHd, had at least one late-flowering QTL, whilst the converse was true of the later-flowering *B. oleracea* var *italica* parent line GDDH33. In the present study, a QTL was located on O7 for which the putative annual parent CA25, unexpectedly, contributes the allele promoting vernalisation, and for which AC498 contains the alternative allele. Some of the AC498 parent plants did actually flower before the end of the trial, contrary to expectation. However, cool wet conditions were experienced during the earlier part of the field trial. This could have provided adequate vernalisation for some of the plants, enabling them to flower without overwintering. As expected, though, only about 50% of the DH lines flowered during the duration of the trial.

The observed differences in maturity across the population may have a pleiotropic affect on the assessment of other traits. All developmental and morphological traits are subject to variations in the underlying ontogeny of an individual. However, we ensured that comparisons were made at the same time across the population, and for leaf traits the same stage of development (leaf number) was sampled.

The outcome of the PCA was used in two ways to clarify the results obtained from the QTL analysis based on the line means. Firstly, where PC-QTL analysis was performed on the line means of individually weighted trait values, very similar results were obtained to those obtained with the component trait data. Secondly, PC-QTL analysis was carried out for each Principal Component, based on the weighted trait means totalled for each DH line. In these cases additional regions of the *Brassica* genome were shown to have significant effects upon the categories of leaf, flowering and axillary bud traits. At these positions individual QTLs may not have been significant previously, yet the effect of combining the data identifies significant QTLs associated with the particular trait group. For example, QTL analysis involving the PCA weightings identified two significant regions for flowering traits. One region on O8 (see Fig. 3) is synonymous with the vernalisation QTL at 24 cM.

However, the second is located at 54 cM on O9 where no significant QTLs were found for flowering traits in this study. Since a QTL has been found for flowering time at 46 cM on O9 using another *B. oleracea* mapping population (Bohuon et al. 1998) this suggests that insufficient data in the present study may have prevented the detection of the flowering-time QTL in this region of the genome.

In the two preliminary field trials in 1992 and 1993 a subset of the lines from the DH mapping population was assessed for a limited number of traits. These data were useful in confirming some of the QTL positions found in the 1998 trial, given that the trials were conducted in different years and environments, and recorded by different scorers. There is much additive genetic variation unaccounted for which may require further field trials in order to resolve, or attribute to the QTLs. Of the eight comparable traits [leaf type (LT), days to flowering (FT), vernalisation requirement (VN), axillary bud distribution (AB), axillary bud type (AT), node number (NN), stem length (SL) and internode length (IN)], and taking into consideration the confidence intervals associated with the relative QTL positions, four (VN, AB, NN, SL) revealed significant QTLs synonymous with the QTLs found in the 1998 trial. Figure 4 shows a comparison of these QTL positions from the 1992, 1993 and the 1998 field-trial data. Confidence intervals are indicated where only a single QTL for that linkage group for a given trait was identified. For each QTL the effect of the parental alleles was the same, e.g. for each QTL for SL on O8 the AC498 parent-line possessed the allele which increased stem length.

Data from the preliminary trials in 1992 and 1993 identified further QTLs in the regions of the linkage groups where PCA-weighted QTLs were detected. Interestingly, there are two examples where the position of a QTL from the preliminary trial data corresponds to the position of PC-QTL, but where no significant QTLs from the larger 1998 trial were located. QTLs for axillary bud type (AT) were detected on O5 at 38 cM (1992 trial) and 40 cM (1993 trial), whilst a PC-QTL for axillary bud traits was detected on the same linkage group at 30 cM. Likewise, a QTL for leaf type (LT) was detected at 36 cM (1992 trial) and 50 cM (1993 trial) on O4, whilst a PC-QTL for leaf characters was detected at 32 cM.

The ability to compare the results of QTL analysis with other studies relies on the alignment of linkage maps through homologous or homoeologous markers, together with trial data for the same or similar traits. A comparison of markers requires access to marker nomenclature synonyms. There will be greater confidence in a comparison of QTL data in terms of position, action and effect where large confidence intervals are associated with QTL positions. Camargo et al. (1995) identified three putative QTLs for petiole length in a population derived from *B. oleracea* vars. *capitata* (cabbage) and *italica* (broccoli). One of these QTLs explained 13% of the additive genetic variation located between the RFLP

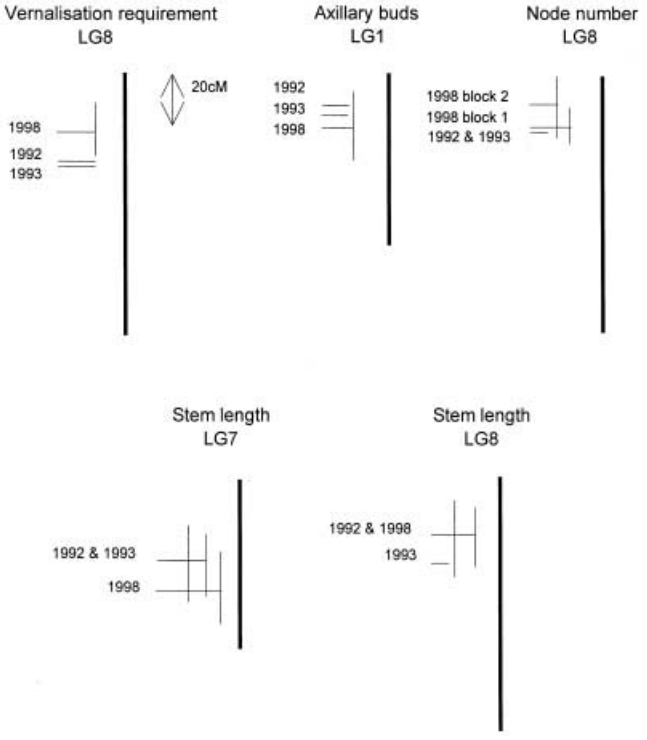

Fig. 4 A comparison of QTL positions for vernalisation requirement (VN), axillary bud distribution (AB), node number (NN) and stem length (SL) from the 1992 and 1993 trial data, together with the 1998 field trial data. Confidence intervals are shown where relevant

loci wg6b2b and wg8a9b on O9. These two markers are synonymous with pW152 and pW143 respectively. These are located on O3 of the present study, between which a significant QTL for lamina petiole length was detected which explained 17% of the additive genetic variation.

A putative QTL for vernalisation was located on O9 of *B. napus* (Ferreira et al. 1995) between the loci wg7f3 and wg6b10. Locus wg7f3 is synonymous with the RFLP pW207. This marker was monomorphic in the present cross. However, it was polymorphic in another *B. oleracea* mapping population, which was used together with the current cauliflower×Brussels sprout cross to produce an integrated linkage map of the *B. oleracea* genome (Sebastian et al. 1999). The marker mapped was located at 23 cM on O8. In the present study a putative QTL for vernalisation was detected on this linkage group at 24 cM, strongly suggesting homology. Other studies have shown this same region in *B. napus* to have homology with a region in *B. rapa* that also has a putative QTL controlling vernalisation (Teutonico and Osborn 1994; Osborn et al. 1997). The additional confidence gained from these comparisons may allow inferences about QTL positions to be made between different populations within a species and between species. This is especially relevant within a genus such as *Brassica* where there are close relationships between its member species based on interspecific hybridisation and polyploidy (U 1935).

This, in turn, may aid the development of breeding programmes through marker-assisted selection.

Variation for many of the traits studied are manipulated and selected in crop-improvement programmes. Knowledge of the number and likely position of loci can provide the information required to select optimal combinations of alleles by the use of MAS. This may be of particular relevance where there exists linkage between an undesirable and a desirable trait in coupling. However, for any trait, there is usually a requirement to confirm the position of the QTL and carry out fine-scale mapping before MAS becomes a viable proposition. In many cases it may be preferable to identify the likely underlying candidate gene(s) in order to establish the extent of allelic variation within the crop contributing to the trait variation. The recent establishment of the complete genome sequence of the related crucifer *Arabidopsis thaliana* and the corresponding development of *Brassica* physical maps and substitution lines, now make map-based cloning of QTL regions a tenable undertaking.

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