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K. Song · M. K. Slocum · T. C. Osborn

Molecular marker analysis of genes controlling morphological variation in *Brassica rapa* (syn. *campestris*)

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Abstract Construction of a detailed RFLP linkage map of *B. rapa* (syn. *campestris*) made it possible, for the first time, to study individual genes controlling quantitative traits in this species. Ninety-five F_2 individuals from a cross of Chinese cabbage cv 'Michihili' by Spring broccoli were analyzed for segregation at 220 RFLP loci and for variation in leaf, stem, and flowering characteristics. The number, location, and magnitude of genes underlying 28 traits were determined by using an interval mapping method. Zero to five putative quantitative trait loci (QTL) were detected for each of the traits examined. There were unequal gene effects on the expression of many traits, and the inheritance patterns of traits ranged from those controlled by a single major gene plus minor genes to those controlled by polygenes with small and similar effects. The effect of marker locus density on detection of QTL was analyzed, and the results showed that the number of QTL detected did not change when the number of marker loci used for QTL mapping was decreased from 220 to 126; however, a further reduction from 126 to 56 caused more than 15% loss of the total QTL detected. The detection of putative minor QTL by removing the masking effects of major QTL was explored.

Key words *Brassica rapa* · *Brassica campestris* · Morphological variation · Quantitative trait loci · RFLP

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K. Song[1] · T. C. Osborn (✉)
Department of Agronomy, University of Wisconsin, Madison, WI 53706, USA

M. K. Slocum[2]
NPI, 417 Wakara Way, Salt Lake City, UT 84108, USA

Present addresses:
[1] Department of Biology, Indiana University, Bloomington, IN 47405, USA
[2] 201 S. Biology Building, University of Utah, Salt Lake City, UT 84112, USA

Introduction

Brassica rapa (syn. *campestris*) is an important species of the *Brassica* genus, which also includes a variety of vegetable and oilseed crops with widely diverse morphotypes such as Chinese cabbage, pak choi, turnip, turnip rape, and sarson. Although there have been active breeding programs in *B. rapa*, very limited information is available on the inheritance of many morphological traits in this species. Using a rapid cycling population of *B. rapa*, Williams (1985) documented the genetic control of some morphological traits, such as anthocyanin synthesis, disease resistance, leaf color, and flower color. However, all of these markers are related to qualitative traits, and the genetic control of many quantitative traits is unknown due to their complex inheritance patterns. The lack of a genetic linkage map in this species also has made it difficult to localize genes controlling both qualitative and quantitative traits.

Restriction fragment length polymorphism (RFLP) markers are very useful for constructing linkage maps and for dissecting the genetic control of morphological traits. RFLP markers have been used to construct genetic maps in various crop species, including *B. oleracea* (Kianian and Quiros 1992; Landry et al. 1992; Slocum et al. 1990), *B. napus* (Ferreira et al. 1994; Landry et al. 1991), and *B. rapa* (Chyi et al. 1992; Song et al. 1991). Detailed RFLP maps make it feasible to identify and detect the effects of genes controlling complex traits, often referred to as polygenic or quantitative traits (Beckmann and Soller 1986; Tanksley et al. 1989). Associations between molecular markers and quantitative traits have been reported in tomato (Nienhuis et al. 1987; Osborn et al. 1987; Paterson et al. 1988, 1991), in maize (Edwards et al. 1987, 1992; Stuber et al. 1987), in soybean (Keim et al. 1990, 1991), and in *B. oleracea* (Kennard et al. 1994). Recently, a detailed genetic linkage map of *B. rapa* was constructed based on 280 RFLP loci (Song et al. 1991). This map provides an opportunity to detect and

measure the effects of genes controlling quantitative traits in this species.

In the study reported here, RFLP loci were used to analyze the inheritance of more than 20 morphological traits in *B. rapa*. Most of these traits were quantitative traits, including leaf, stem, and flowering characteristics. Quantitative trait loci (QTL) underlying these traits were identified and located using RFLP markers, providing new information on the genetic control of these traits. Factors affecting the efficiency of locating QTL, such as the number of marker loci used and the masking effect of major genes on minor genes, were explored.

Materials and methods

Plant materials

A Chinese cabbage cultivar, 'Michihili' (Olds Seed Co., Madison, Wis.), and an accession of Spring broccoli (Crucifer Genetics Coop., Madison, Wis.) were selected as the female parent and the male parent, respectively. These parents represent two diverse groups in *B. rapa* that are polymorphic for both morphological traits and restriction fragment lengths (Figdore et al. 1988; Song et al. 1988). Single plants were used to make F_1 hybrids, and a single F_1 plant was randomly selected and self-fertilized by bud pollination to produce an F_2 population. Ninety-five F_2 plants and 4 plants for each of the parents and F_1 were grown in 30-cm-diameter pots, fed with slow-release fertilizer (Osmocote 10-10-10), and sprayed intermittently with Diazane, Orthene, and Pentac for pest control. Plants were maintained in cold frames in Madison from July 13 to October 20, 1987, and were then moved to a greenhouse.

Fig. 1 RFLP linkage map showing the 220 loci selected for detecting putative QTL. The codes of marker loci are listed on the *right*, and the map distances based on a previous analysis with 280 marker loci (Song et al. 1991) are in cM on the *left*. * indicates marker loci used for the analysis with 126 markers, and ** indicates marker loci used for the analysis with 56 marker loci. *Pub* and *Lob* represent single loci controlling the presence/absence of pubescence and leaf lobes, respectively. Marker loci *underlined* are those showing distorted segregation (significantly different from a 1:2:1 ratio) (Song et al. 1991)

Trait measurement and RFLP genotyping

The parents, F_1 and 95 F_2 plants were measured for 17 morphological traits related to leaf, stem, plant height, and flowering characteristics (Table 1). Eleven additional traits or measurements which were thought to have biological significance were calculated using a subset of the measured traits (Table 1). The leaf characteristics were scored at day 69 after sowing (15- to 18-leaf) using the largest leaf of each plant, and the stem characteristics were measured at the flowering stage (103 days after seeding). All plants were measured on the same day for all traits except days to bud (DB), days to flower (DF), and plant height at flowering (PH2), which were scored at the same developmental stage on all plants.

RFLP genotypes of the 95 F_2 individuals had been determined previously for 280 RFLP loci using genomic DNA clones as probes (Song et al. 1991). For marker- trait analysis, 220 RFLP loci for which the genotypes of most of the F_2 plants were known (>85 of 95 individuals) and subsets of 126 and 56 RFLP loci were selected (Fig. 1), and marker genotypes of the F_2 plants were used to construct new linkage maps with the MAPMAKER II computer program (Lander et al. 1987). These maps had the same marker locus orders as reported previously (Song et al. 1991), although some distances between marker loci were different and the overall map distances were shorter (see Results).

Table 1 Designations of traits and description of trait measurements

Trait designation	Trait description
P1 (Pubescence 1)	Pubescence on leaf surface scored as 1 (present-Michihili) or 0 (absent-Spring broccoli) taken at the eight-leaf stage
P2 (Pubescence 2)	Degree of pubescence on the leaf surface scored on a 0 (no pubescence-Spring broccoli) to 4 (most pubescence-Michihili) scale at the eight-leaf stage
P3 (Pubescence 3)	Same measurement as P2, excluding individuals with a score of 0.
LL (Leaf Length)	Mature leaf length (cm) from based to tip including petioles measured at the 15- to 18-leaf stage
LW (Lamina Width)	Lamina width (cm) across the widest portion of the same leaf used for LL (cm)
LAL (LAmina Length)	Lamina length (cm) derived by LL-PL2
LI (Lamina Index)	Ratio of lamina length to width derived by LAL/LW
NLL1 (Number of Leaf Lobes 1)	Leaf lobes scored as absent (0-Michihili) or present (1-Spring broccoli) scored at the 15- to 18-leaf stage
NLL2 (Number of Leaf Lobes 2)	Number of leaf lobes scored at the 15- to 18-leaf stage
NLL3 (Number of Leaf Lobes 3)	Same measurement as NLL2, excluding individuals without lobes
PL1 (Petiole Length 1)	Petiole length (cm) from stem to the lowest leaf lobe of the same leaf used for LL
PL2 (Petiole Length 2)	Petiole length (cm) from stem to lamina base, including lobes
PL3 (Petiole Length 3)	Same measurement as PL1, excluding individuals without lobes
PL4 (Petiole Length 4)	Same measurement as PL2, excluding individuals without lobes
PL5 (Petiole Length 5)	Petiole length (cm) from the first leaf lobe to based of lamina, derived by PL2-PL1
PL6 (Petiole Length 6)	Petiole length (cm) from the first leaf lobe to base of lamina, derived by PL4-PL3
PW (Petiole Width)	Petiole width (cm) at 1 cm from stem of the same leaf used for LL
PT (Petiole Thickness)	Petiole thickness (cm) at 1 cm from stem of the same leaf used for LL
PI (Petiole Index)	The ratio of petiole width to petiole thickness derived by PW/PT
PH1 (Plant Height 1)	Plant height (cm) measured at the 15–18 leaf stage (vegetative stage)
PH2 (Plant Height 2)	Plant height (cm) from base to the first open flower
SL (Stem Length)	Stem length (cm) measured from base to the point where inflorescence elongates
SD (Stem Diameter)	Stem diameter (cm) at the widest place of the stem
SI (Stem Index)	Ratio of stem length to stem width, derived from SL/SD
NAS (Number of Axillary Shoots)	Number of axillary shoots within the measured stem length (SL)
DB (Days to Bud)	Number of days from sowing seed to appearance of thumb-size bud cluster
DF (Days to Flower)	Number of days from sowing seed to appearance of the first open flower
DBF (Days from Bud to Flower)	Number of days from budding to the first open flower, derived by DF-DB

1A: 19a ** — 11 — 38c — 5 — 105a * — 13 — 86 * — 20 — 100 — 2 — 93b ** — 3 — 10b — 4 — 190a — 4 — 303 — 2 — 37 * — 4 — 70a — 3 — 76a — 1 — 83c — 10 — 52 — 7 — 90b ** — 1 — 4b — 5 — 44b — 10 — 97a — 7 — 129 * — 3 — 196 — 2 — 93a — 3 — 64 — 5 — 21 — 6 — 18b — 1 — 206 ** — 5 — 46a — 2 — 187b — 5 — 16 — 3 — 38b * — 6 — 34 — 13 — 56a — 12 — 115 ** — 39 — 26d ** — 10 — 318a * — 13 — 203b * — 19 — 81 **

2A: 72a ** — 7 — 8a — 5 — 40a * — 12 — 90a ** — 14 — 44c * — 18 — 83a ** — 13 — 49a * — 3 — 104 — 1 — 7b * — 5 — 76c — 12 — 83b **

3A: 312 ** — 10 — 28a * — 4 — 66b * — 6 — 23 * — 10 — 38a ** — 5 — 200a * — 9 — 29a — 2 — 36b * — 3 — 99a — 1 — 305a * — 5 — 133 * — 14 — 185 ** — 16 — 326 * — 3 — 329 * — 16 — 323b — 1 — 7c — 2 — 76b * — 5 — 30b ** — 4 — 113 — 18 — 102 * — 9 — 144 ** — 32 — 40b * — 5 — 4c **

4A: *Lob* — 9 — 48 ** — 13 — 128 * — 5 — 149 * — 7 — 27 — 1 — 33 * — 7 — 325 ** — 10 — 55b * — 24 — 107b ** — 16 — 9b * — 7 — 22b * — 16 — 319 ** — 3 — 10a * — 3 — 190b — 2 — 56b — 3 — 209 — 9 — 71 **

5A: 174 ** — 3 — 22a * — 18 — 67 * — 7 — 91a * — 3 — 97b ** — 3 — 32a — 2 — 94 — 7 — 2b — 3 — 39 * — 7 — 45 — 13 — 211 — 4 — 4a ** — 15 — 306 * — 14 — 199 — 1 — 96c ** — 18 — 316 * — 3 — 302 — 10 — 193 — 13 — 26c ** — 6 — 26b * — 4 — 5b — 7 — 119 * — 13 — 47 * — 12 — 59a ** — 3 — 116a — 3 — 58 — 17 — 88 — 5 — 18a,198

6A: 202b ** — 9 — 207 — 8 — 54c * — 17 — 32c ** — 7 — 327 * — 11 — 79b * — 15 — 54d * — 7 — 54b ** — 11 — 2a — 6 — 44a * — 10 — 197b * — 12 — 70b ** — 8 — 30a * — 3 — 304 — 3 — 212 — 3 — 15a — 2 — 96b — 1 — 96a — 3 — 53b — 3 — 73 ** — 1 — 51a — 1 — 74a — 2 — 98b — 12 — 51c — 1 — 308 **

7A: 19b ** — 15 — 29d — 1 — 148 * — 16 — 105b ** — 13 — 42 * — 8 — 107a * — 4 — 157 — 1 — 117 — 8 — 43 ** — 5 — 79a * — 6 — 32b * — 3 — 54a — 2 — 202a ** — 13 — 131 * — 27 — 309 * — 17 — 24a * — 20 — 20 ** — 6 — 25b

8A: 186 ** — 19 — 46b * — 1 — 187a — 11 — 24c — 2 — 31a — 4 — 172 — 3 — 28b — 2 — 204 ** — 10 — 183 * — 13 — 38d * — 6 — 200b — 2 — 29c ** — 10 — 36a — 6 — 99b — 10 — 12a * — 18 — 53a — 2 — 151 — 1 — 307 ** — 6 — 203a — 15 — 318b * — 13 — 61c — 2 — 161 ** — 12 — 75a * — 1 — 68 — 5 — 130 * — 6 — 124b — 8 — 168 **

9A: 331 ** — 4 — 114 — 3 — 24b — 16 — 136 * — 7 — 153b ** — 20 — 95b * — 20 — 156 ** — 11 — 92 * — 11 — 197a — 9 — 91b * — 3 — 213a — 4 — 315 ** — 3 — 56c — 2 — 25a — 1 — 61a — 3 — 330 * — 3 — 111 * — 5 — 116b * — 13 — *Pub* — 9 — 145 ** — 15 — 317 * — 33 — 154 ** — 9 — 85

10A: 9a ** — 6 — 95a — 9 — 305b * — 10 — 313a * — 10 — 213b ** — 9 — 15b — 1 — 96d * — 24 — 195 ** — 11 — 14 * — 5 — 5a — 2 — 153a — 2 — 141 **

Data analysis

Single loci controlling the two qualitative traits (P1 and NLL1) were mapped with respect to RFLP loci using the MAPMAKER II program. Putative QTL controlling the 26 quantitative traits were identified using the MAPMAKER/QTL program (Lander and Botstein 1989; Lincoln and Lander 1990). Normality of trait distributions were tested using the 'trait' command of the MAPMAKER/QTL program, and Pearson correlation coefficients were calculated between the traits measured by using the CORR procedure of SAS (SAS Institute 1982). On the basis of the genome size of *B. rapa* and the density of 220 marker loci in our map, a LOD score of 2.8 was chosen as the threshold for declaring putative QTL (Lander and Botstein 1989). This LOD threshold corresponds to a false positive level of 5% for the entire genome. Since the false negative level for this threshold is very high, regions with LOD scores between 2.0 and 2.8 were considered to contain possible QTL. In order to determine the effect of number of marker loci on efficiency of detecting putative QTL, subsets of 126 and 56 marker loci were selected (Fig. 1) and analyzed in the same way described above, except that LOD thresholds were adjusted to 2.6 and 2.4, respectively, based on marker densities (Lander and Botstein 1989).

Map positions of putative QTL were determined by the peak LOD scores. The confidence interval for a putative QTL was defined by the region within one log of the peak LOD score. Multiple intervals on one chromosome were considered to represent distinct QTL if (1) the peaks of the intervals were separated by at least 50 cM map distances with a defined valley in between, and (2) the test for multiple QTL gave LOD scores ≥ 2.8 (Paterson et al. 1991). The minimum number of QTL for a particular trait was estimated by the number of intervals significantly associated with that trait across the entire genome. The additive and dominance effects of putative QTL alleles from the Spring broccoli parent and the percentage phenotypic variation explained by putative QTL were calculated for each trait (Lincoln and Lander 1990).

Table 2 Means and standard deviations (SD) of the parents ('Michihili' and Spring broccoli) and their F_1 and F_2 populations for measured traits (*n.a.* not applicable)

Trait[a]	Michihili[b]	Spring broccoli[b]	F_1^b	F_2^c
	Mean (SD)			
P1	1.0(0)	0.0(0)	1.0(0)	0.7 (0.4)
P2	4.0(0)	0.0 (0)	2.5 (0.6)	1.8 (1.4)
P3	n.a	n.a	n.a	2.6 (1.0)
LL	66.8 (10.9)	77.0 (6.0)	68.3 (2.8)	67.9 (8.0)
LW	26.0 (4.2)	25.5 (0)	22.8 (1.7)	23.6 (3.9)
LAL	63.4 (11.1)	35.3 (10.8)	33.3 (4.2)	37.7 (16.2)
LI	2.5 (0.1)	1.4 (0.4)	1.5 (0.2)	1.6 (0.7)
NLL1	0.0 (0)	1.0 (0)	1.0 (0)	0.7 (0.4)
NLL2	0.0 (0)	6.0 (2.8)	9.5 (1.9)	6.1 (4.0)
NLL3	n.a.	n.a.	n.a.	8.0 (2.4)
PL1	3.4 (1.4)	12.5 (2.0)	7.8 (1.7)	9.0 (5.3)
PL2	3.4 91.4)	41.8 (7.0)	35.0 (4.3)	30.1 (16.2)
PL3	n.a.	n.a.	n.a.	10.4 (4.6)
PL4	n.a.	n.a.	n.a.	38.8 (7.0)
PL5	n.a.	n.a.	n.a.	22.1 (13.6)
PL6	n.a.	n.a.	n.a.	28.4 (7.9)
PW	3.3 (0.4)	1.1 (0.1)	2.0 (0.1)	2.3 (1.0)
PT	0.8 (0.1)	1.0 (0.1)	0.9 (0.1)	0.9 (0.2)
PI	4.1 (0.5)	1.2 (0.1)	2.4 (0.4)	2.8 (1.8)
PH1	61.0 (4.7)	82.0 (2.4)	74.3 (2.5)	73.0 (6.8)
PH2	54.3 (2.2)	89.3 (1.7)	84.8 (3.3)	62.3 (12.9)
SL	28.3 (7.4)	36.5 (1.7)	29.0 (1.8)	12.3 (5.5)
SD	3.4 (0.4)	2.4 (0.2)	2.5 (0.2)	4.5 (0.9)
SI	8.4 (2.3)	15.2 (2.0)	11.6 (0.9)	2.9 (1.6)
NAS	0.0 (0)	10.5 (1.3)	11.0 (1.8)	1.8 (2.2)
DB	100.0 (3.1)	41.8 (2.5)	75.5 (3.1)	89.8 (11.9)
DF	114.8 (5.7)	65.3 (3.5)	91.5 (11.7)	117.0 (17.3)
DBF	14.8 (3.2)	23.5 (1.3)	16.0 (1.8)	27.2 (13.6)

[a] See Table 1 for description of traits
[b] Mean (SD) of 4 plants
[c] Mean (SD) of 95 plants

Results

Description of phenotypes

On the basis of means and standard deviations, the two parents, 'Michihili' and Spring broccoli, were very different for most of the morphological traits measured (Table 2). The F_1 plants were intermediate for most traits. P1 and NLL1 were two exceptions for which the presence of pubescence and leaf lobes appeared to be controlled by dominant alleles at single loci. F_2 individuals showed a wide range of segregation for all of the quantitative traits.

Measurements of some related trait were significantly correlated with each other in the F_2 population. For example, petiole length (PL2) was positively correlated with petiole thickness (PT) ($r = 0.76$, $P < 0.0001$) and number of leaf lobes (NLL2) ($r = 0.89$, $P < 0.0001$), and was negatively correlated with lamina width (LW) ($r = -0.66$, $P < 0.0001$) and petiole width (PW) ($r = -0.49$, $P < 0.0001$). As expected, days to flower (DF) was positively correlated with the component traits days to bud (DB) ($r = 0.62$, $P < 0.0001$) and days from bud to flower (DBF).

Different types of phenotypic distributions were observed for traits measured in our F_2 population. The traits lamina length (LL), lamina index (LI), pubescence (P2), number of leaf lobes (NLL2), and petiole length (PL2) had similar types of distributions in which two discrete groups were observed (see Fig. 2A). Petiole index (PI), days to bud (DB), and number of axillary shoots (NAS) had obviously skewed distributions (see Fig. 2B). The remaining traits had normal or near-normal distributions (see Fig. 2C). For traits showing skewed distributions, most of the individuals appeared more like Spring broccoli than 'Michihili'. These traits were usually controlled by QTL with unequal effects, whereas traits with normal distributions were usually controlled by several putative QTL with small and similar effects (Table 3). For traits with skewed distributions, the data were transformed to improve normality. However, the reported results are based on non-transformed data because (1) in some cases the transformation did not improve normality, (2) the transformed units were not biologically meaningful, and (3) the same putative QTL were usually identified using the transformed and non-transformed data. Also, we found that deviation from normality did not seriously affect the results if underlying QTL had large phenotypic effects.

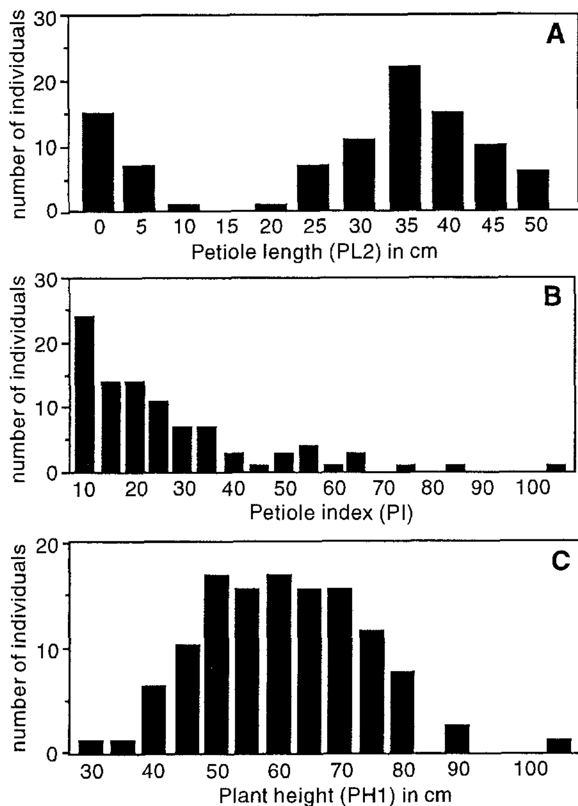


Fig. 2A–C Examples of phenotypic distributions of the 'Michihili' × Spring broccoli F_2 population for various traits. **A** Petiole length (PL2), **B** petiole index (PI), **C** plant height (PH1)

Marker trait analysis

The 220 marker loci used for QTL analyses covered ten linkage groups with a total length of 1593 cM and average spacing of 7.2 cM. On the basis of a LOD threshold of 2.8, 48 intervals contained putative QTL controlling the 26 quantitative traits measured, with a range of 0–5 QTL for each trait (Tables 1 and 3). In addition, 36 intervals had peak LOD scores of 2.07 to 2.79, suggesting the presence of possible QTL (data not shown). Of the 28 traits measured, 2 (P1 and NLL1) were controlled by single major genes, 11 traits were controlled by multiple genes with approximately equal effects on the phenotypes, and the remaining 15 traits were controlled by genes having obvious major and minor effects (Table 3).

Leaf traits

For the pubescence trait P1, 72 of the 95 F_2 plants had hairs (scored as 1), and 23 were hairless (scored as 0). However, continuous variation was observed for the degree of pubescence among the 72 individuals having hairs (trait P3). This observation suggests that the presence of pubescence was controlled by a dominant allele at a single locus, whereas the degree of pubescence was controlled by polygenes. On the basis of segregation for the presence or absence of pubescence, the single dominant gene, designated *Pub*, was mapped directly using MAPMAKER II on the linkage group 9A, flanked by marker loci *116b* and *145* (Fig. 1). By analyzing the data set based on numerical scores of 0–4 (P2), we found a QTL with a major effect at the *Pub* locus and a QTL having a minor effect on linkage group 7A (Table 3, Fig. 3A, B). When the hairless plants were excluded from the data set (trait P3), the major effect at the *Pub* locus was not observed and 3 putative QTL having small effects were uncovered on linkage groups 9A ($\sim$ 100 cM from *Pub*), 7A, and 4A (Table 3, Fig. 3).

The mode of inheritance of leaf lobes was similar to that of pubescence. The F_2 individuals could be divided into two discrete classes based on the presence/absence of leaf lobes (NLL1), whereas the number of lobes among the plants having leaf lobes (NLL3) had a normal distribution. A segregation ratio of 71 (with lobes) to 24 (without lobes) was observed, suggesting a dominant allele at 1 locus controlled the presence of leaf lobe. This gene, designated *Lob*, was mapped at the upper end of linkage group 4A (Fig. 1). Analysis of the same individuals for number of leaf lobes (NLL2) revealed a major gene effect at the *Lob* locus and an additional minor gene on linkage group 7A (Table 3). A small effect was observed at the *Lob* locus for the trait NLL3, and 2 additional QTL were detected on linkage group 7A and 10A (Table 3). These QTL had small and almost equal effects on the phenotype and may represent the minor genes controlling the number of leaf locus.

Several traits related to petiole morphology, such as petiole length (PL1–PL6), petiole width (PW), and petiole thickness (PT), were measured (Table 1). Two QTL were detected for PL1 on linkage groups 4A and 6A, and both had small and equal effects on the phenotype (Table 3). For the trait PL2, only one major gene effect was detected at the *Lob* locus with a LOD score of 38.75, and no minor genes were detected (Table 3). The same major gene effect was found for PL5 (LOD score of 32.34 at the *Lob* locus), and 1 additional QTL having small effects was detected on linkage group 7A (Table 3). When only individuals with lobes were analyzed for petiole measurements (PL3, PL4 and PL6), the major gene effect at the *Lob* locus was not observed, and additional minor genes were detected. Comparison of results from these petiole measurements (Table 3) suggests that petiole length was controlled by a major gene (*Lob*) and several minor genes, depending on how the trait was measured. The major gene mainly affected length of petiole in the region with lobes, probably by controlling the presence or absence of lobes. Other petiole traits, including petiole width (PW), petiole thickness (PT), and the ratio of width to thickness (PI), were controlled by several QTL located on linkage groups 4A, 5A, and 6A (Table 3). Two QTL, located in the interval *22a–67* of linkage group 5A and the interval *32c–327* of 6A, were found to be associated with all 3 of the traits; whereas 1 QTL at the interval *Lob-48* of

Table 3 Summary of putative QTL controlling morphological traits.

Trait[a]	Linkage group	Flanking markers[b]	Peak position	LOD score	Confidence interval[c]	Additive[d]	Dominance[d]	%VAR[e]	Minimum number of QTL
P2	9	Pub-145 (1,2)	Pub	19.84	116b(+8) – Pub(+4)	–1.68	0.73	70.5	2
	7	79a-32b (1,2)	79a + 2 cM	3.53	43 (+2) – 32b(+2)	0.49*	1.08	20.3	
P3	9	153b – 95b(1, –)	153b + 8cM	2.92	136 – 197a(+4)	–0.85	–0.11	27.8	3
	7	32b – 54a(1,2)	32b	3.12	79a – 202a(+10)	0.49*	0.81	23.0	
	4	27 – 33(1, –)	27	2.85	149 – 33(+6)	–0.66	0.32	21.3	
LL	9	136 – 153b(1,2)	136	3.84	Off – 153b(+8)	4.20	3.32	17.0	2
	10	213b – 15b (1,2)	213b + 6cM	3.05	305b(+2) – 96d(+20)	3.67	3.02	15.9	
LAL	4	Lob – 48(1,2)	Lob	44.83	Off – Lob(+4)	–19.00	–14.21	88.9	2
	7	19b – 29d(1,2)	19b	2.88	Off – 19b(+10)	–7.43	–4.30	13.4	
LI	4	Lob – 48(1,2)	Lob	47.96	Off – Lob(+6)	–19.80	–14.42	90.8	2
	7	19b – 29d(1,2)	19b	2.88	Off – 19b(+10)	–7.52	–4.77	13.4	
NLL2	4	Lob – 48(1,2)	Lob	32.90	Off – Lob(+4)	4.76	2.58	82.5	2
	7	19b – 29d(1,2)	19b	3.27	Off – 19b(+10)	1.86	1.47	15.2	
NLL3	4	Lob – 48(1,2)	Lob	3.99	Off – 48(+6)	4.60	2.95	26.6	3
	7	79a – 32b(1, –)	79a	3.61	43(+2) – 79a(+4)	–0.38*	–2.06	20.7	
	10	9a – 95a(1,2)	9a	2.83	Off – 95a(+2)	0.67	1.52	16.9	
PL1	4	Lob – 48(1,2)	Lob + 4cM	5.80	Off – 48(+6)	3.13	4.12	31.8	2
	6	202b – 207(1,2)	202b	5.06	Off – 202b(+10)	–3.71*	–0.75	21.9	
PL2	4	Lob – 48(1,2)	Lob	38.75	Off – Lob(+6)	18.87	13.17	85.6	1
PL3	4	Lob – 48(1,2)	Lob + 4cM	3.71	Off – 48(+8)	2.50	3.29	20.7	1
PL5	4	Lob – 48(1,2)	Lob	32.34	Off – Lob(+6)	16.61	8.90	79.8	2
	7	43 – 79a(1,2)	43 + 2cM	3.86	107a(+2) – 32b	–5.45*	–8.98	19.2	
PL6	9	154 – 85(1,2)	154 + 6cM	5.06	317(+10)-Off	3.41	7.29	33.6	1
PW	4	Lob – 48(1,2)	Lob + 4cM	10.52	Off – 48(+8)	–0.86	–0.55	46.6	3
	5	22a – 67(1,2)	22a + 8cM	3.76	174(+2) – 67(+6)	–0.66	–0.63	26.5	
	6	32c – 327(1,2)	32c	3.45	54c(+4) – 32c(+6)	0.46*	–0.38	15.4	
PT	4	325 – 55b(1, –)	325	2.87	Off – 325(+6)	0.10	0.00	13.3	3
		22b – 319(1,2)	22b + 6cM	5.51	9b(+2) – 190b(+2)	0.14	–0.02	26.4	
	6	54c – 32c(1,2)	54c + 16cM	3.75	54c(+4) – 327(+4)	–0.07*	0.11	18.3	
PI	4	Lob – 48(1,2)	Lob + 4cM	8.07	Off – 48(+6)	–1.47	–1.02	40.5	4
		22b – 319-(1,-)	22b + 4cM	3.34	9b(+2) – 22b(+10)	–1.13	–0.11	18.1	
	5	22a – 67(1,2)	22a + 8cM	4.30	22a – 22a(+14)	–1.35	–1.23	31.1	
	6	32c – 327(1,2)	32c	5.73	54c(+8) – 32c(+4)	0.97	–1.09	24.3	
PH1	4	10a – 190b(1,2)	10a	3.38	22b(+6) – 209(+6)	3.45	2.03	15.1	2
	9	114 – 24b(1,2)	114	3.64	Off – 153b(+18)	3.61	2.41	16.2	
PH2	5	67 – 91a(1,–)	67	2.86	22a(+4) – 91a(+2)	–6.37*	–5.21	13.1	1
DB	3	113 – 102(1,2)	113 + 6cM	6.88	113(+2) – 102(+6)	–9.27	–8.88	40.1	5
	6	54d – 54b(1,2)	54d	4.59	79b(+2) – 44a	–6.57	–5.72	20.0	
	7	79a – 32b(1,2)	79a + 2cM	3.96	117(+4) – 202a(+8)	–6.45	–4.42	18.6	
		20 – 25b(1,2)	20 + 4cM	3.19	309(+6) – off	–3.26	–7.41	14.7	
	8	186 – 46b(1,2)	186	2.86	Off – 186(+16)	–4.88	–6.25	13.8	
DF	3	200a – 29a(1,2)	200a	7.05	38a(+2) – 99a(+2)	–11.87	–9.16	29.7	1
SL	6	207 – 54c(1,2)	207 + 2cM	9.43	202b(+4) – 54c	3.52	–4.46	39.7	3
	7	105 – 42(1,2)	105b + 8cM	3.94	Off – 79a	3.21	–1.84	19.5	
	8	29c – 36a(1,2)	29c	3.28	38d(+4) – 29c(+6)	1.78	–3.81	14.7	
SI	6	207 – 54c(1,2)	207 + 2cM	8.90	202b(+4) – 54c	1.05	–1.16	37.6	3
	7	105b – 42(1,-)	105b + 6cM	2.90	148 – 79a	0.79	–0.52	14.6	
	8	29c – 36a(1,2)	29c	4.11	38d(+2) – 29c(+6)	0.67	–1.14	18.2	

[a] See Table 1 for description of traits

[b] Marker loci flanking a peak LOD score. The numbers in parentheses indicate that LOD ≥ 2.6 and 2.4 were observed in the same interval using the subset of 126 marker loci (1) and 56 marker loci (2), respectively (see text for details)

[c] Map position of one-log confidence intervals designated by marker loci plus cM toward the bottom of the linkage group; 'off' means that the boundary is beyond the end of the linkage group

[d] Additive and dominance effects of the allele from the male parent Spring broccoli in units of the trait measurement (see Table 1). *indicates QTL showing 'cryptic' gene effects (see text).

[e] % VAR indicates the percentage of phenotypic variation explained by the putative QTL

linkage group 4A mainly contributed to the trait PW, while another QTL at *22b-319* of 4A contributed to the trait PT.

Leaf length (LL) was controlled by at least 2 QTL located on linkage groups 9A and 10A (Table 3). Lamina length (LAL) and ratio of lamina length to width (LI) were controlled mainly by a major gene at the *Lob* locus and a minor QTL located on the linkage group 7A. The LAL measurement included the lobed portion of the leaf, and thus the *Lob* locus controlling the presence or absence of lobes probably had a large effect on lamina length.

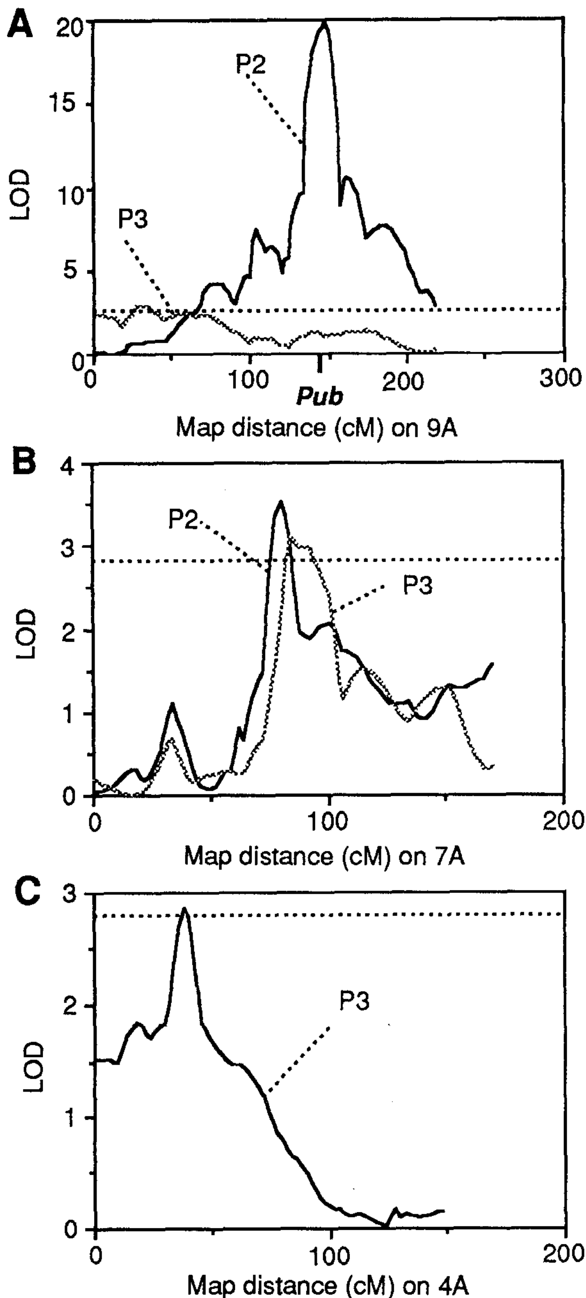


Fig. 3 LOD profiles for QTL controlling the pubescence traits P2 and P3 on linkage groups 9A (**A**), 7A (**B**) and 4A (**C**). The *Y axis* gives LOD (log of the odds ratio) scores for the probability of the presence of QTL for each trait. The *horizontal line* at LOD = 2.8 indicates the threshold selected for declaring a putative QTL. The *X axis* shows the relative position of QTL effects for each linkage group. *Pub* on linkage group 3A marks the position of a gene controlling the presence/absence of pubescence

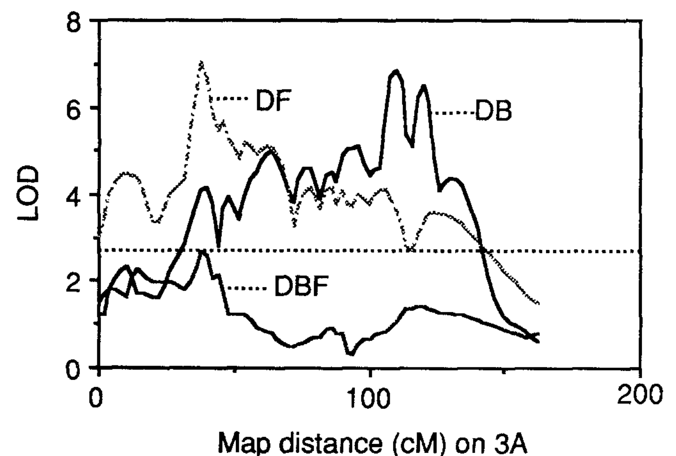
Stem traits

For plant height, two measurements were conducted at different developmental stages: one at the vegetative stage (PH1) and another at the first open flower stage (PH2). A minimum of 2 QTL for PH1 were detected on 4A and 9A, and 1 QTL for PH2 was detected on linkage group 5A (Table 3). All of the QTL controlling PH1 and PH2 showed small and equal effects on the phenotypes. Two and 3 QTL were found for stem length (SL) and the ratio of length to diameter (SI), respectively. There were no significant QTL detected for the traits stem diameter (SD) and number of auxiliary shoots (NAS); however, 3 and 5 possible QTL (LOD scores 2.07–2.79) were observed for these traits (data not shown).

Flowering traits

Genes controlling the traits days to bud (DB), days to flower (DF) and days from bud to flower (DBF) were detected on four linkage groups (Table 3). Five QTL controlling DB were found on linkage groups 3A, 6A, 7A, and 8A, and 1 QTL for DF was located on 3A. For DBF only 1 possible QTL (LOD = 2.67) was detected on 3A in the same interval as the QTL for DF (Fig. 4). Marker loci across this entire linkage group were found to be significantly associated with the traits DB and DF (Fig. 4). A major QTL having a large effect on DB (LOD score = 6.88) was observed at the interval defined by marker loci *113* and *102*; whereas a major QTL for DF (LOD = 7.05) was found in the interval defined by marker loci *200a* and *29a* (Table 3, Fig. 4). These results suggest that genes conveying major effects for the traits DB and DF were located on the same linkage group but in different positions. Significant LOD scores for DB and DF also were observed between these positions in the region from marker locus *29a* to *113* on 3A. A test for multiple QTL indicated the possible existence of other QTL in this region for both traits; however, we could not

Fig. 4 LOD profiles for QTL controlling the flowering traits days to bud (*DB*), days to flower (*DF*), and days from bud to flower (*DBF*) on linkage group 3A. The *Y axis* gives LOD (log of the odds ratio) scores for the probability of the presence of QTL for each trait. The *horizontal line* at LOD = 2.8 indicates the threshold selected for declaring a putative QTL. The *X axis* shows the relative position of QTL effects for each linkage group



be certain if both traits shared a common set of minor genes.

Detection of 'cryptic' gene effects

The additive genetic effects of alleles from Spring broccoli on the traits measured are shown in Table 3. A positive value for a given QTL indicated that the Spring broccoli allele at that locus increased the trait value, whereas a negative value indicated that the Spring broccoli allele decreased the trait value or that the allele from 'Michihili' increased the trait value. In most cases, differences between genotypic class means were consistent with morphological differences between the two parents. For instance, alleles from Spring broccoli usually increased the number of leaf lobes, petiole length, stem length, and plant height, whereas 'Michihili' alleles usually increased trait values for pubescence, lamina length, leaf index, and petiole width. This was particularly true for genes having major effects, such as those controlling P1, NLL1, and PL2. However, for QTL having minor effects, both parents contributed alleles that increased trait values. For example, alleles from Spring broccoli usually increased the petiole length from base to lobe (PL1); however, the Spring broccoli allele located on 6A had a negative additive effect (-3.71 cm) on PL1, that is, the allele from 'Michihili' at this locus increased the petiole length. These 'cryptic' gene effects (alleles from the low parent increasing the trait value) were observed for 8 of the 28 traits analyzed (Table 3).

Effects of marker density on detection of QTL

In order to determine how a reduction in the number of marker loci used would influence the detection of QTL, two subsets of 126 and 56 marker loci were selected (Fig. 1) and used to analyze trait data. Linkage maps based on these subsets of data were constructed using MAPMAKER II, giving total lengths of 1375 cM (10.9 cM average marker spacing) and 1235 cM (22.1 cM average marker spacing) for the sets of 126 and 56 marker loci, respectively. Based on these maps, we conducted QTL analyses for all of the traits examined, and the results were compared to those based on 220 marker loci. With the set of 126 marker loci, the same 48 QTL were detected ($\mathrm{LOD} \geq 2.6$) as those based on analysis with 220 loci ($\mathrm{LOD} \geq 2.8$) (Table 3). Also, the positions of peaks and intervals detected by 126 marker loci were almost identical to those detected by 220 marker loci. Analysis based on 56 marker loci detected 41 ($\mathrm{LOD} \geq 2.4$) of the 48 QTL detected using either 220 or 126 marker loci. LOD scores of the 8 undetected QTL ranged from 2.85–3.61 based on the 220 marker data set (Table 3). For most of the QTL detected by the 56 marker data set, the positions of the interval peaks were shifted 2–8 cM compared to those in data sets of 220 and 126 marker loci (data not shown). When the minimum number of QTL was used as a criterion, the efficiency of detecting putative QTL did not decrease when the number of marker loci was reduced from 220 to 126, but decreased 16.3% after reduction to 56 marker loci. However, QTL with LOD scores > 4 were detected in all three data sets (Table 3).

Discussion

Inheritance of traits examined

Various patterns of inheritance were observed for the morphological traits examined in this population. Inheritance of the traits pubescence (P1-3), leaf lobes (NLL1-3), and petiole length (PL1-6) represented typical major gene plus minor gene systems in which a single major gene controlled the presence/absence of the traits; however, the degree of expression of the trait was determined by several minor genes with similar effects. Inheritance of plant height (PH1 and PH2), leaf length (LL), petiole thickness (PT), and stem diameter (SD) represented typical polygenic systems in which traits were controlled by several genes with small and similar effects. Other traits had inheritance patterns between these two extremes.

Some functionally related traits, such as traits related to petiole morphology (PL, PW, PT, and PI) and the traits related to flowering (DB, DF, DBF), were highly correlated and were controlled by QTL located on the same linkage groups. This also was observed in a similar study of morphological variation in *B. oleracea* (Kennard et al. 1994). In that study, the same set of QTL based on map position appeared to control both days to bud and days to flower. In our study, major QTL on linkage group 3A controlled both traits, but the peak effects were at different map positions. F_2 individuals with different marker genotypes in these regions could be used as parents of segregating progenies to determine if separate linked QTL control these two traits.

Factors affecting detection of QTL underlying complex traits

One of the important features of molecular marker-facilitated analysis of QTL is the ability to dissect complex traits into individual components or Mendelian factors. The results from this study demonstrate the usefulness of RFLP markers in dissecting genetic components of quantitative traits in *B. rapa*. However, there are still unanswered questions about the efficiency of using RFLP markers in determining the number, location, and magnitude of gene effects for a quantitative trait. In practice, QTL mapping with RFLP markers is expensive. Thus, it is important to determine the most efficient way to obtain sufficient information about the genetic control of a quantitative trait.

This study provided information on the effects of changing some parameters in QTL analysis. First, we found that analysis based on marker loci spaced 10.9 cM apart gave almost the same results as those based on marker loci spaced 7.2 cM apart. A further decrease in marker density resulted in the detection of fewer putative QTL at the selected thresholds. Since all of the QTL not detected by reducing the number of marker loci to a spacing of 22.1 cM were those having small effects, additional marker loci were particularly useful for detecting minor genes. Second, the selection of appropriate thresholds for declaring putative QTL seemed very important and dependent upon the specific goal and specific traits to be examined. In our study, 36 possible QTL for 17 traits were considered to be insignificant based on a LOD threshold of 2.8. This number is quite large compared to the total number of 48 significant QTL. Although more of these loci will be false positives than those exceeding the 2.8 LOD threshold, many of these might be real QTL with minor effects on the trait. The improvement of quantitative traits through plant breeding often involves the accumulation of favorable QTL having small effects on phenotypes. Therefore, a less stringent threshold might be appropriate for detecting minor QTL in an initial search. Additional testing with larger populations, more environments, and other populations will determine the value of targeting these loci for selection in a breeding program.

Another factor affecting the detection QTL is the method of measuring variation for a trait. In our study, the presence or absence of 2 traits, pubescence and leaf lobes, was controlled by single loci with dominant alleles conferring the presence of the traits. Plants that were homozygous recessive for these alleles did not provide information on the degree of pubescence or the number of leaf lobes, and thus they may have obscured our ability to detect minor gene effects in the QTL analysis of P2 and NLL2. By analyzing subsets of the population including only individuals with the presence of the trait (P3 and NLL3), we were able to detect additional putative minor genes controlling the degree of trait expression. A potential problem with this type of approach is the reduction in sample size by eliminating a portion of the population. Thus, the existence of minor genes detected by this method should be confirmed by analyzing a second, larger population. Another possible approach is to measure the trait in a way that excludes a portion of the phenotypic variation controlled by a major gene. An example of this in our study was the petiole measurement that excluded the portion of the petiole containing lobes (PL1). These approaches seem especially useful for analyzing populations in which the presence or absence of traits is controlled by major genes.

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References

Beckmann JS, Soller M (1986) Restriction fragment length polymorphisms and genetic improvement of agricultural species. Euphytica 35:111–124

Chyi Y-S, Hownecke ME, Sernyk JL (1992) A genetic linkage map of restriction fragment length polymorphism loci for *Brassica rapa* (syn. *campestris*). Genome 35:746–757

Edwards MD, Stuber CW, Wendel JF (1987) Molecular-marker-facilitated investigation of quantitative trait loci in maize. I. Numbers, genomic distribution and types of gene action. Genetics 116:113–125

Edwards MD, Helentjaris T, Wright S, Stuber CW (1992) Molecular-marker-facilitated investigations of quantitative trait in maize. 4. Analysis based on genome saturation with isozyme and restriction fragment length polymorphism markers. Theor Appl Genet 83:765–774

Ferreira ME, Williams PH, Osborn TC (1994) RFLP mapping of *Brassica napus* using F_1-derived doubled haploid lines. Theor Appl Genet (in press)

Figdore SS, Kennard WC, Song KM, Slocum MK, Osborn TC (1988) Assessment of the degree of restriction fragment length polymorphism in *Brassica*. Theor Appl Genet 75:833–840

Keim P, Diers BW, Olson TC, Shoemaker RC (1990) RFLP mapping in soybean: association between marker loci and variation in quantitative traits. Genetics 126:735–742

Keim P, Diers W, Shoemaker RC (1991) Genetic analysis of soybean hard seededness with molecular markers. Theor Appl Genet 79: 465–469

Kennard WC, Slocum MK, Figdore SS, Osborn TC (1994) Genetic analysis of morphological variation in *Brassica oleracea* using molecular markers. Theor Appl Genet 87:721–732

Kianian SF, Quiros CF (1992) Generation of a *Brassica oleracea* composite RFLP map: linkage arrangements among various populations and evolutionary implications. Theor Appl Genet 84:544–554

Lander ES, Botstein D (1989) Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121: 185–199

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) Mapmaker: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Landry B, Hubert N, Etoh T, Harada JJ, Lincoln SE (1991) A genetic map of *Brassica napus* based on restriction fragment length polymorphism detected with expressed DNA sequences. Genome 34:543–552

Landry B, Hubert N, Crete R, Chang MS, Lincoln S, Etoh T (1992) A genetic map of *Brassica oleracea* based on RFLP markers detected with expressed DNA sequences and mapping of resistance genes to race 2 of *Plasmodiophora Brassicae* (Woronin). Genome 35:409–420

Lincoln SE, Lander ES (1990) Mapping genes controlling quantitative traits using MAPMAKER/QTL. Whitehead Institute for Biomedical Research, Technical Report, Cambridge, MA

Nienhuis JT, Helentjaris T, Slocum M, Ruggero B, Schaefer A (1987) Restriction fragment length polymorphism analysis of loci associated with insect resistance in tomato. Crop Sci 27:797–803

Osborn TC, Alexander DC, Fobes JF (1987) Identification of restriction fragment length polymorphisms linked to genes controlling soluble solid content in tomato fruit. Theor Appl Genet 73: 350–356

Paterson AH, Lander ES, Hewitt JD, Peterson S, Lincoln SE, Tanksley SD (1988) Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment length polymorphisms. Nature 335:721–726

Paterson AH, Damon S, Hewitt JD, Zamir D, Rabinowitch HD, Lincoln SE, Lander ES, Tanksley SD (1991) Mendelian factors underlying quantitative traits in tomato: Comparison across species, generations, and environments. Genetics 127:181–197

SAS Institute Inc (1982) SAS user's guide: basics and statistics. SAS Institute, Cary, N.C.

Slocum MK, Figdore SS, Kennard WC, Suzuki JY, Osborn TC (1990) Linkage arrangement of restriction fragment length polymorphism loci in *Brassica oleracea*. Theor Appl Genet 80: 57–64

Song KM, Osborn TC, Williams PH (1988) *Brassica* taxonomy based on nuclear restriction fragment length polymorphisms (RFLPs). 2. Preliminary analysis of subspecies within *B. rapa* (syn *campestris*) and *B. oleracea*. Theor Appl Genet 76: 593–600

Song KM, Suzuki JY, Slocum MK, Williams PH, Osborn TC (1991) A linkage map of *B. rapa* (syn. *campestris*) based on restriction fragment length polymorphism loci. Theor Appl Genet 82: 296–304

Stuber CW, Edwards MD, Wendel JF (1987) Molecular-marker-facilitated investigation of quantitative trait loci in maize. II. Factors influencing yield and its component traits. Crop Sci 27: 639–648

Tanksley SD, Young ND, Paterson AH, Bonierbale MW (1989) RFLP mapping in plant breeding: new tools for an old science. Bio/Technology 7: 257–264

Williams PH (1985) Crucifer Genetics Cooperative: resource book. Crucifer Genetics Cooperative, Madison, Wis.