University of Alberta

Association of molecular markers with qualitative and quantitative traits in *Brassica juncea.*

by

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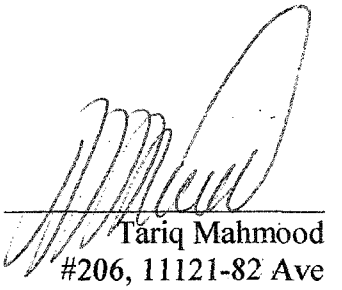

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled "**Association of molecular markers with qualitative and quantitative traits in *Brassica juncea***" submitted by **Tariq Mahmood** in partial fulfillment of the requirements for the degree of doctor of philosophy in Plant Science.

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To my Dad and late Mum

Abstract

Doubled haploid populations, developed from a cross and its reciprocal between a non-canola *B.* juncea cultivar (RLM-514) and a canola quality breeding line, were analyzed to construct an RFLP genomic map of *B. juncea*, and to study the genetics and mapping position of qualitative and quantitative traits.

The RFLP genomic maps developed from the two DH populations were homogenous, thus showing the absence of sex-based differences of recombination frequencies in *B. juncea*. In the combined map, 280 loci were assembled into 18 linkage groups, 20 into small segments, and sixteen remained unlinked. The *B. juncea* genome was highly duplicated and rearranged when compared with the genomes of other *Brassica* species.

At least 65 QTLs significantly affected yield and yield-contributing parameters. Strong QTL x environment and genotype x environment interactions were observed. QTLs associated with days to first flowering, pod length, number of pods per main raceme and 1000-grain weight, were more stable than those associated with days to maturity, seed number per pod and yield, across environments.

Two QTLs were associated with erucic acid content. The QTLs showed epistasis, and this model explained approximately all of the variation in the population. The same QTLs also affected oleic, linoleic, linolenic and eicosenoic acids but in an opposite direction i.e. alleles from non-canola quality and canola quality parents decreased and increased respectively the levels of these acids. For linolenic acid content, three

additional QTLs were identified. A new model for the inheritance of eicosenoic acid content has been proposed.

The glucosinolate profile of *B. juncea* consisted mainly of 2-butenyl and 3-propenyl glucosinolates. Major QTLs associated with individual glucosinolates were stable across environments, but disappeared when the data were analyzed using total glucosinolate content, probably because of a strong negative correlation between the individual glucosinolates. Breeding strategies have been proposed appropriate to the QTLs associated with individual traits in the study.

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List of symbols and abbreviations

RFLP	Restriction fragment length polymorphism
RAPD	Random amplified polymorphic DNA
DH	Doubled haploid
QTL	Quantitative trait loci
DNA	Deoxyribonucleic acid
FAO	Food and Agriculture Organization
n	Haploid number of chromosome
LEP	Low-gluc, low-erucic acid parent
HEP	High-gluc high-erucic parent
LOD	Log of the odds
LK	Linkage group
BN	*Brassica napus*
BR	*B. rapa*
BO	*B. oleracea*
S	DH population derived from straight
R	DH population derived from reciprocal cross
C	R and S populations together
χ^2	Chi-square
F_1	First filial generation
F_2	Second filial generation
F_6	Sixth filial generation
MAS	Marker assisted selection
r_{ij}	Correlation coefficient between trait i and trait j
σ_{ij}/	Covariance of traits i and j
σ_i	Stnadard deviation of trait i
h^2	Heritability
σ^2_G	Genotypic variance
σ^2_P	Phenotypic variance
σ^2_E	Environmental variance

CHAPTER 1

INTRODUCTION

Plant breeding is both an art and science dealing with the changing of genetic architecture of plants with respect to their economic use. Thousands of improved crop varieties have been released throughout the world during the 20th century. In Canada, the development of canola is an excellent example of plant breeding achievement. The history of plant breeding is as old as agriculture itself. Plant breeding began when farmers first selected seeds for planting their crops. Over centuries, conscious or unconscious selection made by farmers, coupled with natural selection, led to the development of the present land races. The first gradual and noticeable change towards organized plant breeding as a specialized profession occurred in the late 19th century. Until then, plant breeding practices simply involved selecting superior cultivars from the existing variants, and plant breeders paid little attention to hybridization as a source of creating variation. The rediscovery of Mendel's work (Mendel 1865) in the early 20th century, provided the scientific basis for plant breeding, and plant breeders began emphasizing hybridization for creating novel combinations. Concepts such as resistance breeding, introgression, hybrid and synthetic varieties were well established before 1970.

The *Brassica* oilseeds, *Brassica napus*, *B. rapa* and *B. juncea*, have responded particularly well to plant breeding efforts, and are one of the world's most important sources of edible vegetable oils. These are commonly known as rapeseed and mustard species. Major producing areas include Canada, China, Northern Europe and the Indian subcontinent (Downey 1990). The species contain two characteristic components, erucic

acid in the oil and glucosinolates in residual meal. High concentrations of erucic acid in the oil may be associated with health risks (Beare et al. 1959, Raine and Uksela 1959). High glucosinolate contents cause problems in the digestive tracts of non-ruminent livestock (Fenwick et al. 1983). Over time, these two components have been genetically manipulated to safer levels, and these cultivars are now commonly referred to as "double low" or "double zero". In Canada, the name, "Canola" is reserved to describe these cultivars.

The commercially cultivated species in the genus *Brassica* are *Brassica rapa*, *Brassica oleracea*, *Brassica nigra*, *Brassica juncea*, *Brassica napus* and *Brassica carinata*. Earlier studies have shown that *B. rapa* (AA, 2n=20), *B. nigra* (BB 2n=16) and *B. oleracea* (CC, 2n=18) are diploid species. However, *B. juncea* (AABB, 2n=36), *B. napus* (AACC, 2n=38) and *B. carinata* (BBCC, 2n=34) are considered to be amphidiploids, originated from the diploid progenitors in different combinations (U, 1935) (Fig. 1.1). Such relationships were confirmed later by cytogenetics (Parkash and Hinata 1980), nuclear DNA contents (Verma and Rees 1974), chloroplast DNA analysis (Palmer et al. 1983; Erickson et al. 1983), artificial synthesis of amphidiploids by crossing the diploid parents followed by chromosome doubling (Song et al. 1993; Axelsson et al. 2000) and conservation of RFLP loci in resynthesized and naturally occurring amphidiploids and the diploid progenitors (Parkin et al. 1995; Axelsson et al. 2000).

In North America, it is essential that the *Brassica* oilseeds produced for the edible oil market be of canola type i.e. the oil contain erucic acid less than 2% of the total fatty acid and the air-dried, oil-free meal contain less than 30 micromoles /g of total

glucosinolate. Canada is among the three largest *Brassica* oilseed producing countries in the world, with total harvested area of 5,564,300 hectares and total production of 8,798,300 tonnes in 1999 (FAO Yearly Statistics 1999).

In Canada, *B. napus* and *B. rapa* are the only canola quality species grown commercially for oil and meal, whereas *B. juncea* is a condiment crop. However, this species is receiving major attention for its potential of being transformed into a canola quality crop (Downey 1990; Woods et al. 1991). Each of the above species has different desirable traits that the others lack, or for which there is only poor expression. *Brassica napus* is a later maturing species compared to *B. rapa*. *B. napus* has high yield potential, and is tolerant to white rust diseases. It also has high oil and protein contents and an excellent fatty acid profile. However, *B. napus* is not shattering resistant (Downey 1990; Woods et al. 1991). *B. juncea* is more heat resistant and drought tolerant than other species. There is a wide range of maturity within *B. juncea*. Canadian condiment mustards are more resistant to seed shattering and higher yield potential than either canola species and are highly resistant to blackleg disease caused by *Leptosphaeria maculans*. However, *B. juncea* has a high level of glucosinolates and contains a significant content of erucic acid, thus making the seed unsuited for edible oil processing in western countries (Woods et al. 1991). However, there is an urgent need to develop canola quality *B. juncea* with an oil content and fatty acid profile similar to other canola species. The potential for this new crop in warm dry areas of the southern prairies is substantial, with the possibility that by its introduction, an additional 4-6 million acres could be utilized for canola production across the prairies (G.R. Stringam, personal communication).

Developments in molecular biology are particularly playing a major role in crop improvement today, and have provided two important tools to plant breeders: genetic transformation and DNA markers (Edwards 1992; Tanksley et al. 1989; Koornneef and Stam 2001). Until recently, only those genes available within the gene pool of a particular crop and its related species were available for breeding. However, with improved transformation and cloning techniques, plant breeders can have available potentially any gene to solve difficult problems e.g. resistance to insects using *Bacillus thuringiensis* genes, herbicide resistance, modification of fruit ripening, engineering male sterility (see review by Koornneef and Stam 2001).

With the re-discovery of Mendel's laws (Mendel 1865), morphological markers have been mapped in various organisms such as Drosophila (*Drosophila melanogaster*) (Bridges 1935), maize (*Zea mays*) (Emerson et al. 1935) and tomato (*Lycopersicon esculentum*) (MacArthur 1934). These simply inherited morphological markers have been employed to track polygenic traits (e.g. seed weight by seed color (Sax 1923)). Morphological markers are limited in their availability, affected by environment, and explain a small proportion of the total phenotypic variation (Tanksley et al. 1989). Isozymes have also been successfully used in plant breeding as genetic markers (Tanksley and Rick 1983). However, due to insufficient numbers of the isozyme markers, the potential of genetic mapping in plant breeding was not fully exploited untill DNA markers were developed (Tanksley et al.1989). DNA markers are genetically neutral, potentially unlimited, and not affected by environment. Molecular maps have been developed for major crops such as, rice (*Oryza sativa*) (Kurata et al. 1997), tomato (Ganal et al. 1991), wheat (*Triticum aestivum*) (Reide and Anderson 1996), soybean

(*Glycine max*) (Mian et al. 1996), maize (Beavis and Grant 1991). Molecular markers have been identified in various crops for traits of economic importance *e.g.*, insect resistance in tomato (Nienhuis et al. 1987), aluminum tolerance in wheat (Riede and Anderson 1996), agronomic traits in rice (Xio et al. 1996), and seed weight in soybean (Mian et al. 1996).

Molecular maps are available for the *Brassicas*, (Kearsey et al. 1996; Lagercrantz and Lydiate 1995; 1996; Cheung et al. 1997; Landry et al. 1991; Foisset et al. 1996; Parkin et al. 1995), and have been used for different purposes e.g. to tag QTLs for different fatty acids (Hu et al. 1995; Jourdren et al. 1996a & b; Ecke et al. 1995; Thorman et al. 1996), glucosinolate contents (Uzunova et al. 1995), resistance to *Albugo candida* (Kole et al. 1996; Ferreira et al. 1995), study the effect of sex on the recombination fraction (Kearsey et al. 1996), and explore the homology, evolution and extent of genome duplication in different *Brassicas* (Langercrantz and Lydiate. 1995; 1996).

Efforts are currently under way to convert *B. juncea* into a canola crop (Stringam and Thiagarajah 1995; Love et al. 1990; Cheung et al. 1997), and there is an urgent need to establish a molecular map of *B. juncea* to facilitate selection of desirable traits. An array of doubled haploids derived from the F_1 of an University of Alberta canola quality line crossed with the East Indian mustard line RLM-514, were used for this study. The objectives were to:

1. Construct a detailed linkage map of *B. juncea* using doubled haploid population.
2. Elucidate the genetics of various agronomic and quality traits.
3. Study the stability of the identified QTLs for the traits across years and locations.

The results of these experiments should help breeders in general, and *Brassica* breeders in particular design their breeding programs and select desirable traits more effectively.

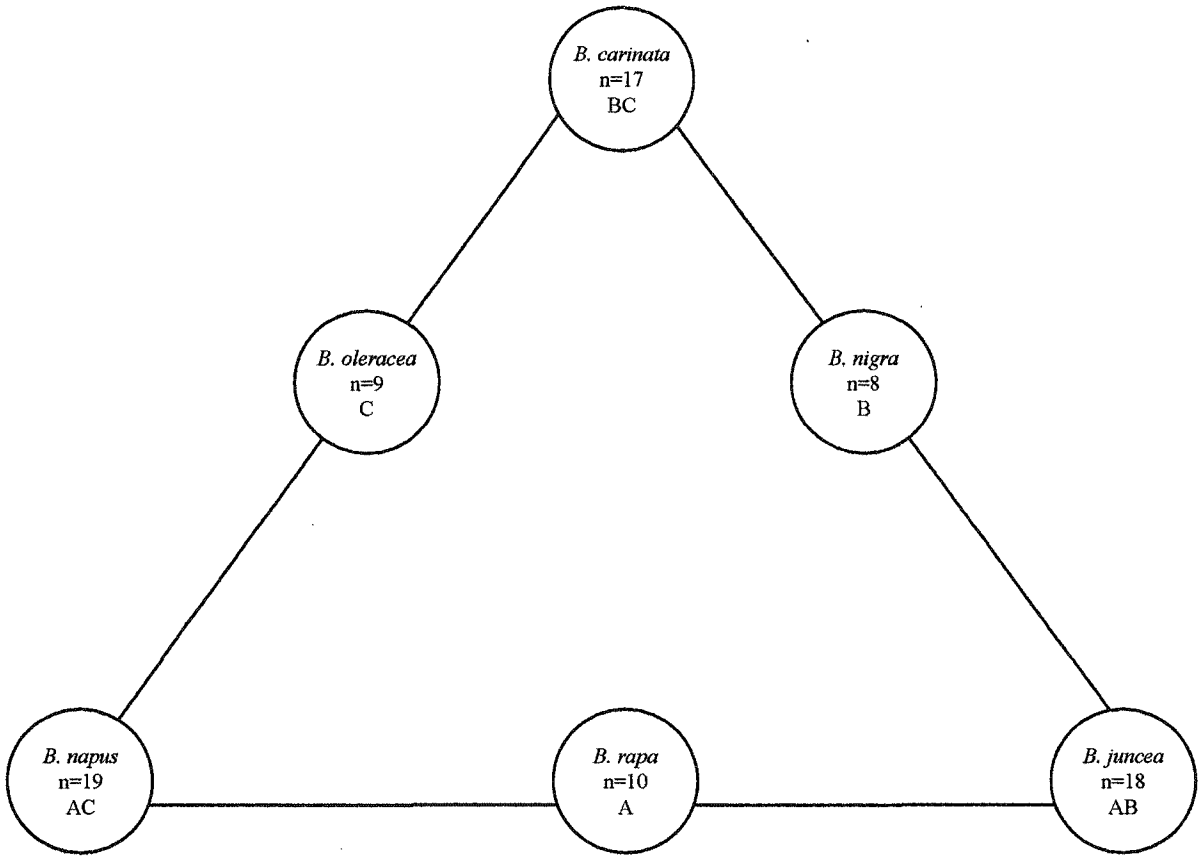

Fig. 1.1. Diagrammatic representation of the genomic relationships among the *Brassica* (after U, 1935).

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Chapter 2

RFLP linkage analysis of reciprocal DH populations of *Brassica juncea.*

2.1 Introduction

Since the development of the first human molecular map (Botstein et al. 1980), molecular markers have been extensively used in mapping genomes of crops. Whole genomes, whole chromosomes or any specific segment on any chromosome (Michelmore et al. 1991) can be mapped rigorously and accurately. Molecular maps have been published for various crops such as wheat (Wang et al. 1995), barley (*Hordeum vulgare*) (Graner et al. 1991), rice (McCouch et al. 1988), maize (Bentolila et al. 1992), lentil (*Lens culinaris*) (Havy and Muehlbauer 1989), citrus (Durham et al. 1992), potato (*Solanum tuberosum*) (Gebhardt et al. 1989), sugar beet (*Beta vulgaris*) (Pillen et al. 1992) and soybean (Apuya et al. 1988). In *Brassicas,* molecular maps are available for species such as *B. rapa* (Chyi et al. 1992; Teutonico and Osborn 1994), *B. napus* (Landry et al. 1991; Ferreira et al. 1994; Cloutier et al. 1995; Parkin et al. 1995; Sharpe et al. 1995; Uzunova et al 1995; Foisset et al. 1996; Kelly et al. 1997; Parkin and Lydiate 1998), *B. oleracea* (Kianian and Quiros. 1992; Landry et al. 1992; Kearsey 1996; Slocum et al. 1990; Camargo et al. 1997), *B. juncea* (Cheung et al. 1997; Axelsson et al. 2000), *B. nigra* (Truco and Quiros 1994; Lagercrantz and Lydiate. 1995; Lagercantz 1998). These maps have been used for various purposes i.e. to tag qualitative and quantitative traits (Butruille et al. 1999), exploit genetic diversity, (Thorman et al. 1994), study genome

evolution (Langercrantz and Lydiate 1996), and investigate sex-dependent differences of recombination frequency (Kearsey et al 1996).

Genetic maps are based on crossing over between genetic markers. Crossing over is not a random process. Therefore, genetic distances do not necessarily correspond to physical distances. Crossing over could be affected by various factors such as distance of loci from centromeres, intra-specific or inter-specific crosses (Paterson et al. 1990), specific genes (Baker et al. 1976; Karp and Jones 1982), environment (Elliot 1955) and sex (Langercrantz and Lydiate 1995). In the presence of sex-dependent differences in recombination rates, the incorrect choice of sex of parents in a cross (male vs female) can seriously affect especially marker-assisted back crossing, thus reducing the chances of recovering the genotype of the recurrent parent and eliminating linkage drag (Young and Tanksley 1989).

In animals, sex-based differences in recombination rates are common, and usually the heterogametic parent exhibits reduced recombination frequency (Dunn and Bennet 1967; Donis-Keller et al. 1987). In extreme cases, there could be no recombination at all, as in male Drosophila (*Drosophila melanogaster*) and female silkworm (*Bombyx mori*). However, hot spots for high recombination frequency have been observed in some heterogametic parents (Dunn and Bennet 1967; Lindahl 1991).

Most plants are hermaphroditic. However, distinguishable differences in recombination frequency in male and female meioses are present in plants such as maize (Robertson 1984). Earlier investigations for such differences have been limited to specific segments of chromosomes, because of the availability of only morphological markers and difficult chiasma formation studies (Robertson 1984). With available molecular markers,

these problems have been resolved, and whole genomes can be studied for such differences (de Vincente and Tanksley 1991; van Oijen et al. 1994; Lagercrantz and Lydiate 1995; Kearsey et al. 1996; Ganal and Tanksley1996; Wang et al. 1995; Kelly et al.1997).

Brassica juncea, an amphidiploid (AABB), is believed to contain the genomes of two diploid ancestors, *B. rapa* (AA) and *B. nigra* (BB) (U, 1935). The amphdiploid nature of *B. juncea* has been confirmed by cytogenetics (Parkash and Hinata 1980), nuclear DNA contents (Verma and Rees 1974), and chloroplast DNA analysis (Palmer et al. 1983; Erickson et al. 1983). In addition, artificial synthesis of *B. juncea* by crossing the diploid parents followed by chromosome doubling (Song et al. 1993; Axelsson et al. 2000) reveals conservation of RFLP loci in resynthesized and naturally occurring *B. juncea* and the diploid progenitors *B. rapa* and *B. nigra* (Axelsson et al. 2000).

Brassica juncea is normally a non-canola oilseed (high erucic acid and high glucosinolate contents). Because of its special characteristics such as blackleg resistance, drought tolerance, shattering resistance, and high yield potential (Woods et al. 1991), Canadian condiment mustard has attracted the attention of plant breeders for its conversion into canola type (Cheung et al. 1997; Love et al., 1990).

The primary objective of the present study was to develop a detailed RFLP genomic map of *B. juncea* using doubled haploid progeny, analyze sex-dependent differences in recombination frequency, determine the genetics of qualitative and quantitative traits, and associate RFLP markers to these traits for future use in marker assisted selection. The results of this study can be applied to hasten the conversion of condiment *B. juncea* to canola type.

In this paper, we report the RFLP genomic map, indistinguishable recombination rates in male and female meiosis, and the rearranged and highly duplicated genome of *B. juncea.*

2.2 Materials and methods

2.2.1 Plant material

The plant material used in this study originated from a cross and its reciprocal between two *B. juncea* lines, a non-canola cultivar (designated as high-erucic acid, high-gluc parent, HEP), and a canola line (designated as low-erucic acid, low-gluc parent, LEP) (Fig. 2.1). The non-canola cultivar is an introduction (RLM-514) from India, highly embroygenic in microspore culture with excellent agronomic traits.

A canola-quality *B. juncea* line (Selection 1058) (Love et al. 1990) was crossed with non-canola mustard at the Agriculture and Agri-Food Canada (AAFC) Research Station at Beverlodge by Dr. D. Woods. The F_2 seed of this cross was planted by the Canola Breeding Group at the U of A, and selection made for a yellow-seeded canola quality line with high oil content. An F_6 plant from this selection was used as the canola quality line (LEP, low erucic-gluc parent) in the cross (Fig 2.1) made in 1990. No seed was available from accessions 91-818-3 and 91-819-3. We obtained self-pollinated seed from only accession 92-117 (four plants) and 92-118 (three plants). The self-pollinated seed of four plants of accession 92-117 and three plants of 92-118 were grown to obtain DNA representing the HEP and LEP lines respectively. Four lines of HEP and three of LEP were used in the RFLP analysis.

Sixty-one doubled haploid (DH) lines were produced from a single F_1 plant (original cross) (designated as the S population), and fifty-one from seven F_1 plants

(reciprocal cross) (designated as the R population) (Fig 2.1) (Thiagaragah and Stringam 1993). The R and S populations together were designated as the C population. Self-pollinated seed of the DH lines were grown in 1998 for DNA extraction

2.2.2 DNA extraction, southern hybridization and clones

DNA extraction, restriction enzyme digestion, gel electrophoresis, and alkaline transfer were carried out as described by Sharpe et al. (1995). RFLP clones (names starting with ec, wg, tg) were provided by T.C. Osborn, University of Wisconson, USA. RFLP probe d3t7 was developed by A.G. Good, University of Alberta, Canada. The remainder of the RFLP probes were ESTs , as described by Sillito et al. (2000).

2.2.3 Linkage analysis

Linkage analysis was carried out by using Mapmaker version 3.0 (Lander et al. 1987). Initially, a LOD score of 5 and a distance of 10cM were used to form the initial linkage groups. Order, Sequence and Try commands were used to construct marker positions of individual groups. Wherever necessary, LOD score and distance were then reduced to 3 and 40 cM respectively to bridge the large gaps between markers. Double cross overs, especially in short intervals, were double checked. Kosambi mapping function (Kosambi 1944) was used to convert recombination frequencies into map distances.

2.2.4 Selection of probes

A total of 229 probes and five restriction enzymes (EcoRI, BamH, HindIII, XbaI, EcoRV) were employed to find the RFLPs between the parental lines (Table 2.1). The percentage of informative probes varied from 40% to 54% for the enzymes, with an average of 47%. Considering all the enzymes together, 69% (159) of the probes were

informative. From these probes, 132 were selected to map RFLPs in the two populations on the basis of easily-scorable bands.

2.3 Results

2.3.1 Genetic map

Three hundred and sixteen loci were scored from the 132 useful probes. However, some of the loci were not used in the two populations due to a larger proportion of data missing. Overall, 276 loci could be mapped in the R population and 307 in the S population. The maps derived from the two populations were homogenous (discussed below); hence, the two populations were combined to obtain a single map. Of 316 loci mapped in the C population, 280 were assembled into 18 linkage groups (LK1-LK18), 20 into seven small segments (A-E) and sixteen remained unlinked (Fig.2.2). The majority of RFLP loci were characterized by two alleles. However, 85 of 316 loci were scored as null for HEP or LEP due to missing bands. The symbols NP (null for HEP) or NM (null for LEP) were assigned to these loci (Fig. 2.2). These symbols were used because bands from HEP and LEP were designated as plus (+) and minus (-) respectively during scoring.

2.3.2 Duplication

Of the 132 clones used for probing, 316 loci were mapped. On average, 2.40 loci were scored per probe. Of 132 probes, 2 (1.5%), 8 (6.1%), 16(12.1%) 23(17.4%), 48(36.6) detected six, five, four, three and two polymorphic loci respectively. Only 35 clones (26.5%) gave only one polymorphic locus.

2.3.2.1 Intra-chromosomal duplication

Intra-chromosomal duplications are illustrated in Table 2.2. Of the 97 (73.48%) probes that showed duplication, only 10 (10.31%) depicted intra-linkage duplication in seven different linkages groups. Only probe wg4d5 was triplicated, while all other duplicated probes had two polymorphic bands (Table 2.2, Fig 2.2). Of the seven linkage groups, four (LK4, LK5, LK16 & LK17) had only one intra-chromosomal duplication. Three linkage groups (LK1, LK2 and LK14) had two intra-chromosomal duplications each (Table 2.2, Fig. 2.2). The distance between the loci ranged from 1.8cM (wg4d5a-wg4d5e on LK14) to 65.1cM (ec5a7a-ec5a7b on LK5).

2.3.2.2 Inter-chromosomal duplication

Approximately 89.7% (87/97) of the clones that detected duplication, involved inter-chromosomal duplication. At least fifteen rearrangements were observed among different linkage groups (Table 2.3). The difference between the genetic distances of rearranged segments varied from 0.2 cM to (LK6 & LK2) to 49.2 cM (LK6 & LK17). LK6 had inversions with five other linkage groups. LK3 and LK9 were involved in four and three inversions respectively (Table 2.3).

2.3.3 Comparison to the maps of related *Brassica* species

The *B. juncea* (BJ) map was compared to genomic maps of related *Brassica* species, and only comparisons having at least three common loci, were considered (Figs. 2.3, 2.4 and 2.5).

In relation to a composite *B. napus* (BN) map (Butruille et al. 1999) and based on 73 common RFLP probes, 23 comparisons were possible (Fig 2.3). The BJ linkage group LK6 had homology with four BN linkage groups (N2, N12, N3 and N13). The BJ linkage

groups LK1, LK12, LK14 and LK17 had common loci with BN linkage groups N4, N7, N8 and N14 respectively. No generalization regarding the distances between the conserved loci could be made in the two maps (Fig 2.3). No homology could be found between the BJ linkage groups LK11, LK13, LK15, LK16, LK18, and BN linkage groups N15, N17 N18, N19. From this comparative analysis, the BJ linkage groups LK1, LK3, LK4, LK5, LK6, LK10, LK12 and LK14 were identified as BN linkage groups N4, N5, N1, N10, N2, N3, N7 and N8 respectively (A genome). The BJ linkage groups LK7 and LK9 appeared to have evolved from the fusion of BN N6 and N9. The remainder of the BJ linkage groups (LK2, LK8, LK11, LK13, LK15-18) probably belong to the B genome.

A total of ten similarities were observed when the *B. juncea* map was aligned with a *B. rapa* (BR) map (Teutonico and Osborn 1994), using RFLP loci detected by 47 common probes (Fig. 2.4). The BR linkage groups LG3 and LG5 had homology with two BJ linkage groups each (LK8, LK10 and LK7, LK5 respectively). The BJ linkage groups LK7 and LK5 had common loci with two BR linkage groups each (LG1, LG5 and LG5, LG8 respectively). All BJ linkage groups except LK8, having common loci with the BR linkage groups, belonged to the A genome in *B. juncea* (Figs. 2.3, 2.4).

Based on RFLP loci detected by 55 common probes, conserved regions were detected in the *B. juncea* map and a *B. oleracea* (BO) genomic map (Camargo et al. 1997) (Fig 2.5). A total of seven comparisons were observed. BO linkage group LG1 had homology with three BJ linkage groups LK6, LK10 and LK5 (Fig 2.5). BJ LK8, the only linkage group associated with the B genome in *B. juncea*, had common loci with the BO linkage group.

By comparing the BJ map to the maps of the related species (BN, BR, BO), in approximately 50% of the cases, the gene order was conserved; in the remaining cases, rearrangements were observed. In general, the distances in the conserved regions were greater in *B. rapa* and *B. oleracea* than those in the *B. juncea*.

2.3.4 Homology between the A and B genome in *B. juncea*

The relationships between A and B genomes is shown in Fig 2.6. Linkage groups LK2, LK11 and LK8 appeared to be homeologous to LK6, LK12 and LK5 respectively. Linkage groups L18 and LK13 had conserved regions with LK1. No relationships could be found between the other linkage groups (Fig 2.6).

2.3.5 Residual heterozygosity

Residual heterozygosity can be implied if parents show polymorphism for a certain locus and that locus exhibits a non-segregating pattern in the DH population, and vice versa. Of 132 probes, 18 (13.1%) probes showed residual heterozygosity in the progeny of parental lines.

2.3.6 Segregation distortion

Segregation distortions were observed in both R and S populations. Out of 307 loci scored in the S population, 121 (39.4%) showed significant segregation distortion ($p<0.05$). Of 121 distorted loci, 91 (75.2 %) were skewed towards HEP and 30 (24.8%) towards LEP (Fig. 2.2). The distorted loci skewed towards the LEP and HEP differed significantly ($\chi^2=30.8$, $p<.001$). In the R population, 23.2% (64/276) of the loci showed significant segregation distortion ($p<0.05$). Approximately 45.3% (29/64) of the distorted loci skewed towards LEP and 54.7% (40/64) towards HEP in the R population (Fig. 2.2). Non-significant differences were observed between the distorted loci skewed towards

LEP and HEP (χ^2=0.56, p=0.25-0.5). The segregation distortion in the two populations differed significantly (χ^2=17.56, p<.001). In the two populations, the percentage of the markers skewed towards HEP showed no statistical difference (χ^2=3.23, p=0.05-0.1) but the percentage of markers skewed towards LEP differed significantly (χ^2=6.0, p<.05). Thirty-six loci showing distortion were common in the two populations.

The distorted loci showed a tendency of forming clusters on the linkage groups. Approximately nine major clusters (on LK1, LK5, LK9, LK10, LK13, LK14, LK15 and LK17) were observed in the S population. Linkage groups LK14, LK15 and LK17 consisted almost entirely of distorted loci (Fig.2.2). In the R population, only four clusters could be found (on LK1, LK5, LK8 and LK17), and the largest LK1 contained almost all the distorted loci (Fig. 2.2). LK11 was the only linkage group having loci without genetic distortion both in the R and S populations. Loci showing skewed segregation towards the LEP and HEP were found only on LK9. Linkage groups LK1, LK3, LK4, LK6, LK7, LK12 and LK13 had LEP-skewed distorted loci, and the remaining linkage groups showed HEP-skewed distorted loci in either of the populations. Linkage analysis of distorted loci was confirmed by a chi square test of independence (Foisset et al. 1996; Lorieux et al. 1995; Bentolila et al. 1992).

2.3.7 Reciprocal recombination differences

The difference in the frequency of meiotic recombination in F_1s from the original cross and its reciprocal was investigated in three different ways: (a) on an individual interval basis, (b) on a whole linkage group basis, and (c) on a whole genome basis. There were 233 intervals flanked by loci common in both S and R populations. The χ^2 test of heterogeneity (α=5%) was applied to determine the homogeneity of the two maps

(Table 2.4). Ten DH lines were randomly removed from the S population to adjust its size to that of the R population. All the χ^2 values were non-significant for all linkage groups, whole genome and individual intervals except nine intervals (3.9%). All linkage groups and unlinked segments except unlinked segment C exhibited non-significant χ^2 values for heterogeneity(Table 2.4, Fig. 2.2).This strongly suggests that the two maps derived from S and R populations are essentially the same, and can be integrated into a single map.

2.4 Discussion

This is the first RFLP genomic map of *B. juncea* developed from reciprocal DH populations. To our knowledge, we are the first to test for sex-based differences of recombination fractions in *B. juncea*. By using χ^2 test (A=0.05) 3.9% (9/233) intervals were found to be significantly different for recombination fraction in the R and S populations. A higher proportion of intervals (8/83) differed for recombination in the F_2 and doubled haploid populations derived from a cross in maize for unknown reasons (Bentolila et al. 1992). Therefore, our results strongly suggest the absence of sex-based differences of recombination fraction in *B. juncea*. Our results are supported by the findings of no differences in the recombination in the male and female meioses in *B. napus* (Kelly et al. 1997). However, contrasting results have been published for *B. nigra* (Lagercrantz and Lydiate 1995) and *B. oleracea* (Kearsey et al. 1996). Indistinguishable and distinguishable patterns of recombination in different *Brassicas* might be species specific (polyploidy level, phylogentic origin) (Lagercrantz and Lydiate 1995).

As in the *Brassicas*, there is no exact pattern of sex-based differences in the recombination rates in other species. Maternal/paternal recombination differences have

been observed in species such as maize (Robertson 1984), wheat (Wang et al. 1995), tomato (de Vincente and Tanksley 1991; van Oijen et al. 1994; Ganal and Tanksley 1996), and Rye (*Secale cereale*) (Reeves and Thompson 1956). However, recombination fraction is apparently not sex-dependent in other crops such as barley (*Hordeum vulgare*) (Sall and Nilsson 1994), and pearl millet (*Pennisetum glaucum*) (Busso et al. 1995).

The findings of the present study have important implications in genetic analysis and breeding strategies. As recombination in the present study was independent of male or female meiosis, either of the parents could be used as male or female in any breeding program. Moreover, integrated maps of *B. juncea* could be developed from different crosses without consideration of male or female meioses. Map based cloning requires that loci be mapped very finely and precisely in a particular interval. My results suggest that the direction of the cross would not, therefore, affect the fine mapping for mapped based cloning in *B. juncea.*

Polymorphisms detected in any species could be a function of the type of parental lines (i.e. genetic divergence between them), number of restriction enzymes used, and the type of probes used (Fidgore et al. 1988; Landry et al. 1991). Approximately 69% (159/224) of the probes were useful in detecting polymorphic bands between the parental lines (Table 2.1). A similar level of polymorphism has been observed in other *Brassicas* i.e. *B. napus* (Ferreira et al. 1994; Landry et al. 1991), *B. juncea* (Cheung et al. 1997), *B.nigra* (Truco and Quiros 1994), *B. oleracea* (Landry et al. 1992), *B. rapa* (Chyi et al. 1992). The *Brassicas* are more polymorphic than crops such as lettuce (*Lactuca sativa*), tomato, barley, sugar beet (*Beta vulgaris*) and soybean (Landry et al. 1987; Helentjaris et al. 1986; Bernatzky and Tanksley 1986; Graner et al. 1991; Pillen et al. 1992; Apuya et

al. 1988) but have shown similar polymorphisms when compared to rice, maize and potato (*Solanum tuberosum*) (McCouch et al. 1988; Gebhardt et al. 1989; Helentjaris 1985).

Of the 159 probes that detected polymorphisms between the parents in the present study, 10.5%(24/229) produced polymorphic RFLP loci with one restriction enzyme only; 59.0% (135/229) depicted the same polymorphism with more than one restriction enzyme. This shows that the majority of the detected polymorphisms resulted from deletions, insertions or rearrangements rather than point or small mutations. Similar findings have been reported in other *Brassicas* (Landry et al. 1991; 1992; Ferreira et al. 1994; Cheung et al. 1997).

In the present study, the parents were true breeding inbred lines but not doubled haploid lines. The LEP has a complex pedigree with introgression from *B. rapa* (Love et al. 1990) (Fig. 2.1). The *Brassicas* have a strong tendency to retain residual heterozygosity, despite repeated self-pollination. (personal communication of B.S. Landry with K. Downey (Landry et al. 1991). This explains why residual heterozygosity was observed in the present study. Similar findings have been reported in other *Brassicas*, where inbred lines were used as parents e.g. 9% (12/230) in *B. napus*, 13% (12/92) in *B. juncea* (Landry et al. 1991; Cheung et al. 1997). The best approach to eliminating this problem of residual heterozygosity is to use doubled haploid parental lines for mapping experiments.

Segregation distortions have been reported in *Brassicas* i.e. *B. rapa* (Song et al. 1991), *B. napus* (Landry et al. 1991; Ferreira et al. 1994; Coloutier et al. 1995), *B. oleracea* (Slocum et al. 1990; Kianian and Quiros 1992; Landry et al. 1992,), *B. juncea*

(Cheung et al. 1997), *B. nigra* (Truco and Quiros 1994). Segregation distortion is also common in other species e.g. rice (McCouch et al 1988), potato (Bonierbale et al. 1988; Gebhardt et al. 1989), maize (Bentolila et al. 1992), citrus (Durham et al. 1992), lentil (*Lens culinaris*) (Havy and Muehlbauer 1989). Segregation distortion has been attributed to genetic divergence of parents (Helentjaris et al. 1986; Paterson et al. 1990), factors affecting gametic selection during *in vitro* androgenesis and plant regeneration during the production of DH lines (Orton and Browers 1985; Guiderdoni et al. 1991), and environmental and random effects (Ferreira et al. 1994). In the present study, segregation distortion differed significantly in the DH populations derived from the original cross and its reciprocal. The proportion of the markers skewed towards LEP showed a significant difference in the populations. This suggested that maternal influence might affect the segregation distortion. In the present study, as far as intra-specific crosses are concerned, the highest percentage of segregation distortion has been observed in the *Brassicas*. This could have resulted from the greater genetic divergence of the two parents. In the S population, nine clusters of genetically distorted loci were observed in the present study (Fig. 2.2). A similar number (7-8) of clusters has been reported in *B.napus* and *B. juncea* (Cheung et al. 1997; Cloutier et al. 1995; Uzunova et al. 1995). This suggests that there might be some common regions in these *Brassicas* that are more susceptible to segregation distortion. Comparative mapping among these amphidiploids using the same set of probes might add new insight into these "hot regions" for genetic distortion.

Some of the loci have been scored null for HEP and LEP. Such loci have been observed earlier in the *Brassicas* (Slocum et al. 1990). This could be due to small restriction fragments not being retained on the gel, or weak hybridization of the probe to

the diverged sequences arising from complex chromosome rearrangements (Slocum et al. 1990).

The *Brassica* genome is highly duplicated, and its duplication is randomly distributed throughout the genome (Fig. 2.2). *Brassica* genome duplication is highly underestimated in every study by ignoring the monomorphic bands. In the present study, the highest level of duplication in *Brassicas* (73%) was reported. The underestimation of duplication even in the present study can be judged from the fact that all clones except ATTS6147, G8B7T7, wg1f10, and wg3e9 produced at least more than four bands (monomorphic and polymorphic). A high level of duplication has been reported in the *Brassicas* such as *B. nigra* (Lydiate and Lagercrantz 1996), *B. rapa* (Song et al. 1991), *B. oleracea* (Slocum et al. 1990). The level of duplication detected in a study could be affected by plant material, probes, experimental conditions, and the scoring of bands (Teutonico and Osborn 1994).

Intra-chromosomal duplication is a common phenomenon in the *Brassicas*. In *B. juncea*, 6.5% and 9.3% of the mapped probes and loci respectively were involved in intra-chromosomal duplication. About 34% (78/230) of the useful probes were involved in the inter-chromosomal duplication (Cheung et al. 1997). A similar level of intra and inter-chromosomal duplications has been observed for *B. napus, B. rapa, B. nigra* (Song et al.1990; Slocum et al. 1990; Landry et al. 1991; Chyi et al. 1992; Lagercrantz and Lydiate 1995; Uzunova et al. 1995). However, contrary results regarding inter- and intra-chromosomal duplication were reported for *B. oleracea* (Landry et al. 1992).

Numerous rearrangements were observed in the genetic map, and genetic distance among the rearranged segments varied considerably (Table 2.3). This variability could be

due to factors other than differences in recombination in these regions i.e. additions, deletions, mutations, translocations or other rearrangements. While comparing the map of *B. juncea* to those of the related *Brassica* species, and the A and B genomes in the present study, rearrangements were frequently observed (Figs. 2.3-2.6). It appears that some common segments were distributed among the genomes but their systematic arrangements (Lagercrantz and Lydiate 1996) were unclear. These results can be explained by assuming rearrangements as well as mutations after or before polyploid speciation. Moreover, interpretations of polymorphism analysis and null genes observed in the present study pointed towards a complex genome structure in *B. juncea*. Similar results/conclusions have been reported in *B. juncea* (Cheung et al. 1997; Song et al. 1995).

However, contrasting results have been reported by Axelsson et al. (2000), indicating that the genetic map of resynthesized *B. juncea* was colinear not only to natural occurring *B. juncea* but also to its diploid progenitor species *B. rapa* and *B. nigra*. No homoeologous crossing over was observed. This suggests that the genome of *B. juncea* and its diploid progenitor remained essentially unchanged since polyploid speciation. Similar conservation of A and C genomes has been observed in *B. rapa; B. oleracea,* naturally occurring and resynthesized *B. napus* after amphidiploid formation (Parkin et al. 1995; Parkin and Lydiate 1998).

Differing conclusions regarding the semi-conservative and conservative nature of the *Brassica* genome might be attributable to different parental materials, probes, mode of propagation of artificially resynthesized amphidiploid *Brassicas*, experimental conditions in these studies and the ability/inability to identify linkage groups as

chromosomes. To our knowledge, there are no reports on the genetic control of homoeologous recombination in the *Brassicas*, as in wheat (i.e. Ph gene in wheat (Riley et al. 1956). Results of such investigation would be valuable in explaining the complexity of the *Brassica* genome.

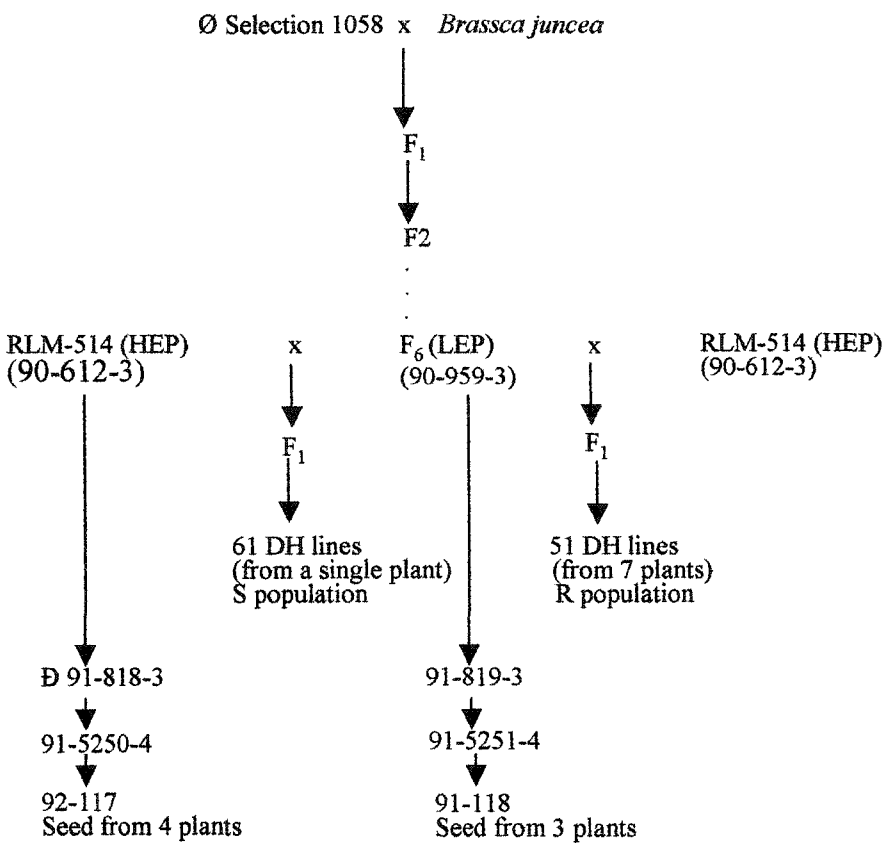


Fig. 2.1. Crossing scheme of parental lines.
HEP = high-erucic acid, high-gluc parent, LEP = low-eruic acid, low-gluc parent, Ø = *B. juncea* canola quality line developed by Love et al. (1990), Ð = Accession numbers used by the Canola Breeding group at the University of Alberta, S population = population derived from the original cross, R population = population derived from the reciprocal cross.

Fig. 2.2. Genetic linkage map of *Brassica juncea*. Eighteen linkage groups have been labeled as LK1-LK18 (arranged on the basis of length), and seven unlinked segments as A-G. Right side and left side of each LK corresponds to map derived from R and S populations. Distances within brackets represent map distances between loci from C population. Intervals followed by ! indicate the recombination fraction in the interval is significantly higher than that for the same interval in the other population. Null loci are followed by NP (null for HEP) and NM (null for LEP). * and ** shows loci deviated from 1:1 ratio at 5% and 1% levels of significance respectively. ‡ shows that distorted loci are skewed towards LEP, otherwise towards HEP. Dup. Loci refers to the proportion of duplicated loci on each linkage group. Distances are in cM. At the bottom of each linkage group, total and aggregate distances are given for C, R and S populations. Dots show that the distance in the particular interval is greater than 40cM, and the distances for such interval have not been added to total distance.

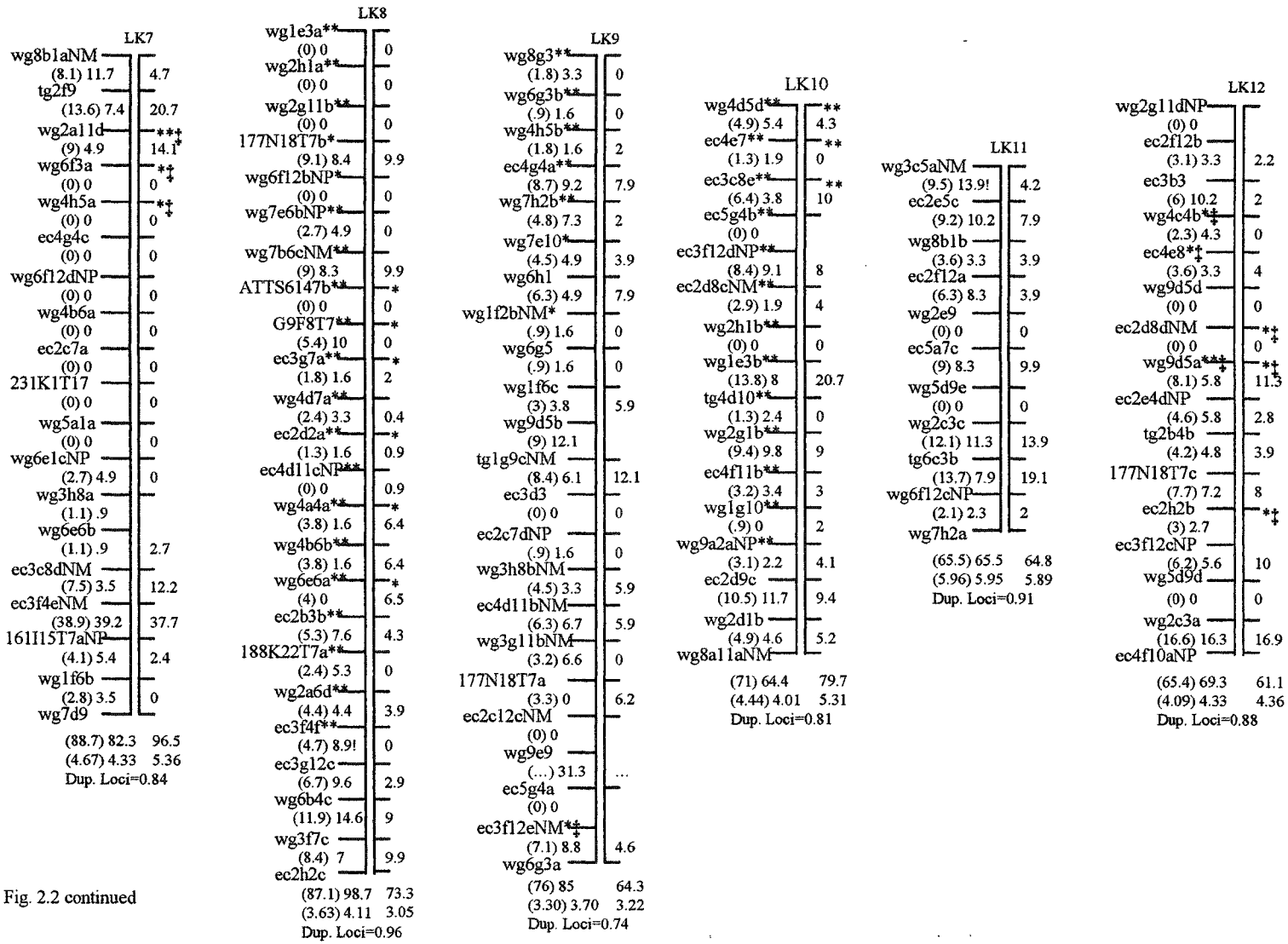
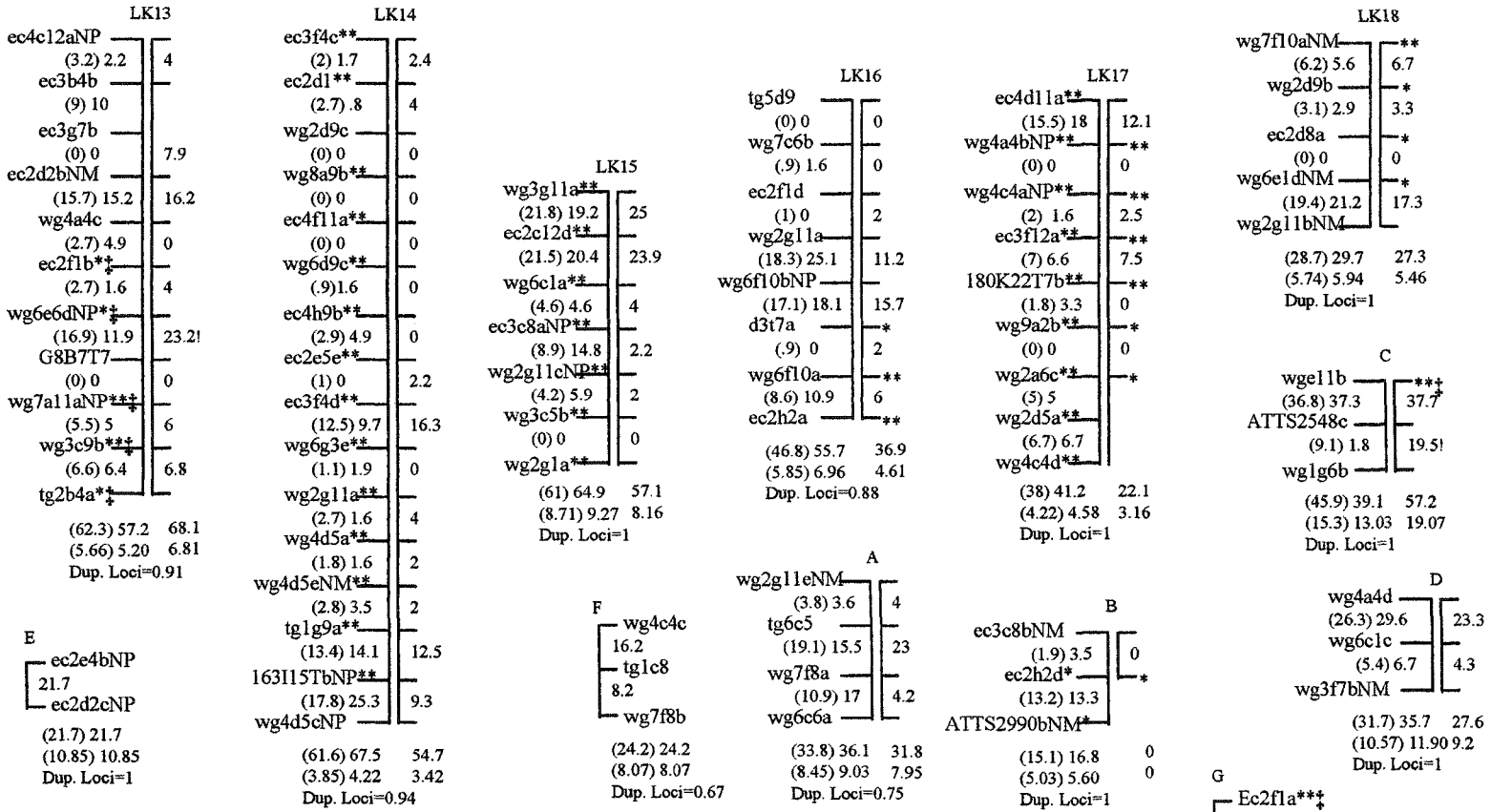


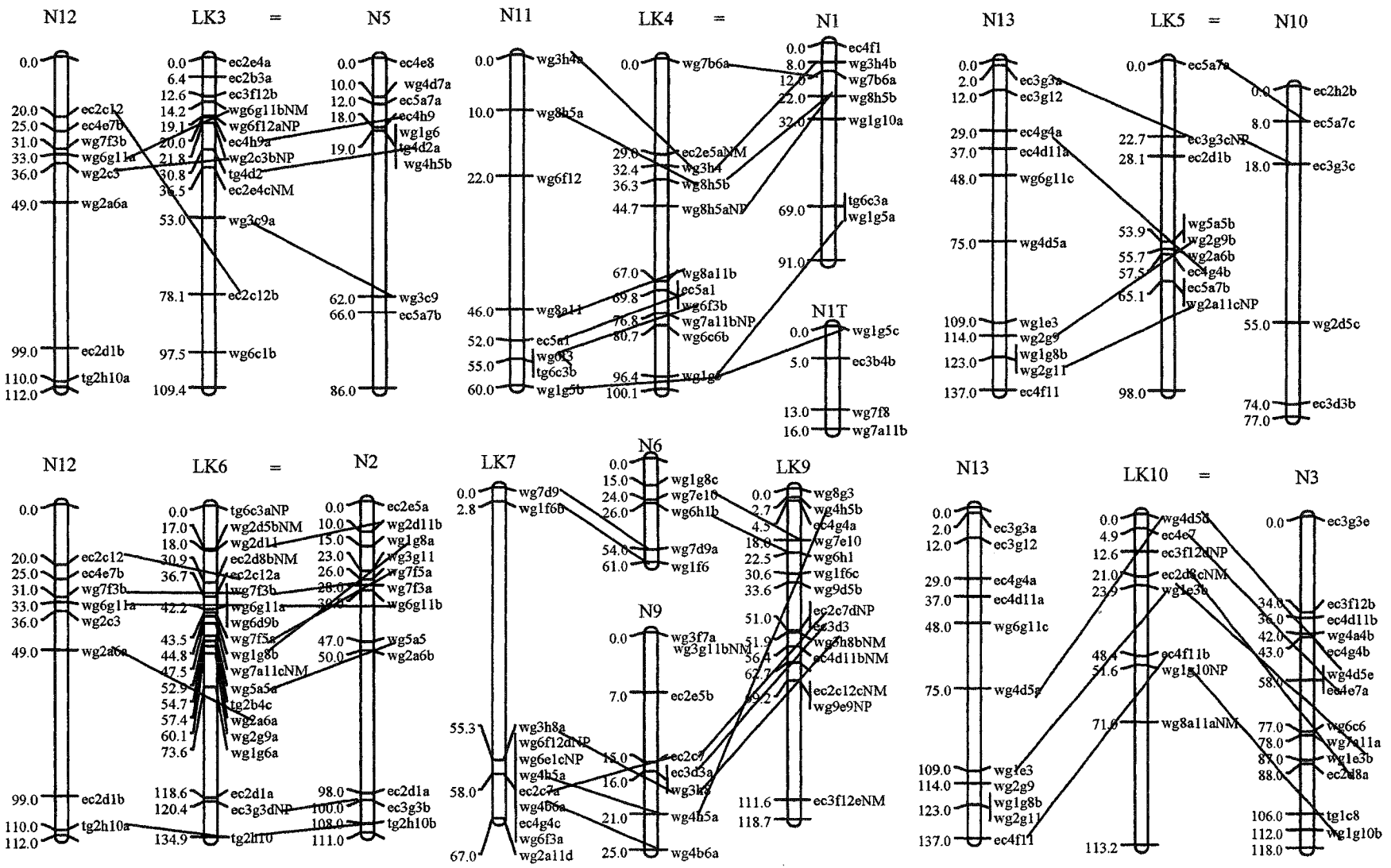
Fig. 2.2 continued



Unlinked loci: ATTS6147aNP*, ATTS2548aNP*‡, wg7b6b*, wg9g5cNM, wg9d5e, wg4d5bNP, ec2e5bNM, ec2e5d*, wg5a1b, wg6b4b, wg1f6a**‡, ec3g12bNM, ec3f4b, wg7h2cNM*‡, wg6e1bNM*‡, wg6g3c

Fig. 2.2 Continued

Fig. 2.3. Comparisons of the linkage maps of *B. juncea* and *B. napus*. Only loci detected by common RFLP probes, and only those linkage groups having at least three common loci in the two maps are shown. N = linkage groups of *B. napus* (Butruille et al. 1999), LK = linkage groups of *B. juncea*, N1-N10 = linkage groups of *B. napus* belonging to the A genome, N11-N19 = linkage groups of *B. napus* belonging to the C genome.



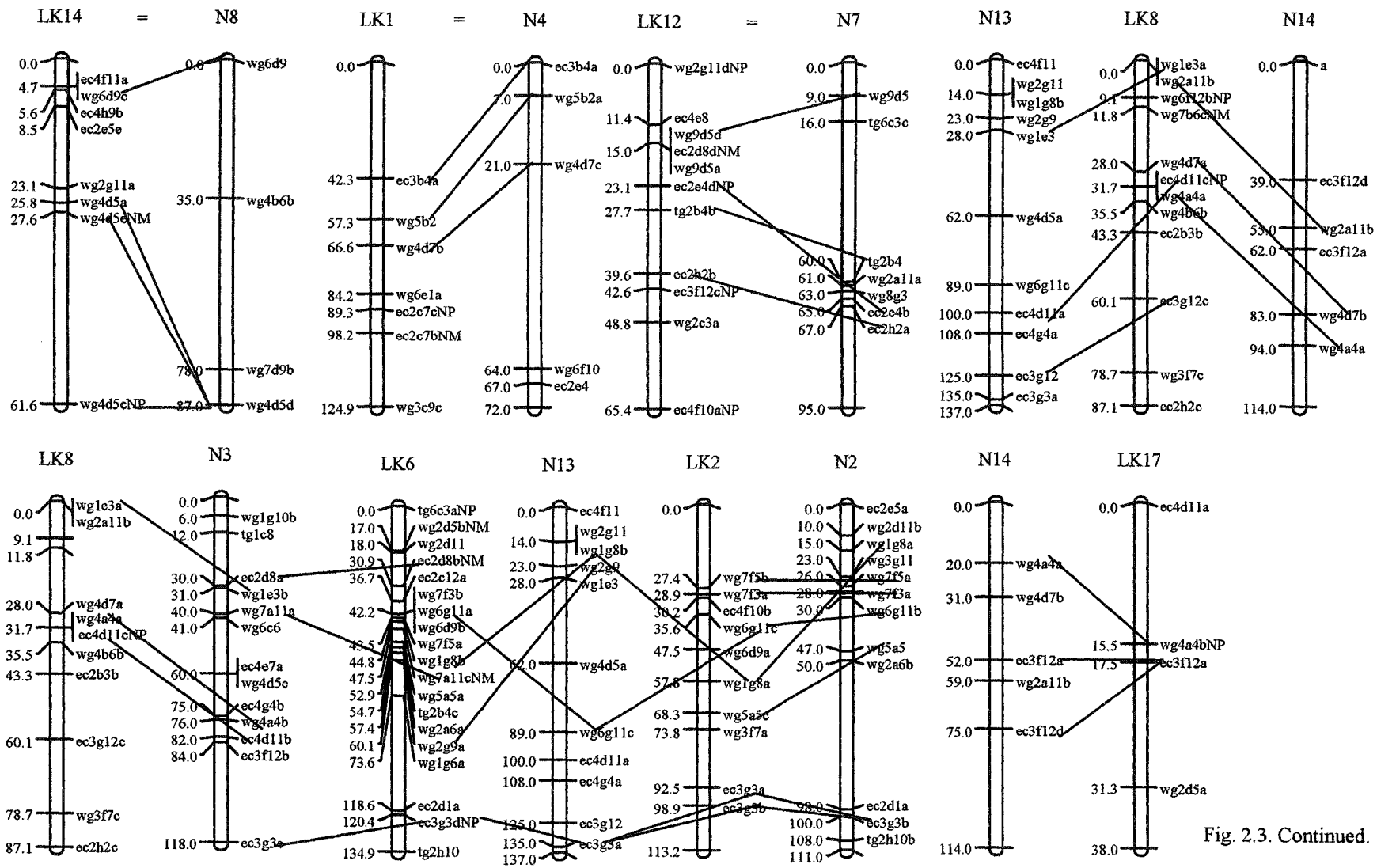


Fig. 2.3. Continued.

Fig. 2.4. Comparison of the linkage maps of *B. juncea* and *B. rapa*. Only loci detected by common RFLP probes, and only linkage groups having at least three common loci in the two maps are shown. LG = linkage groups of *B. rapa* (Teutonica and Osborn 1994), LK= linkage groups of *B. juncea*.

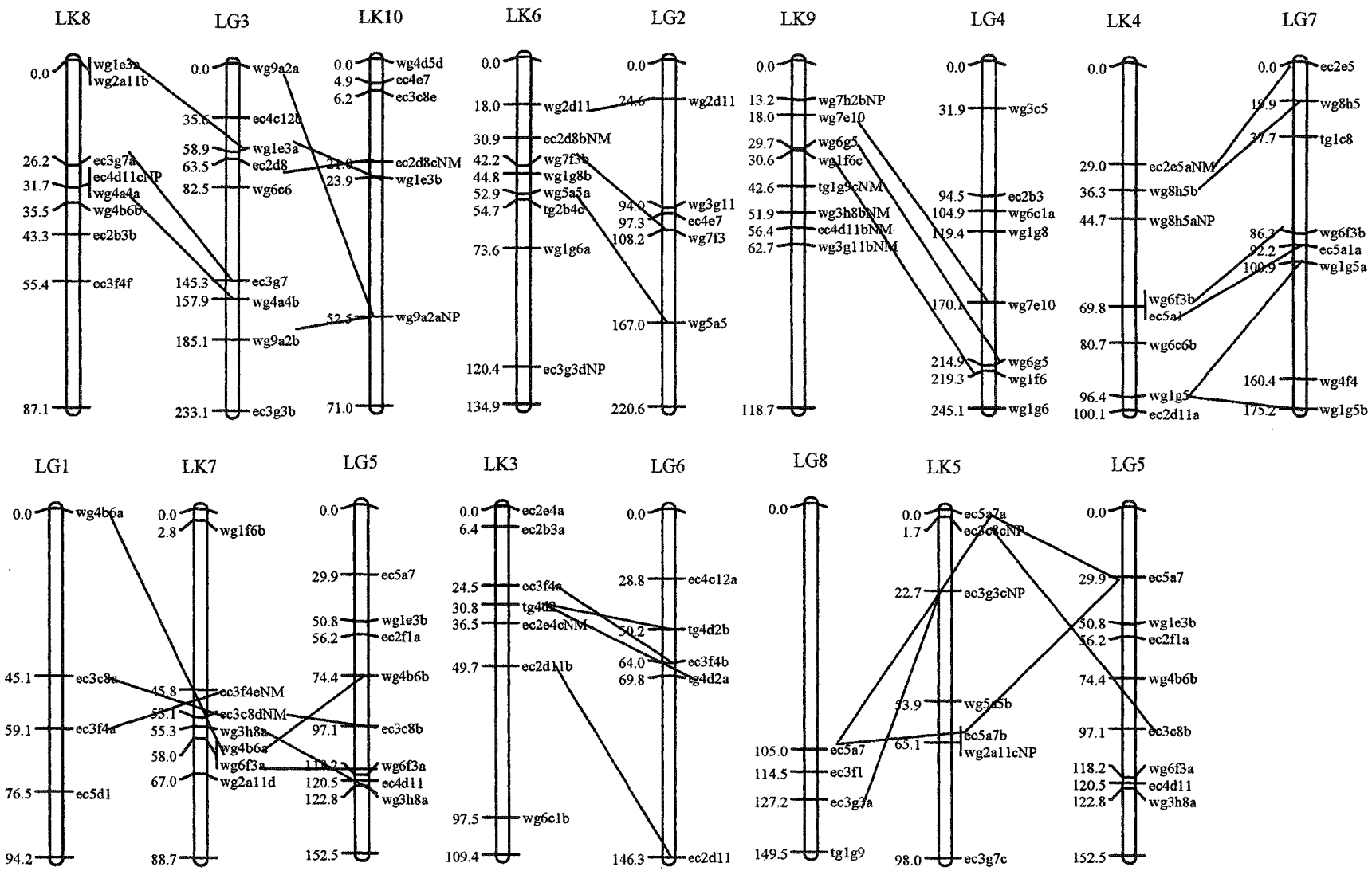
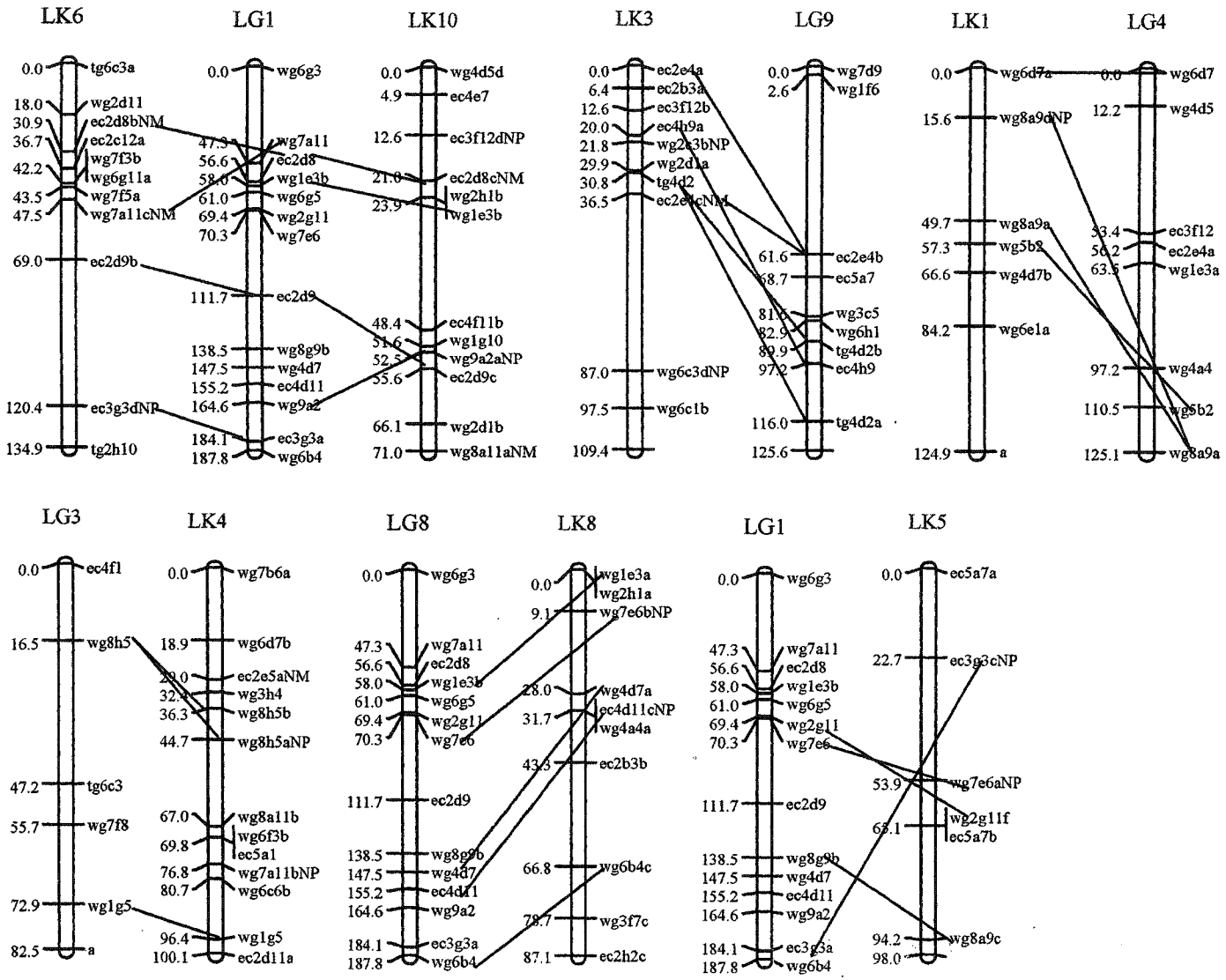


Fig. 2.5. Comparison of the linkage maps of *B. juncea* and *B. oleracea*. Only loci detected by common RFLP probes, and only linkage groups having at least three common loci in the two maps are shown. LG = linkage groups of *B. oleracea* (Camargo et al. 1997), LK= linkage groups of *B. juncea*.



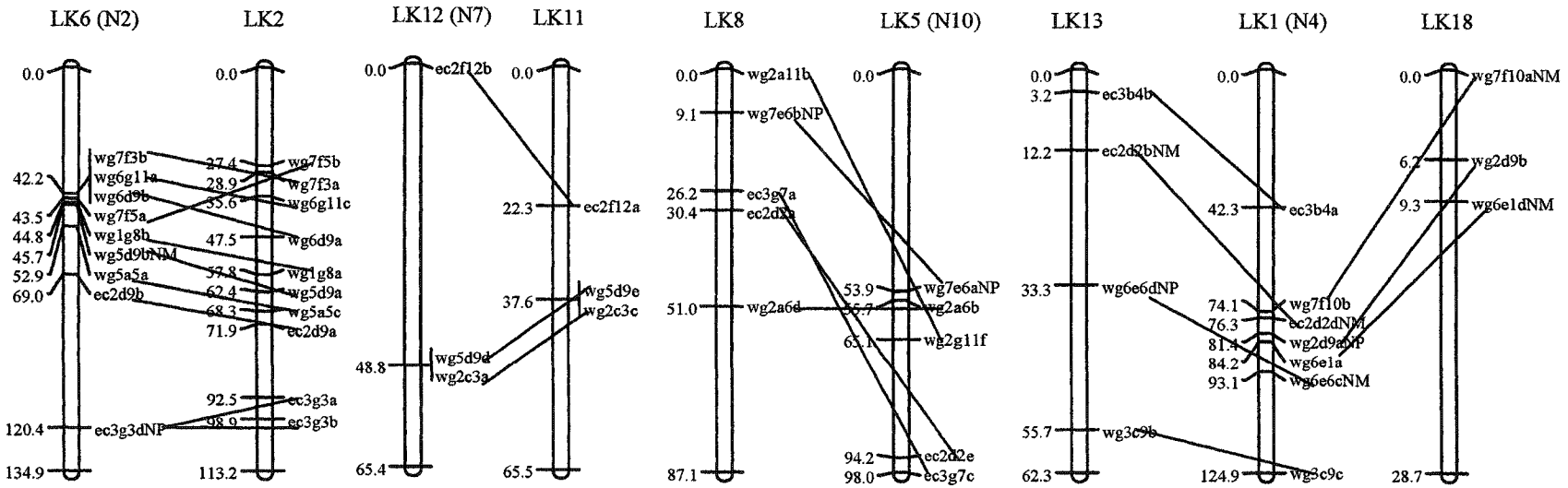


Fig. 2.6. Comparison of the A and B genome of *B. juncea.*

Table 2.1. Degree of polymorphism detected by various RFLP probes and restriction enzymes in *B. juncea.*

Probes	EcoRI	BamH	HindIII	XbaI	EcoRV	Overall
Used	222	229	224	223	138	229
Polymorphic	100	92	106	107	75	159
Monomorphic	122	137	118	116	63	70
Polymorphic(%)	45.05	40.18	47.32	47.98	54.35	68.99

Table 2.2. Intra-chromosomal duplications in *B. juncea.*

LK	Duplicated lci	Distance (cM)	Duplicated copies in others LKs
1	wg8a9a & d ec2c7c & b	34.1 8.9	(c) 5, (b) 14 (a) 7, (d) 9
2	wg5d9a & c ec3g3a & b	62.4 6.4	(b) 6, (d) 12 (e) 11 (e) 5, (d) 6
4	wg8h5a & b	8.4	-
5	ec5a7a & b	65.1	(c) 11
14	ec3f4c & d wg4d5a, e & c	 9.5 1.8, 34	(a) 3, (e) 7, (f) 8, (b) U & (d) 10, (b) U
16	wg6f10a & b	18	
17	wg4c4 a & d	22.5	(b) 12, (c) F

U, = unlinked, LK= linkage group, a, b, c, d, e, f = copies of the loci detected by the same probe,

Table 2.3. Rearrangements among linkage groups in the genetic map of *B. juncea*.

LK		Distance
8	wg6f12bNP-ec2b3b	34.2
3	ec2b3a-wg6f12aNP	12.7
8	177N18T7b-ec4d11c	31.7
9	ec4d11bNM-177N18T7a	9.5
6	ec2c12a-wg6g11a	11.3
3	wg6g11bNM-ec2c12b	58.2
6	wg5d9bNM-tg2b4c	9
12	Tg2b4b-wg5d9d	21.1
6	wg7f3b-wg7f5a	1.3
2	wg7f5b-wg7f3a	1.5
6	tg6c3a-wg5d9bNM	45.7
11	wg5d9e-tg6c3b	12.1
6	wg2d5bNM-wg2a6a	54.2
17	wg2a6c-wg2d5a	5
3	ec3f12b-ec2c12b	59.8
9	ec2c12cNM-ec3f12eNM	31.8
3	wg6f12aNP-wg2c3bNP	2.7
11	wg2c3c-wg6f12cNP	25.8
9	ec4g4a-wg1f2bNM	24.3
5	wg1f2aNP-ec4g4b	14
5	wg2g11f-wg8a9c	29.1
14	wg8a9b-wg2g11a	18.4
12	ec2d8dNM-ec3f12cNP	33.8
10	ec3f12dNP-ec2d8cNM	8.4
12	wg4c4b-ec3f12cNP	33.5
17	ec3f12a-wg4c4d	20.5
12	wg2g11dNP-ec2d8dNM	15
18	ec2d8a-wg2g11bNM	19.4
10	wg4d5d-ec4f11b	48.4
14	ec4f11a-wg4d5a	22.9

LK = linkage group

Table 2.4. Chi-square test for heterogeneity for two maps developed from populations derived from a cross and its reciprocal in *B. juncea*.

LK	(χ^2) Dev	(χ^2) Het	df	Intervals significantly differing for recombination fraction in two populations
1	2.86	22.54	12	wg6d7a-wg8a9dNP, wg5b2-tg1g9bNP
2	2.98	13.07	17	
3	0.02	12.37	18	
4	0.75	9.4	6	
5	3.06	10.58	11	179F6T7B-ec3g3cNP
6	1.09	24.8	19	wg2g9a-wg6b4aNP, wg1g6a-ec2d1a
7	0.37	7.55	15	
8	0.05	21.09	22	ec3f4f-ec3g12c
9	0.08	11.08	15	
10	0.31	7	12	
11	0.00	14.41	9	wg3c5aNM-ec2e5c
12	0.64	3.56	10	
13	0.69	11.08	7	wg6e6dNP-G8B7T7
14	0.20	10.24	14	
15	3.48	2.81	5	
16	0.95	4.05	6	
17	0.80	0.95	5	
18	0.19	0.07	3	
19	0.27	2.71	2	
21	0.47	4.18	1	ATTS2548c-wg1g6b
22	0.33	0.05	1	
Over All	0.30	209.86	232	

LK = linkage group, (χ^2) Dev = chi-square value for deviation from 1:1 ratio, (χ^2) Het = chi-square value for heterogeneity, df = degree of freedom.

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Chapter 3

Comparative mapping of QTLs for agronomic traits in mustard (*Brassica juncea*) in different environments using doubled haploid populations.

3.1 Introduction

In *Brassicas*, as in other crops, the expression of agronomic traits results from the interaction of multiple genes and the environment. The polygenic nature of these traits results in continuous variation, rather than discrete classes, and the traits are difficult to analyze genetically. Early attempts to track these polygenic parameters used simply inherited morphological markers (e.g. seed weight by seed color (Sax 1923) and flowering time by flower color (Rasmusson 1935). However, there are few phenotypic markers available, they tend to be affected by environment, and usually explain a small proportion of the total phenotypic variation. Hence, these markers are not well suited for extensive study of quantitative traits (Tanksley et al. 1989). Recent advances in molecular markers have allowed us to develop detailed genetic maps, with which we can now determine the number of QTLs controlling a quantitative trait, their gene action, phenotypic and pleiotropic effects, stability in different environments and interaction with other QTLs. Veldboom et al (1996a) reported QTLs associated with yield (a polygenic parameter highly influenced by environment) in corn in both stress and non-stress environment.

In North America, *Brassica napus* and *Brassica rapa* are the only canola species grown (erucic acid <%2, glucosinolate <30 micromoles /g of oil free meal). However, *Brassica juncea,* has a number of superior characteristics (i.e. high yield potential, early maturity, excellent drought tolerance, blackleg resistance) as compared to canola species

(Downey 1990; Wood et al. 1991) but is not canola quality. The Canola Breeding Group at the University of Alberta has developed a canola quality version of this species, however, this *B. juncea* canola type is poor agronomically as compared to condiment *B. juncea.*

Growing season conditions are very important for the development of the *Brassicas*, especially where there is possibility of drought (as in Australia) or frost (as in Canada) at the end of the growing season. Therefore, early maturity, without a yield-compromise, is highly desirable. Days to first flowering, last flowering, flowering period and maturity are important maturity determinants and inter-related. Molecular markers have been associated with flowering time in the *Brassicas* (Ferreira et al. 1995; Teutonico and Osborn1995); however, there are no such reports on other maturity determinants as days to last flowering, maturity and flowering period.

High seed yield is a major breeding objective for the *Brassicas* cultivars, and the most expensive and difficult trait to follow. Components associated with seed yield are, number of pods per plant, pod length, number of seed per pod and seed weight. In *B. napus*, pod length has been reported to be controlled by two dominant genes acting in a complimentary manner (Chay and Thurling 1989 a,b), and by three genes with additive gene action (Bing 1996). Pods play an important role in transporting nutrients and photosynthates to developing seeds (Norton and Harris 1975; Brar and Thies 1977). Previous studies have shown that seed yield is positively correlated with pod number, pod length, and seed number per pod (Thurling 1974, 1991; Shabana et al. 1990). Seed number per pod and seed-weight have positive association with pod length (Chay and Thurling1989a, b; Thurling 1991). Indirect selection for high seed yield through yield

components has been suggested (Thurling 1991). Unfortunately, genotype x environment interactions often cause changes in their relationships (Bing 1996). Various breeding methods such as bulk, pedigree, single seed descent, and doubled haploidy have been proposed to improve seed yield in the *Brassicas* (Downey and Rakow 1987; Thurling 1991). These methods are subjected to available resources and the breeder's personal choice and experience, rather than on the information about the interrelationships of these traits.

Marker assisted selection (MAS) is an important tool in the hands of plant breeders for the effective selection of complex traits such as yield. Using DNA markers, QTLs associated with agronomic traits have been mapped in all major crops, including maize (Veldboom and Lee 1996a &b; Berke and Rocherfoed 1995), rice (Xiao et al.1996; Lu et al.1999), soybean (Mansur et al. 1993; Lee et al. 1996), sunflower (Leon et al. 1995), and tomato (Paterson et al. 1991). *Brassicas* are the only major oilseeds lagging behind in this respect. Few studies dealing with agronomic traits in the *Brassicas* have been reported (Butruille et al.1999)

QTLs, expressed consistently across environments, are best suited for a marker-assisted selection program. Conflicting results have been published regarding the consistency of QTLs in different environments. Paterson et al. (1991) mapped 29 putative QTLs for morphological parameters in a tomato population grown in three diverse environments; only four were identified in all environments. Bubeck et al. (1993) reported different QTLs for different environments for gray leaf spot disease resistance in maize. A total of 44 QTLs were found affecting yield components and plant height in rice in three trials, however, only 17 were common in more than one environment (Zhuang et

al. 1997). Veldboom and Lee (1996a) found that 50% of the total QTLs detected for yield and yield components in maize in two different locations were common. Lu et al. (1996) identified 22 QTLs for six agronomic traits by growing DH populations of rice in three environments. QTLs for spikelet and filled grains per panicle were consistent across environments but inconsistent for heading date and plant height. Consistent QTLs for plant height and lodging and inconsistent ones for maturity across environments have been reported in soybean (Lee et al.1996).

The objective of the present study was to study the genetics of yield and yield-associated traits in *B.* juncea. The molecular markers, tightly linked with QTLs for yield and yield-associated traits, can be used for effective marker assisted selection to improve the agronomic traits of canola type *B. juncea.*

3.2 Materials and methods

3.2.1 Plant material

The plant material used in this study has been described in the chapter 2. Seeds, arising through (controlled) self-pollination, of parental and DH lines were sown in field trials in 1999 at the Edmonton Research Station and Ellerslie. The same lines were used for field trials during 2000 at the Edmonton Research Station, Kelsey and Ellerslie using seed harvested from the 1999 trial.

3.2.2 Experimental design

A randomized complete block design with three replications was used at each site. Taking into consideration the large size of the experiment, each replication was randomly divided into four sets. Two parental lines and 112 DH lines were randomly nested into four sets. Each set contained RLM-514 (HEP) as check. Thus one set contained 30 entries

and three sets 39 entries each. Each set contained the same DH lines in each replication but in a different arrangement due to randomization. The data was analyzed according to the following models:

a) By location and year

$$Y_{ijkm}=\mu + R_i + S_j + RS_{ij} + L(S)_{k(j)} + \in_{ijkm}$$

b) Across years by location

$$Y_{ijkmn}=\mu + Yr_n + R(Yr)_{i(n)} + S_j + L(S)_{k(j)} + YrL(S)_{nk(j)} + \in_{ijkmn}$$

c) Across locations by year

$$Y_{ijkml}=\mu + Lc_l + R(Lc)_{i(l)} + S_j + L(S)_{k(j)} + LcL(S)_{lk(j)} + \in_{ijkml}$$

Where R=replications, i=1to3, S=sets, j=1 to 4, L=lines, k=1 to 40, Yr=years, n=1 to 2, and Lc=locations, l=1 to 3.

Each plot consisted of four rows, 6m long and 0.3m apart. For each DH line, seed rate was adjusted according to its 1000-grain weight to ensure a uniform plant population. Before sowing, seed was treated with Furadon 5G for protection against beetle attack. Hand weeding was practiced throughout the season. To exclude border effects, the plot length was reduced to 5m by cutting 0.5m from back and front of each plot after seed set was complete.

Five plants were selected at random from the inner two rows and the number of pods on the main raceme counted. The first five pods formed on the main raceme of the selected plants were removed for measuring pod length and counting the number of seeds

per pod. Pods were sampled from the basal portions of the raceme since pods at this portion have more fertile ovules than the pods on the apical portion of the raceme (Bouttier and Morgan 1992), and are less variable than those on other parts of the plant. Pod length, defined as the distance between the pedicellar end connecting pod and base of the beak, of each sampled pod was measured (mm). Seed numbers per sampled pod were counted visually. Plant height was recorded after pod fill from the center of each plot. Each plot was harvested mechanically, and yield recorded. Bulk seed from each plot was used as a source for 1000-kernel weight. Where more than one observation was taken (e.g. number of pods/main raceme, seed number/pod), an average value was used to represent the DH lines.

Days to first flowering were recorded when at least 70% of plants in a plot were flowering. Days to last flowering were taken when flowering ceased on the main racemes of at least 70% of plants in a plot. Flowering period was determined by subtracting days to first flowering from days to last flowering. Days to maturity were taken by checking coloration and loss of moisture from seed at the base of the main raceme.

3.2.3 Trait analysis

Pheotypic correlations among different traits were determined using the formula $r_{ij}= \sigma_{ij}/\sigma_i\sigma_j$, where σ_{ij} is the covariance of traits i and j, σ_i and σ_j are the standard deviations for traits i and j respectively. Heritability was determined by the formula $h^2=\sigma^2_G/\sigma^2_P=\sigma^2_G/(\sigma^2_G+\sigma^2_E)$, where σ^2_G is the genotypic variance, σ^2_P phenotypic variance and σ^2_E environmental variance. Variances and covariances were computed using SAS/SAT 6.0 (SAS Institute Inc. 1989). The number of genes controlling a quantitative trait was determined by the following method (Snape et al.1984).

$k = ((Range)/2)^2/DH$ genetic variance

k = number of genes controlling a certain parameter.

3.2.4 RFLP and QTL analysis

An RFLP linkage map of *B. juncea* has been constructed. The map consisted of 18 linkage groups (280 loci), seven segments (20 loci) and 16 unlinked loci (Chapter II). MapQTL (version 3.0) (Van Ooijen and Maliepaard 1996) was employed for QTL analysis using MQM approach (Jansen and Stam 1994). This approach has two steps. First step involves finding putative QTLs using multiple regression or interval mapping. A LOD value of 2.4 was chosen as the threshold to declare the presence of a putative QTL. In the second step, markers close to QTLs were selected as co-factors, thus leading to a multiple-QTL model.

3.3 Results

In 1999, the trial at Ellerslie site was lost due to herbicide spray drift. At the Edmonton Research Station, the trial was damaged by hailstorm several days before harvesting. Yield data were recorded, but could not be used in statistical analysis due to very high coefficient of variability (CV). A cold and wet period occurred at Kelsey during flowering. Kelsey 2000 was included in the analysis, however, this environment could be considered as stressful.

3.3.1 Trait statistics

Analysis of variance indicated that the DH lines differed highly significantly for each trait in each environment. Least square means of each line were calculated and used for QTL analysis (data not shown). Parental means, population means and ranges were computed for each parameter (Table 3.1). Transgressive segregation was observed for

each parameter. Maximum transgressive segregation was observed for pod length in Ellerslie 2000; two parents differed by .4 mm, and population extremes by 25.7mm. Thousand-grain weight showed minimal transgressive segregation at the Edmonton Research Station 1999, where parental lines and population extremes differed by 1.6g and 1.8g per 1000-grain respectively (Table 3.1).

3.3.2 QTLs for different traits

3.3.2.1 Days to first flowering

Five QTLs significantly affecting days to flowering (FF) were found, three in each of the Edmonton Research Station 1999, the Edmonton Research Station 2000 and Kelsey 2000, two in Ellerslie 2000 explaining about 54.2%, 36.0%, 27.2% and 24.6% of total phenotypic variation respectively (Table 3.2, Fig. 3.1). In the mean environment, a new QTL FF2 appeared which was lacking in all environments. QTL FF18 was common in all environments, QTL FF8a and FF1 in three and two environments respectively. QTL FF8b appeared only in the Edmonton Research Station 2000.The HEP (high erucic parent) alleles increased days to flowering at QTLs FF1 and FF2. At all other QTLs, the LEP alleles caused an increase in days to flowering. The proportion of phenotypic variation explained individually by these QTLs varied from 8.5 to 28.9% and collectively from 24.6 to 54.2% (Table 3.2, Fig. 3.1).

3.3.2.2 Days to last flowering

Three QTLs affecting days to last flowering (LF) were identified in the Edmonton Research Station 1999, five in the Edmonton Research Station 2000, three in Ellerslie 2000 and four in Kelsey 2000. A total of 10 QTLs were mapped in all environments, and explained about 31.8-45.2% of total phenotypic variation. Of 10 QTLs, only two were

identified in the mean environment, explaining about 34.4% of total phenotypic variation. Interestingly, at QTL LF12 in the Edmonton Research Station 2000, the HEP alleles decreased days to flowering. However, at the same locus in Ellerslie 2000 and Kelsey 2000, the LEP alleles reduced days to flowering. Differential expression of another QTL FF8b was observed in the Edmonton Research Station 2000 and Kelsey 2000. Two QTLs, approximately 33cM apart, were mapped on linkage group 5. At QTL LF5a, the HEP alleles increased days to last flowering. However, the HEP reduced days to last flowering at QTL LF5b (Table 3.2).

3.3.2.3 Flowering period

Eight QTLs were found to significantly affect flowering period (FP) at least in one environment. Individually, these QTLs explained about 6.1-20.3% and collectively, about 27.6-50.0% of the total phenotypic variation in different environments (Table 3.2, Fig. 3.1). In the Edmonton Research Station, QTL FP3 appears to consist of two tightly linked QTLs (Table 3.2). Two QTLs were found on linkage group 5. At the QTL FP5a, the LEP alleles reduced the flowering period. However, at FP5b, the LEP increased the flowering period. The QTL FP12 showed differential expression, exhibiting a positive additive effect in the Edmonton Research Station 1999 and the Edmonton Research Station 2000 but a negative effect in Ellerslie 2000 and Kelsey 2000. The QTL FP12 was consistent in all environments, but could not be detected in the mean environment. Only one QTL, FP3, was observed in the mean environment (Table 3.2).

3.3.2.4 Days to maturity

Nine QTLs were mapped that affected days to maturity (MT) in different environments. Out of nine QTLs, only two QTLs were detected in the mean environment

(Table 3.2). These QTLs explained individually about 6.7-19.8%, and collectively about 20.5-45.2% of the total phenotypic variation. The QTL MT8a expressed differentially in Ellerslie 2000 and Kelsey 2000. Two QTLs were identified on linkage group 5. At both of these QTLs MT5a and MT5b, the LEP alleles contributed to days to maturity (Table 3.2).

3.3.2.5 Pod length

Eight QTLs significantly affecting pod length (PL) were detected in four environments (Table 3.2, Fig. 3.1). The QTLs explained individually about 7.6-16.7% and together about 36.3-46.1% of the total phenotypic variation in different environments. Of eight QTLs, five were detected in the mean environment, explaining about 48.4% of the total phenotypic variation. The QTL PL12 was consistent in all environments. The LEP alleles increased pod length at QTLs PL2, PL4 and PL8. At other QTLs, the alleles increasing pod length was contributed by the HEP (Table 3.2).

3.3.2.6 Seed number per pod

Eight QTLs were identified which influenced seed number per pod (SN) in four different environments (Table 3.2, Fig. 3.1). The proportion of phenotypic variation explained by individual QTLs varied from 8.0 to 16.5%. The proportion of the total phenotypic variation explained by these QTLs in different environments ranged from 23.1 to 45.4%. Out of eight QTLs, only two were detected in the mean environment. The QTL SN8 was consistent in three environments. In Kelsey, two QTLs, SN12a and SN12b were found on the same linkage group. The LEP and HEP alleles increased seed number at SN12b and SN12a respectively. At SN8, SN2 and SN12b, the LEP alleles increase seed number. At other QTLs, the HEP alleles contributed to seed number (Table 3.2).

3.3.2.7 Number of pods per main stem

Eight QTLs were mapped for number of pods per main raceme (NP) (Table 3.2, Fig. 3.1). They individually explained about 7.5-24.1% of the total phenotypic variation. Two QTLs were detected in the Edmonton Research Station 1999, four in the Edmonton Research Station 2000, three in Ellerslie 2000 and four in Kelsey 2000 explaining about 29.5%, 38.1%, 34% and 52.4% of the total phenotypic variation respectively. A new QTL NP2 appeared in the mean environment. Out of eight QTLs, five were identified in the mean environment. The QTL NP3 was consistent in three environments. The QTLs NP4, NP8 and NP15 were common in two environments (Table 3.2).

3.3.2.8 Plant height

Five QTLs were found affecting plant height (PH) significantly, at least in one environment (Table 3.2, Fig. 3.1). No QTL was detected in Kelsey 2000. However, QTLs PH18 and PH4 were common in three environments and PH6 in two environments. These QTLs were also observed in the mean environment explaining 45.7% of the total phenotypic variation. Individually, the QTL PH4 showed a maximum phenotypic variation of 19.4% in the Edmonton Research Station 1999. Total phenotypic variation explained by these QTLs in different environments ranged from 35.2% to 46.8%. At PH16, the HEP alleles increased plant height. However, at PH6, PH5, PH4 and PH18, the LEP alleles contributed to plant height (Table 3.2).

3.3.2.9 1000-grain weight

QTL GW12 significantly affected 1000-grain weight (GW) in all environments, and could explain one-third of the total phenotypic variation in the population. At GW12,

HEP alleles increased seed weight in all environments except Kelsey 2000. In Kelsey 2000, HEP alleles caused a reduction in seed weight (Table 3.2).

3.3.2.10 Yield

Three QTLs affecting yield (YL) significantly were identified in Kelsey 2000 (Table 3.2). The QTLs explained approximately 41.0% of the total phenotypic variation. Individually, these QTLs explained about 9.1-17.2% of the total variation. At all QTLs, the LEP alleles increased seed yield. No QTL for yield was found in any other environment (Table 3.2).

3.3.3 QTL x environment interactions

QTL x environment interactions were determined by the stability of QTLs in different environments. Strong QTL x environment interactions were observed (Table 3.2). Out of 65 QTLs mapped for ten parameters, only four were consistent in all environments, 7 in three environments and 14 in two environments. These 65 QTLs appeared 105 times in different combinations in different environments, and the proportion of the phenotypic variation explained by these QTLs individually varied from 6.1 (FP18 in the Edmonton Research Station 1999) to 30.9% (GW12 in the Edmonton Research Station 1999) with an average of 12.4%, and collectively from 20.5 (days to maturity in Kelsey 2000) to 54.2% (days to first flowering in the Edmonton Research Station 1999). Approximately, 39% of the total QTLs could be identified in the mean environment. In the mean environment, two new QTLs were identified, which were lacking in the individual environment. In the mean environment, the proportion of phenotypic variation associated with these QTLs individually ranged from 6.1 (FF2, NP2) to 24.4% (LF18) with an average of 12.1%. The proportion of total phenotypic

variation explained by these QTLs collectively varied from 13.8 (flowering period) to 57.3% (days to first flowering) (Table 3.2).

Apart from the stability of different QTLs in different environments, differential environmental effects of the QTLs LF8b, GW12, LF12, FP12 and MT8a were observed in different environments (discussed below) (Table 3.2).

3.3.4 Genotype x environment interactions

Significant genotype x year and genotype x location interactions were observed for all parameters except plant height (Tables 3.4 and 3.5). Strength of genotype x environment interaction was determined by calculating correlations among rankings of DH lines in different environments. Highly significant correlations were observed between the rankings of DH lines in 1999 and 2000 in the Edmonton Research Station for all parameters except days to maturity (Table 3.6). Rankings of different lines in different locations in 2000 showed a positive and significant correlation for pod length, number of pods per main raceme and plant height. Yield and 1000-grain weight were positively and significantly correlated among rankings of DH lines in the Edmonton Research Station 2000 and Ellerslie 2000 but non-significantly among rankings of DH lines in other locations. Seed number per pod had non-significant correlations among rankings of DH lines in the Edmonton Research Station 2000 and Kelsey 2000 but positive and significant among rankings at other locations. For flowering parameters, days to first flowering and last flowering had significant and positive correlation among ranking of DH lines in the Edmonton research Station 2000 and Kelsey 2000. However, rankings among DH lines for days to maturity in Kelsey 2000 and Ellerslie 2000 were negatively and significantly associated. For all other parameters at all locations, these correlations

were non-significant. The heritability of these parameters in different years at the same location was always greater than that in different locations in the same year (Table 3.7). This suggested that these parameters, especially ones associated with flowering were more influenced by genotype x location interactions than by genotype x year interactions.

3.3.5 Maternal effects

Maternal effects were observed for parameters such as days to first flowering, last flowering & maturity, flowering period and pod length. The effects were influenced by environment, and the results are summarized in Table 3.1.

3.3.6 Epistatic realtionships

Markers linked to the 65 QTLs were tested for digenic relationships among themselves. Very few alleles showed epistasis, and results have been summarized in Appendix I. Epistasis appear to be influenced by environments. In the present study, every trait showed epistasis in at least one of the environments.

3.4 Discussion

3.4.1 Transgressive segregation

Transgressive segregation was observed for all parameters in all environments. It has been noted that the smaller the difference between parents, the greater the transgressive segregation, and vice versa (Table 3.1). The genetic basis of transgessive segregation has yet to be experimentally determined, but it is suggested to be due to complimentary gene action from two parents (Xiao et al. 1996). Except for yield and 1000-grain weight, the HEP and LEP alleles increased phenotypic values for all traits in at least one environment. For example, four QTLs (PL2, PL4, PL9 & PL12) were mapped for pod length at the Edmonton Research Station 2000. The HEP alleles

increased pod length at the QTLs PL9, PL12 and the LEP alleles at the QTLs PL2, PL4 respectively.

The smallest amount of transgressive segregation was observed for 1000-grain weight. It appeared that the QTLs contributed by LEP parent were too small to be observed at high threshold values. Transgressive segregation provides an excellent opportunity for detecting QTLs in populations where parental lines do not differ widely from each other (Xiao et al. 1996).

3.4.2 Clustering of genes

Clustering of genes has been reported in all major crops (Mansur et al.1993; Leon et al. 1995; Xiao 1996; Veldboom and Lee 1996a; and Tinker at al. 1996), and a similar pattern was observed in the present study (Fig. 3.1). QTLs for days to first flowering, last flowering, maturity, flowering period and plant height, were mapped on linkage group 18 between loci wg7f10aNM and wg2d9b within a distance of 6.2 cM (Fig. 3.1). QTLs for days to last flowering, flowering period, pod length, seed number/pod, number of pods/main raceme, and 1000-grain weight, clustered on linkage group 12 between loci wg9d5a and ec4f10aNP. QTLs mapped for different parameters were also identified on linkage groups 3, 4, 8 & 15 (Fig 3.1). It is interesting to note that these parameters did not appear to be inherited independently (Table 3.3), thus providing a strong basis for clustering of QTLs in certain linkage groups.

3.4.3 Differential environmental effects

Differential environmental effects of the same QTL in different environments have been reported (Tinker et al. 1996), and was observed in the present study. The QTL GW12 for 1000-grain weight had a negative additive effect at the Edmonton Research

Station 1999, the Edmonton Research Station 2000 and Ellerslie 2000, but a positive effect in Kelsey 2000. The LEP alleles increased flowering period at the QTL FP12 in the Edmonton Research Station 1999 and the Edmonton Research Station 2000. However, at Ellerslie 2000 and Kelsey 2000, the LEP alleles reduced flowering period at the QTL. A similar pattern was observed for the QTL LF12. All of these QTLs were clustered on LK12. The QTLs MT8a and LF8b, all mapped on LK8, and showed differential environmental effects for days to maturity, and last flowering respectively.

Differential environmental effects can help explain certain results in the present study. Highly significant genotype x year and genotype x location interactions were observed for 1000-grain weight. However, correlations between rankings of DH lines in the Edmonton Research Station 1999, the Edmonton Research Station 2000 and Ellerslie 2000 were positive and highly significant with correlation co-efficients being ≥.7 (Table 3.6). However, no correlation was observed between rankings of DH lines in the Edmonton Research Station 2000, and in Kelsey 2000 and Ellerslie2000 and Kelsey 2000 (Table 3.6). This could be due to differential environmental effects of the QTL GW12 alleles in these environments. Flowering period was negatively and significantly correlated with 1000-grain weight in the Edmonton Research Station 1999, the Edmonton Research Station 2000 and Kelsey 2000 (Table 3.3). The correlation between these two parameters was positive and significant in Ellerslie 2000 and non-significant in the mean environment. This could be attributed to differential environmental effects of the QTLs GW12 and FP12. The QTL alleles behaved similarly in EL00 but differently in the Edmonton Research Station 1999, the Edmonton Research Station 2000, and Kelsey

2000. Similar reasoning could be given to explain different correlations between days to last flowering and 1000-grain weight in different environments.

3.4.4 Correlation and similar genomic regions

A general picture of correlations among yield and yield-associated traits is given in Table 3.3. Days to first flowering were negatively correlated with 1000-grain weight in all environments except in Ellerslie 2000. Pod length had no relationship with plant height except in the Edmonton Research Station 1999. The fluctuations in these relationships could be attributed to genotype x environment interactions (Bing 1996). Correlations among different traits result from genetic and environmental effects. Genetic effects are due to linkage of genes or pleiotropy (Falconer, 1981). In QTL mapping, it is difficult to distinguish between linkage and pleiotropy (Veldboom and Lee 1996a). It has been demonstrated that correlated traits had QTLs often mapped in similar genomic regions (Veldboom and Lee1996a &b; Abler et al. 1994; Paterson at al. 1991). A similar pattern was observed in the present study. Plant height, days to first flowering, last flowering and maturity were positively and significantly correlated among themselves (Table 3.3). QTLs PH18, FF18, LF18 and MT18 were identified on LK18 between loci wg7f10aNM and wg2d9b, and QTLs FF8, LF8 and MT8 on LK8 (Fig. 3.1). As mentioned earlier, differential environmental effects of of QTL GW12, FP12, LF12 alleles in different environments explained why 1000-grain weight was positively and negatively associated with flowering period and days to last flowering in different environments. Pod length and 1000-grain weight were positively correlated in all environments (Table 3.3), and the QTLs GW12 and PL 12 clustered on LK12 (Table 3.1, Fig.3.1). However, the correlation coefficient was minimal in Kelsey 2000. The HEP

alleles increased pod length and grain weight at QTLs PL12 and GW12 in all environments except in Kelsey 2000. In Kelsey 2000, HEP alleles increased pod length at PL12, but reduced grain weight at GW12.

3.4.5 Number of QTLs detected and of genes controlling quantitative parameters

Butruille et al. (1999) reported that the number of QTLs detected for parameters such as days to flowering, plant height, 1000-grain weight and yield in *Brassica napus* were in the range of the number of effective factors calculated by using biometrical methods. In the present studies, similar patterns were observed in a few cases. This is, however, an ambiguous relationship. Simply changing critical LOD values for declaring the presence of a QTL, can change the number of QTLs detected for a parameter. Also, underlying conditions for calculating the number of effective factors i.e. equal effects of individual alleles, absence of epistasis, opposite extremes of segregating populations containing all increasing and decreasing alleles in small populations, literally can not be met.

3.4.6 Strategies for marker-assisted selection

Strong genotype x environment interactions were observed in the present study. Approximately 39% of all QTLs detected in different environments could be mapped in the mean environment. Strong inconsistencies in QTLs expression across environments have been well documented (Paterson et al. 1991; Bubeck et al. 1993; Zhuang et al. 1997; Lu et al. 1996; Lee et al.1996). However, it was observed that QTL x environment interactions were trait dependent (Lu et al. 1996; Lee et al.1996). A similar pattern was observed in the present study. Traits such as pod length, 1000-grain weight, number of pods per main raceme and days to first flowering showed least QTL x environment

interactions; for these traits, approximately 69% of the QTLs appearing in different environments could be mapped in the mean environment. For days to first flowering, number of pods per main raceme and 1000-grain weight, approximately 80%, 63%, 63% and 100% of the QTLs expressing in different environments were identified in the mean environment. Strong QTL x environment interactions were observed for traits such as days to last flowering, flowering period, maturity, seed number per pod and yield. Of 38 QTLs mapped for these traits in different environments, only 7 (approximately 19%) could be detected in the mean environment.

Differential environmental effects of the same QTL in different environments could have serious implications in marker-assisted selection. Veldoom and Lee (1996a) recommended that QTLs detected in the mean environment be used in an efficient MAS. They suggested that averaging over different environments would reduce environmental noise to a great extent, thus leading to better resolution of QTLs. In the present study, it was found that differential environmental effects could seriously underestimate not only the number of QTLs identified for any parameter but also the effect associated with these QTLs. The QTL FP12 showed differential environmental effects. It showed positive additive effect in the Edmonton Research Station 1999 and the Edmonton Research Station 2000 but negative in Ellerslie 2000 and Kelsey 2000. The QTL FP12 could not be identified in the mean environment (Table 3.2). The same QTL showed significant positive additive effect in the mean of the Edmonton Research Station 1999 and the Edmonton Research Station 2000, and significant negative effect in the mean of Ellerslie 2000 and Kelsey 2000 (data not shown). QTLs LF12, LF8b and MT8a showed similar patterns to that of FP12. QTL GW12 showed negative additive effect in the Edmonton

Research Station 1999, the Edmonton Research Station 2000 and Ellerslie 2000, but positive effect in Kelsey 2000. The QTL was identified in the mean environment but the proportion of the total phenotypic variation explained by the QTL GW12 in the mean environment was minimal as compared to that in any individual environment (Table 3.2).

For MAS for any parameter in any crop, one must look at the number of QTLs detected, stability and proportion of total phenotypic variation explained by these QTLs across environments and linkage of detected QTLs with the QTLs of other parameters (Ribaut et al. 1997). Yield was the most sensitive parameter observed in this study (Table 3.2), therefore, direct selection for yield using MAS would not be the best strategy for the QTLs identified in this study. Indirect selection for yield has been previously proposed for the *Brassicas* (Thurling 1974).

Days to first flowering were positively correlated with days to last flowering and maturity (Table 3.3). It was also the most stable parameter in all environments (Table 3.2), and could be taken as a good indicator for total growing season. Number of pods per main raceme and 1000-grain weight showed positive association with yield. Pod length had no correlation with yield in the present study but was positively correlated with 1000-grain weight. QTLs for any parameter could be effectively used in MAS for yield, even if the parameter was contributing indirectly to yield (Raibut et al. 1997). Therefore, the best strategy for improving yield would be to use consistent QTLs for 1000-grain weight, pod length, number of pods per main raceme, and days to first flowering. Inclusion of morphological characters and yield components in the selection index has been previously proposed (Thurling 1974 and 1991).

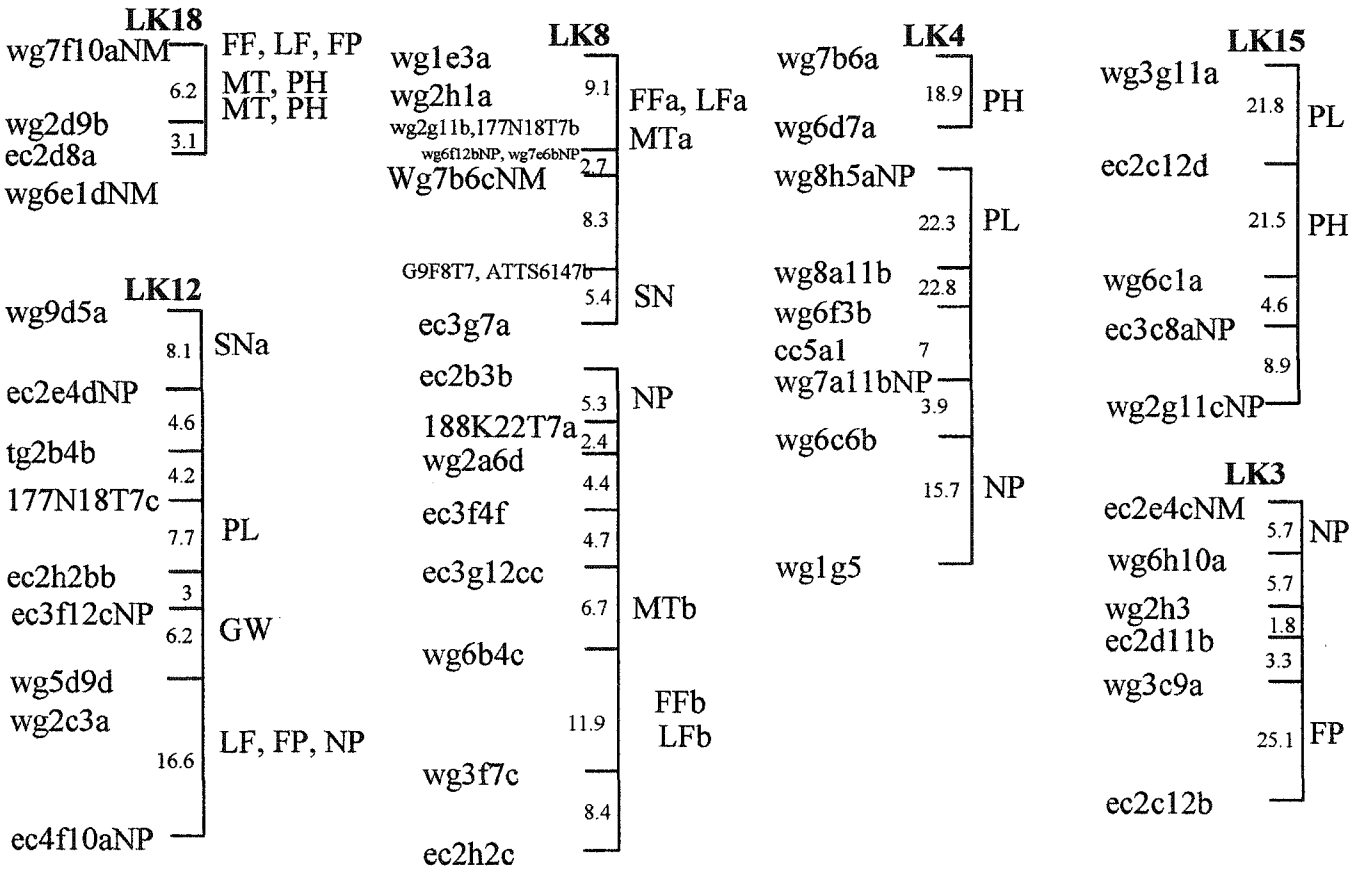

Fig. 3.1. Areas of clustering in *B. juncea* linkage map for traits of agronomic importance.
FF = days to first flowering, LF = days to last flowering, FP = flowering period, MT = days to maturity, PL = pod length, SN = seed number per pod, NP = number of pods per main stem, PH = plant height, GW = 1000-grain weight, LK = linkage group.

Table 3.1. Population and parental means, standard deviation, range and maternal effects for yield and yield-associated traits in *B. juncea* in different environments.

FF = days to first flowering, LF= days to last flowering, MT = days to maturity, , FP = flowering period, PL = pod length, SN = seed number per pod, PH = plant height, NP = number of pods per main raceme, GW =1000-grain weight, PMEAN = population mean, ENV = environment, ERS99 = Edmonton Research Station, ERS00 = Edmonton Research Station 2000, EL00 = Ellerslie 2000, KE00 = Kelsey 2000, LEPM = low erucic acid parent mean, HEPM = high erucic acid parent mean, ME = mean environment, SD = standard deviation, MT EFF = maternal effect, NS = non-significant, *, **, *** = significant at 5%, 1% and .1% levels respectively.

		FF (days)	LF (days)	FP (days)	MT (day)	PL (mm)	SN	PH (cm)	NP	GW (gram)	YIELD (gram)
ERS99	PMEAN	47.27	74.60	27.33	110.09	42.38	14.59	112.80	28.53	2.93	.
	SD	2.68	4.74	3.37	3.71	4.49	1.64	10.10	3.32	0.32	.
	RANGE	41.67-52.02	64.67-85.69	19.67-33.95	101.90-121.95	32.79-53.21	10.43-18.83	85.26-138.33	18.64-37.93	2.17-3.92	.
	LEPM	51	80.33	29.33	118.33	45.42	13.87	109	29.01	2.40	.
	HEPM	42.08	65.00	22.92	114.00	43.37	13.32	100.17	24.55	3.95	.
	MT EFF	NS	*	**	**	*	NS	NS	NS	NS	NS
ERS00	PMEAN	53.90	75.32	21.42	108.47	45.07	16.14	141.22	38.28	3.11	27.87
	SD	1.87	4.33	3.60	2.39	3.17	1.33	5.61	3.98	0.28	4.17
	RANGE	48.67-59.33	68.0-83.33	14.0-27.67	103.67-114.33	37.08-55.11	12.84-23.69	130.0-154.33	27.53-50.20	2.37-3.93	14.16-36.80
	LEPM	58.67	83.00	24.33	115.00	43.01	16.35	139.00	41.50	2.67	16.88
	HEPM	50.58	69.83	19.25	109.00	43.49	15.10	131.17	32.03	4.06	29.34
	MT EFF	NS	NS	NS	**	NS	NS	NS	NS	NS	NS
EL00	PMEAN	54.81	77.76	22.96	111.12	43.16	16.06	140.67	39.91	2.85	29.75
	SD	1.63	2.86	2.79	3.14	3.98	1.21	6.83	3.31	0.24	4.36
	RANGE	51.33-59.67	71.0-85.0	15.67-30.0	105.33-118.33	30.60-56.29	13.16-19.82	127.67-158.67	31.13-47.67	2.29-3.58	20.34-43.67
	LEPM	57.69	83.67	23.67	120.33	42.41	16.39	137.67	43.54	2.40	18.36
	HEPM	55.22	78.42	26	112.42	42.76	14.82	129	31.54	3.45	29.68
	MT EFF	**	NS	NS	*	**	NS	NS	NS	NS	NS
KE00	PMEAN	54.93	86.99	32.07	124.68	39.64	11.17	141.87	30.69	3.08	27.16
	SD	2.16	3.26	3.69	3.77	2.85	1.55	7.33	4.00	0.25	4.63
	RANGE	51.0-59.33	77.67-95.33	24.33-41.67	117.0-132.67	32.79-49.61	7.91-14.94	124.67-160.0	22.07-43.27	2.50-3.88	17.56-37.49
	LEPM	58.00	89.00	31.00	127.33	37.52	14.27	133.33	35.80	2.45	23.34
	HEPM	52.00	80.17	28.17	114.17	40.41	10.65	134.50	28.55	3.41	20.00
	MT EFF	NS	NS	NS	NS	*	NS	NS	NS	NS	NS
ME	PMEAN	52.73	78.67	25.94	113.59	42.56	14.49	134.22	34.35	2.99	28.26
	SD	1.62	2.54	1.97	1.76	3.1	0.96	5.85	2.72	0.21	2.87
	RANGE	49.0-55.83	74.08-85.23	21.83-30.97	110.0-117.56	35.45-52.15	11.73-17.26	123.83-149.33	27.44-42.18	2.49-3.5	19.91-36.05
	LEPM	56.34	84.00	27.08	120.25	42.09	15.22	129.75	37.46	2.48	19.53
	HEPM	49.97	73.35	24.08	112.40	42.51	13.47	123.71	29.17	3.72	26.34
	MT EFF		**	**	***	**	NS	NS	NS	NS	NS

Table 3.2. Genetics of QTLs for yield and yield associated traits in *B.* juncea in different environments.

Trait	Env	QTL	Dis (cM)	Loci	LOD	σ_P^2	Totσ_P^2	ADD	NO. QTL	K
FF	ERS99	FF8a!	10	wg6b4c-wg3f7c	6.10	13.4	54.2	♦1.02	3	4-5
		FF1	5	wg6e6cNM-ec2c7bNM	4.58	11.9		•-1.09		
		FF18	5	wg7f10aNM-wg2d9b	10.01	28.9		1.52		
	ERS00	FF8b	5	177N18T7b-wg6f12bNP	3.28	12.2	36	0.68	3	12-13
		FF4	15	wg8h5aNP-wg8a11b	3.21	14.1		0.72		
		FF18	0	wg7f10aNM-wg2d9b	2.72	9.7		0.60		
	EL00	FF8a	5	wg3f7c-ec2h2c	3.91	13.1	24.6	0.61	2	15-16
		FF18	0	wg7f10aNM-wg2d9b	3.59	11.5		0.58		
	KE00	FF8a	5	wg3f7c-ec2h2c	2.99	9.8	27.2	0.70	3	4-5
		FF1	0	wg6e6cNM-ec2c7bNM	2.82	8.9		-0.74		
		FF18	0	wg7f10aNM-wg2d9b	2.78	8.5		0.67		
	ME	FF8b	5	177N18T7b-wg6f12bNP	3.43	7.4	57.3	0.47	5	
		FF8a	5	wg3f7c-ec2h2c	5.84	12.6		0.61		
		FF1	0	wg6e6cNM-ec2c7bNM	4.2	8.8		-0.57		
		FF2	5	ec3g3a-ec3g3b	2.97	6.1		-0.53		
		FF18	5	wg7f10aNM-wg2d9b	8.64	22.4		0.82		
LF	ERS99	FF8a	0	wg6b4c-wg3f7c	3.02	7.7	44.3	1.38	3	5-6
		LF3	15	wg3c9a-ec2c12b	4.80	16.1		-1.89		
		FF18	0	wg7f10aNM-wg2d9b	7.45	20.5		2.27		
	ERS00	LF8b	0	wg7e6bNP-wg7b6cNM	3.4	9.1	45.2	1.39	5	3-4
		LF6	0	tg2b4c-wg2a6a	2.70	7.2		1.18		
		LF12	0	ec2d8dNM-wg9d5a	3.45	9.1		1.35		
		LF4	5	ec5a1-wg7a11b	2.40	6.7		1.17		
		FF18	0	wg7f10aNM-wg2d9b	4.74	13.1		1.63		
	EL00	LF5a	5	ec3c8cNP-ATTS2990a	2.66	10.4	31.8	-0.93	3	17-18
		LF12	5	wg2c3a-ec4f10aNP	3.76	13		-1.05		
		LF2	0	wg5d9cNP-wg7f5b	2.62	8.4		0.94		
	KE00	LF8b	5	177N18T7b-wg6f12bNP	3.01	10.5	43	-1.1	4	9-10
		LF5b	10	wg1f2aNP-wg7e6aNP	3.17	9.8		1.93		
		LF12	15	wg2c3a-ec4f10aNP	4.23	13.4		-1.19		
		FF18	0	wg7f10aNM-wg2d9b	2.87	9.3		1.03		
	ME	LF3	10	wg3c9a-ec2c12b	2.42	10	34.4	-0.8	2	
		LF18	5	wg7f10aNM-wg2d9b	6.85	24.4		1.30		
FP	ERS99	FP3	15	wg3c9a-ec2c12b	3.53	15.5	50.0	-1.77	5	5-6
		FP3	0	wg6c3dNP-wg6c1b	3.21	8.5		-1.07		
		FP12	0	177N18T7c-ec2h2b	2.58	7.1		0.90		
		FP11	10	tg6c3b-wg6f12cNP	4.76	12.8		-1.21		
		FP18	0	wg7f10aNM-wg2d9b	2.61	6.1		0.88		
	ERS00	FP3	15	wg3c9a-ec2c12b	2.75	13.6	27.6	-1.33	2	3-4
		FP12	5	wg9d5a-ec2e4dNP	3.49	14.0		1.37		
	EL00	FP5a	5	ATTS2990a-179F6T7B	3.04	9.6	29.8	-0.90	3	17-18
		FP12	5	wg2c3a-ec4f10aNP	3.98	13.3		-1.04		
		FP18	0	wg7f10aNM-wg2d9b	2.41	6.9		-0.77		
	KE00	FP8	5	177N18T7b-wg6f12bNP	5.51	20.3	48.4	-1.72	4	7-8
		FP5b	10.4	wg1f2aNP-wg7e6aNP	3.84	10.7		2.81		
		FP12	15	wg2c3a-ec4f10aNP	2.72	8.8		-1.09		
		FP1	5	ec2d2dNM-wg2d9aNP	2.64	8.6		1.26		

	ME	FP3	5	229N15T7-ec3f12b	3.49	13.8	13.8	-0.74		
MT	ERS99	MT2	10	wg5d9cNP-wg7f5b	3.29	14.1	22.8	1.49	2	11-12
		MT18	5	wg7f10aNM-wg2d9b	2.63	8.7		1.12		
	ERS00	MT8b	5	wg7b6cNM-ATTS6147b	5.15	19.8	45.2	1.16	3	7-8
		MT5b	5	ec4g4b-wg2g11f	2.50	13.4		2.29		
		MT1	5	wg6e1a-ec2c7cNP	3.57	12.0		-0.98		
	EL00	MT8a	5	ec3g12c-wg6b4c	4.66	12.7	39.7	1.19	4	7-8
		MT5a	5	179F6T7B-ec3g3cNP	4.33	12.2		1.17		
		MT18	5	wg7f10aNM-wg2d9b	2.72	8.1		0.91		
		MT11	9	ec5a7a-wg5d9e	2.48	6.7		0.8		
	KE00	MT8a	0	ec3g12c-wg6b4c	2.49	8.9	20.5	-1.18	2	7-8
		MT12	10	wg2c3a-ec4f10aNP	2.59	11.6		-1.29		
	ME	MT8b	0	177N18T7b-wg6f12bNP	4.14	14	27.5	0.67	2	
		MT18	5	wg7f10aNM-wg2d9b	3.79	13.5		0.66		
PL	ERS99	PL9	5	ec3f12eNM-wg6g3a	3.07	8	46.1	-1.34	4	5-6
		PL12	0	ec3f12cNP-wg5d9d	4.53	12.9		-1.65		
		PL2	10	wg3f7a-ec3g3a	4.2	13.8		1.68		
		PL4	10	wg8h5aNP-wg8a11b	3.26	11.4		1.55		
	ERS00	PL9	5	ec3f12eNM-wg6g3a	3.44	10	39.9	-1.04	3	13-14
		PL12	5	177N18T7c-ec2h2b	5.72	16.7		-1.3		
		PL11	5	ec2f12a-wg2e9	4.60	13.2		-1.17		
	EL00	PL12	5	177N18T7c-ec2h2b	3.42	11.8	39.8	-1.39	3	13-14
		PL4	15	wg8h5aNP-wg8a11b	3.07	11.8		1.38		
		PL15	15	wg3g11a-ec2c12d	3.94	16.2		-1.61		
	KE00	PL8	5	G9F8T7-ec3g7a	3.33	9.6	36.3	0.95	4	11-12
		PL12	0	ec2h2b-ec3f12cNP	2.58	7.6		-0.80		
		PL15	10	ec2c12d- wg6c1a	2.77	10.7		-0.95		
	****	PL14	5	ec3f4d-wg6g3e	2.50	8.4		-0.86		
	ME	PL9	7.1	ec3f12eNM-wg6g3a	3.39	8.3	48.4	-0.94	5	
		PL12	0	ec2h2b-ec3f12cNP	5.79	16.4		-1.28		
		PL2	10	wg3f7a-ec3g3a	2.65	9		0.92		
		PL4	10	wg8h5aNP-wg8a11b	2.44	7.1		0.84		
		PL15	15	wg3g11a-ec2c12d	2.40	7.6		-0.87		
SN	ERS99	SN8	0	ec2d2a-ec4d11cNP	4.24	11.1	40	0.59	3	8-9
		SN6	5	wg1g6a-ec2d1a	3.63	13.6		-0.62		
		SN16	5	wg6f10a-ec2h2a	5.24	15.3		-0.68		
	ERS00	SN8	0	wg4b6b-wg6e6a	4.94	16.5	26.7	0.58	2	50-51
		SN2	10	wg6g11c-wg6d9a	2.92	10.2		0.44		
	EL00	SN5	0	ec5a7a-ec3c8cNP	2.56	11.1	23.1	-0.40	2	9-10
		SN12a	5	ec3b3-wg4c4b	3.45	12.0		-0.44		
	KE00	SN8	0	wg7e6bNP-wg7b6cNM	5.24	13	45.4	0.62	4	11-12
		SN3	5	wg3c9a-ec2c12b	2.93	9.5		-0.49		
		SN12a	0	wg9d5a-ec2e4dNP	3.32	8		-0.51		
		SN12b	5	wg2c3a-ec4f10aNP	5.05	14.9		0.66		
	ME	SN8	0	G9F8T7-ec3g7a	4.93	16.9	24.9	0.44	2	
		SN6	0	wg6d9b-wg7f5a	2.52	8.2		-0.28		
NP	ERS99	NP3	0	ec2e4cNM-wg6h10a	4.37	16.1	29.5	-1.34	2	13-14
		NP15	10	ec2c12d-wg6c1a	2.93	13.4		1.23		
	ERS00	NP8	5	ec2b3b-180K22T7a	3	7.9	38.1	1.21	4	11-12
		NP6	0	tg6c3aNP-wg2d5bNM	2.73	11.1		1.35		
		NP3	0	ec2e4cNM-wg6h10a	3.46	8.9		-1.28		
		NP15	15	wg3g11a-ec2c12d	3.16	10.2		1.37		

	EL00	NP3	0	ec2e4cNM-wg6h10a	2.86	9.3	34	-1.02	3	9-10
		NP10	5	wg2g1b-ec4f11b	3.82	14.1		-1.27		
		NP4	10	wg6c6b-wg1g5	2.72	10.6		-1.1		
	KE00	NP8	5	ec2b3b-180K22T7a	2.43	7.5	52.9	1.14	4	15-16
		NP12	10	wg2c3a-ec4f10aNP	5.76	24.1		1.93		
		NP4	10	wg7b6a-wg6d7b	3.09	11.1		-1.37		
		NP17	5	ec4d11a-wg4a4bNP	2.84	10.2		-1.40		
	ME	NP8	5	ec2b3b-180K22T7a	4.43	10.3	57	0.96		
		NP3	0	ec2e4cNM-wg6h10a	6.85	17.4		-1.14		
		NP12	0	ec2h2b-ec3f12cNP	3.41	8		0.78		
		NP2	0	ec3g3b-wg2e11a	2.41	6.1		-0.67		
		NP4	10	wg6c6b-wg1g5	2.68	7.5		-0.76		
		NP15	5	ec2c12d-wg6c1a	2.83	7.7		0.79		
PH	ERS99	PH16	10	wg6f10bNP-D3t7a	2.78	10	44.8	-3.28	3	15-16
		PH4	10	wg7b6a-wg6d7b	4.92	19.4		4.57		
		PH18	0	wg7f10aNM-wg2d9b	4.81	15.4		4.14		
	ERS00	PH6	5	wg7a11cNM-wg5a5a	4.98	15.7	35.2	2.24	3	6-7
		PH4	5	wg7b6a-wg6d7b	3.13	12.0		2.03		
		PH18	0	wg7f10aNM-wg2d9b	2.41	7.5		1.58		
	EL00	PH6	5	wg7a11cNM-wg5a5a	5.16	14.2	46.8	2.60	4	7-8
		PH5	5	wg1f2aNP-wg7e6aNP	2.46	10.3		3.27		
		PH4	10	wg8h5aNP-wg8a11b	3.39	13.1		2.51		
		PH18	0	wg7f10aNM-wg2d9b	3.24	9.2		2.14		
	KE00	-	-	-					-	18-19
	ME	PH6	5	wg7a11cNM-wg5a5a	3.92	11.2	45.7	1.97	3	
		PH4	5	wg7b6a-wg6d7b	5.09	19.3		2.69		
		PH18	0	wg7f10aNM-wg2d9b	4.94	15.2		2.35		
GW	ERS99	GW12	5	ec3f12cNP-wg5d9d	8.67	30.9	30.9	-0.18	1	7-8
	ERS00	GW12	5	wg2c3a-ec4f10aNP	6.22	26.2	26.2	-0.14	1	9-10
	EL00	GW12	5	wg2c3a-ec4f10aNP	6.42	23.3	23.3	-0.11	1	10-11
	KE00	GW12	10	wg2c3a-ec4f10aNP	4.58	21.6	21.6	0.11	1	15-16
	ME	GW12	0	ec3f12cNP-wg5d9d	4.56	18.7	18.7	-0.09	1	
YL	ERS00			-						25-26
	EL00			-						14-15
	KE00	YL12	10	wg2c3a-ec4f10aNP	3.97	14.8	41.1	1.77		7-8
		YL13	5	ec2d2bNM-wg4a4c	2.42	9.1		1.4		
		YL11	5	ec2e5c-wg8b1b	5.12	17.2		1.93		

σ_P^2 = %age of total phenotypic variance explained by individual loci, Totσ_P^2 = %age of total phenotypic variance explained by all QTLs detected in an environment, LOD =Lod value associated with detected QTL, No. QTL = total number of QTL detected in an environment, ADD additive effect associated with detected QTL, K number of genes controlling a parameter as determined by formula given by Snape et al. (1984), !QTLs were named relative to traits, number associated with QTL indicated linkage group on which QTL was mapped. If more than one QTL were identified on the same linkage group, an alphabet followed the name of QTL.

♦positive additive effect showed that LEP alleles contributed to parameter at associated QTLs

•negative additive effect showed that contributing alleles were coming from HEP

Table 3.3. Phenotypic correlations among yield and yield-associated traits in *B. juncea* in different environments.

		LF	FP	MT	PL	SN	PH	NP	GW	YL
ME	FF	.52****	-.1****	.3****	0	-.05NS	.69****	.39****	-.06*	-.09**
ERS99		.67****	.13*	.21***	.02NS	-.13*	.41****	.14*	-.21***	-
ERS00		.56****	.1NS	.1NS	.07NS	.06NS	.24****	.23****	-.3****	-.38****
EL00		.39****	-.19****	.14**	.05NS	-.01NS	.13*	.05NS	.09NS	.07NS
KE00		.13*	-.46****	.17**	.05NS	.08NS	.14**	-.03NS	-.17**	-.03NS
ME	LF		.79****	.67****	-.25****	-.49****	.40****	-.07*	-.05NS	-.14****
ERS99			.82****	.24****	.02NS	-.1NS	.29****	.24****	-.21****	-
ERS00			.89****	.11*	-.02NS	.14**	.39****	.33****	-.26****	-.17**
EL00			.82***	-.04NS	.1*	.07NS	.02NS	0	.16**	.07NS
KE00			.82****	.34****	-.13*	-.23***	.29****	-.06NS	-.25****	-.19***
ME	FP			.56****	-.29****	-.53****	-.03NS	-.35****	-.01NS	-.12****
ERS99				.16**	0	-.03NS	.06NS	.21****	-.13*	-
ERS00				.07NS	-.01NS	.14**	.33****	.26****	-.14**	0
EL00				-.13*	.08NS	.04NS	-.1NS	.09NS	.17**	0
KE00				.21****	-.14*	-.26****	.18***	-.03NS	-.13*	-.16**
ME	MT				.34****	.61****	.24****	.27****	.03NS	.13****
ERS99					-.03NS	-.11*	.07NS	.15**	.2***	-
ERS00					.05NS	.11	.03NS	0	-.04NS	.1NS
EL00					-.11*	0	.09NS	.14*	-.12*	-.09
KE00					-.01NS	-.14*	.16**	.22****	-.34***	-.12*
ME	PL					.48****	.04NS	.11****	.18****	.03 NS
ERS99						.31****	.13*	-.3****	.24****	-
ERS00						.25****	.08 NS	-.11*	.17**	.01 NS
EL00						.34****	.04 NS	-.13*	.26****	-.05NS
KE00						.39***	.03NS	.03 NS	.11*	.02NS
ME	SN						-.01 NS	.42****	-.13****	.19****
ERS99							.21***	-.07 NS	-.21***	-
ERS00							.14**	.02 NS	-.03 NS	.08 NS
EL00							-.04NS	.13*	-.1NS	.11*
KE00							-.13*	.31****	.03 NS	.21****
ME	PH							.42****	.02 NS	.03NS
ERS99								.2***	-.15**	-
ERS00								.25****	-.058 NS	.02NS-
EL00								.31****	.01NS	.02 NS
KE00								-.03 NS	.02NS	.08 NS
ME	NP								-.14****	.16****
ERS99									-.16**	-
ERS00									-.33****	-.04 NS
EL00									-.29****	.03 NS
KE00									.19***	.21****
ME	GW									.18****
ERS99										-
ERS00										.24****
EL00										.3****
KE00										.23****

*, **, *** and **** = significance level of 5%, 1%, .1% and .01% respectively, rest of abbreviations have been explained in previous tables.

Table 3.4. Genotype x year interactions for yield and yield-associaed traits in *B. juncea* at the Edmonton Research Station during 1999 and 2000.

SOV	DF	FF	LF	FP	MT	PL	SN	PH	NP	GW
YEAR	1	****	****	****	****	****	*	****	****	****
REP(YEAR)	4	****	NS	****	***	NS	**	****	*	****
SET	3	****	****	****	NS	**	NS	****	***	****
SET X YEAR	3	****	****	****	NS	*	***	****	****	****
LINES(SET)	113	****	****	***	****	****	****	****	****	****
LINES(SET) X YEAR	113	****	****	****	****	****	****	NS	**	**
R^2		.96	.90	.89	.68	.75	.65	.83	.84	.86

SOV = source of variation, DF = degree of freedom respectively,
rest of abbreviations have been explained in previous tables.

Table 3.5. Genotype X location interactions for yield and yield-associated traits in *B. juncea* at the Edmonton Research Station, Ellerslie and Kelsey during 2000.

SOV	DF	FF	LF	FP	MT	PL	SN	PH	NP	GW	YL
LOC	2	****	****	****	****	****	****	NS	****	****	***
REP(LOC)	6	**	NS	NS	NS	NS	*	**	****	****	****
SET	3	****	*	NS	NS	****	****	****	****	****	*
SET X LOC	6	****	****	****	****	****	****	****	****	****	****
LINES(SET)	113	****	****	****	****	****	****	****	****	****	****
LINES(SET) X LOC	226	****	****	****	****	**	****	NS	****	****	****
R^2		.79	.88	.85	.94	.73	.82	.55	.81	.80	.57

LOC = locations,
rest of abbreviations have been explained in previous tables.

Table 3.6. Correlations among rankings of DH lines over years and locations for yield and yield-associated traits in *B. juncea.*

Site/year	FF	LF	FP	MT	PL	SN	PH	NP	GW	YL
ERS99 & ERS00	.55****	.75****	.58****	0.16NS	.63****	.29**	.51****	.53****	.74****	-
ERS00 &EL00	.09NS	-.02NS	-.11NS	.08NS	.70****	.31**	.58****	.65****	.7****	.22*
ERS00 & KE00	.54****	.2*	.04NS	-.07NS	.56****	.12NS	.51****	.37****	.007NS	-.011 NS
KE00 & EL00	.15NS	.03NS	.15NS	-.22*	.51****	.26**	.62****	.26****	.12NS	.11 NS

Table 3.7. Heritability for yield and yield-associated traits in *B. juncea* over years and locations.

	G X L	G X Y	ERS99	ERS00	EL00	KE00
FF	.24	.65	.84	.68	.19	.69
LF	.11	.68	.83	.84	.15	.62
FP	.02	.44	.69	.75	.18	.56
MT	.03	.15	.49	.43	.53	.65
PL	.41	.49	.82	.32	.57	.52
SN	.09	.16	.69	.15	.51	30
PH	.30	.35	.37	.48	.42	.18
NP	.27	.40	.46	.48	.47	.36
GW	.34	.74	.80	.76	.59	.48
YL	.12	.03	-	.19	.28	.40

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Chapter 4

Mapping loci controlling the fatty acid profile in *Brassica juncea*.

4.1 Introduction

In canola species, oleic acid (18:1), linoleic acid (18:2), linolenic acid (18:3) and erucic acid (22:1) are the main constituent fatty acids that determine the quality of the oil. However, the traditional definition of canola cultivars requires low erucic acid levels (<%2) and low glucosinolate (<30 micromoles/g of oil-free seed). High erucic acid oil has been found associated with cardiac problems in rats (Beare et at. 1963). By inference, high levels of erucic acids may also be undesirable for human consumption (Vles 1974). However, oil with high erucic acid is used in industrial applications *i.e.* paints, lubricants, nylon. Oleic and linoleic acids are considered to be neutral fatty acids, and to maximize their content is one of the major objectives in any *Brassica* breeding program. Linolenic acid is undesirable because its three double bond structure predisposes it to oxidation, resulting in off flavor and reduced shelf life (Galliard 1980). On the other hand, this fatty acid has been associated with the lowering of LDL in the blood, thus contributing to lower risk of heart disease.

In North America, *B. rapa* and *B. napus* are the only canola species currently grown. *Brassica juncea* has superior agronomic characteristics to either canola species (i.e. higher yield potential, greater drought tolerance, superior blackleg resistance) (Downey 1990; Woods et al. 1991), and a canola version of this species has been developed (Love et al. 1990; Thiagarajah and Stringam 1993). Conventional canola oil has 55-65% oleic acid, 14-18% linoleic acid and 8-12% linolenic acid. *B. juncea* oil has

50-60% erucic acid, 10-15% oleic acid, 10-15% linoleic acid and 14-16% linolenic acid. To convert non-canola type *B. juncea* into a canola type, the entire fatty acid profile requires alteration, as was the case with *B. napus* (Downey 1990; Woods et al. 1991).

In *B. juncea*, erucic acid levels are controlled by two genes acting in an additive manner (Kirk and Hurlstone 1983). To date, there is no report on the inheritance of any other fatty acids in *B. juncea*, however, similar studies have been carried out in other canola species. Except for erucic acid, the inheritance of oleic, linoleic and linolenic acids is not well defined, although the biosynthetic pathways have been elucidated. A simplified diagram for the formation of these fatty acids is shown below:

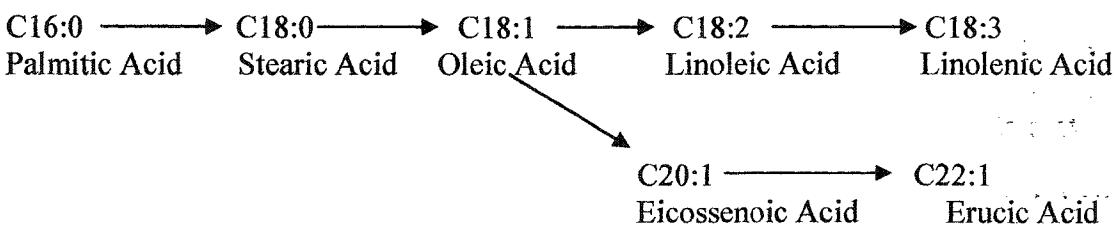

Fig. 4.1 Biosynthetic pathways for major fatty acids in *Brassica* (modified from Downey and Rakow 1987).

In *B. napus*, erucic acid content is also controlled by two genes acting additively (Dorrel and Downey 1964; Harvey and Downey 1964; Stefansson and Hougen 1964; Kondra and Thomas 1975; Jonsson 1977; Siebel and Pauls 1989; Chen and Beversdorf 1990) and the genes have been cloned (Roscoe et al. NCBI Accesssion U50771). At least five alleles governing erucic acid content in the *Brassicas* have been identified (Annand and Downey 1981; Krzymanski and Downey 1969). In *B. napus*, the genes controlling erucic acid content also control the inheritance of eicosenoic acid content acting in a dominant manner (Kondra and Stefansson 1965). Erucic acid content is controlled by two

genes acting additively in *B. carinata* (Getinet et al. 1997). In *B. napus*, levels of oleic, linoleic and linolenic acids are controlled by two, two and three genes respectively (Chen and Beversdorf 1990). Environmental factors such as day length, soil conditions and especially temperature may also greatly influence the level of expression of all fatty acids (Craig 1961; Harvey and Downey 1964; Pleines and Friedt 1989).

Marker-assisted selection (MAS) is an important tool for plant breeders to increase the efficiency of breeding programs, especially for traits controlled by many genes. QTLs associated with different fatty acids in the *Brassicas* have been identified. (Ecke et al. 1995; Thorman et al. 1996, Jourdren et al. 1996 a&b; Hu et al. 1995). Thorman et al. (1996) and Jourdren et al. (1996b) found two QTLs associated with erucic acid content in *B. napus*, explaining nearly all phenotypic variation in the mapping population. Thorman et al. (1996) found a QTL accounting for 47% of linolenic acid variation in *B. napus*. The gene (FAD3, omega-3 desaturase) has been cloned in *Arabidopsis* (Arondel et al. 1992). . Hu et al. (1995) and Jourdren et al. (1996a) developed RAPD markers linked to genes controlling linolenic acid content in *B. napus*. By converting a RAPD marker into an RFLP marker, Hu et al. (1995) found that the percentage of the total phenotypic variation explained by the marker increased from 12.8 to 26. Rajcan et al. (1999) developed two RAPD markers (RM 350& RM 574) very tightly linked to two unlinked linolenic acid genes. The markers showed epistasis, and explained about 37% of the total phenotypic variation in the trait.

The purpose of the present study was to investigate the genetics of fatty acids in *B. juncea* using doubled haploid populations. The molecular markers tightly linked with the QTLs associated with different fatty acid content, can then be utilized for marker

assisted selection to align the fatty acid profile of *B. juncea* more closely to those of existing canola species.

4.2 Materials and methods

4.2.1 Plant material

The plant material has been described earlier (Chapter 2).

4.2.2 Experimental design

The layout of the experiment has been described in the chapter 3. Seeds, arising through (controlled) self-pollination, of parental and DH lines were planted in field trials. In 1999, the trial was conducted at the Edmonton Research Station and Ellerslie using three replications. Each plot consisted of four rows, 6m long and 0.3m apart. The Ellerslie site was lost due to herbicide spray drift. In 2000, the trial was conducted at three sites, the Edmonton Research Station, Ellerslie and Kelsey. In 2000, two replications were seeded, and the plot size was reduced to three rows, 4m long and 0.3m apart due to shortage of self-pollinated seed.

4.2.3 Trait analysis

Pheotypic correlations among different traits were determined by using the formula $r_{ij}= \sigma_{ij}/\sigma_i\sigma_j$, where σ_{ij} is the covariance of traits i and j, σ_i and σ_j are the standard deviations for traits i and j respectively. Heritability was determined by the formula $h^2=\sigma^2_G/\sigma^2_P=\sigma^2_G/(\sigma^2_G+\sigma^2_E)$, where σ^2_G is the genotypic variance, σ^2_P phenotypic variance and σ^2_E environmental variance. Variances and covariances were computed using SAS/SAT 6.0 (SAS Institute Inc. 1989).

4.2.4 Fatty acid analysis

Self-pollinated seeds from parental and DH lines were obtained, and analyzed for fatty acid profile by using the 5508 method, ISO, 1990.

4.2.5 RFLP and QTL analysis

RFLP and QTL analyses have been described earlier (chapters 2 and 3).

4.3 Results

4.3.1 Maternal effect

No statistical difference in fatty acid profiles was found between the DH populations developed from the reciprocal crosses (data not shown).

4.3.2 Erucic acid

The high erucic parent (HEP), low erucic parent (LEP) and F_1 had erucic acid content of 50.8%, 0.6% and 34.5% respectively. The 112 DH lines were distributed into three classes: low (0-5%), medium (25-40%) and high (40-55%) with 22, 55 and 35 lines respectively (based on the average of four environments, Edmonton Research Station 1999 & 2000, Ellerslie 20000 and Kelsey 2000), and followed a 1:2:1 ratio (χ^2=3.05, P=.25-.10). This indicated that erucic acid content in *B. juncea* was controlled by two genes acting in an additive manner, and confirmed the findings of Kirk and Hurlstone (1983). For simplicity, the genes are represented as E_{1a} and E_{1b}. Thus, the HEP, LEP and F_1 had $E_{1a}E_{1a}E_{1b}E_{1b}$, $e_{1a}e_{1a}e_{1b}e_{1b}$, and $E_{1a}e_{1a}E_{1b}e_{1b}$ genotypes, respectively. Two QTLs, associated with erucic acid content, were identified in the present study. Individually, one QTL explained approximately 48.9-55.0% of the phenotypic variance in the population in different environments (Table 4.1). This QTL was tightly linked with RFLP locus ec4h9b, and was designated as E_{1a} (Table 4.1, Fig. 4.2). The other QTL explained

approximately 30.9-33.6% of the phenotypic variation in population across all environments (Table 4.1), was tightly linked to the RFLP locus wg3c5aNM, and designated as E_{1b} (Fig. 4.2). A multi-locus model including the effects of both QTLs explained approximately 79.8-88.6% of the variation in the population across all environments (Table 4.1). A very strong epistatic interaction between the two loci was found using two-way analysis of variance. This model explained approximately 97-98%(R^2) of the variation in the population (Table 4.2).

Erucic acid content segregation followed a qualitative inheritance model. The genotypic classes were designated as follows: for low class $e_{1a}e_{1a}e_{1b}e_{1b}$ (alleles from the LEP), for the high class $E_{1a}E_{1a}E_{ib}E_{1b}$ (alleles from the HEP) and for the intermediate class either $E_{1a}E_{1a}e_{1b}e_{1b}$ or $e_{1a}e_{1a}E_{1b}E_{1b}$.

The locus E_{1a} (ec4h9b) showed a highly significant distortion (χ^2=8.06, P<0.005) towards the HEP in the S population but followed a 1:1 ratio (χ^2=0.75, P=0.25-0.5) in the R population. However, locus E_{1b} (wg3c5aNM) followed a1:1 ratio in both the S and R populations (χ^2=0.33, P=0.5-0.75, χ^2=0.017, P=0.99-1).

The locus E_{1a} and E_{1b} explained more than half and one third of the variation in the population respectively (Table 4.1). However, the group ($E_{1a}E_{1a}e_{1b}e_{1b}$) had higher erucic acid content than that of the group ($e_{1a}e_{1a}E_{1b}E_{1b}$), and the difference between the two groups was significant in every population across all environments (Table 4.3). This indicated that the two loci contributed unequally to the erucic acid content.

4.3.3 Oleic acid

The HEP, LEP and F_1 had 9.17%, 42.7% and 14.8% oleic acid, respectively. The recombinant DH population segregated into three classes; lower class (7-11%) comprised

28 lines, medium class (11-25%) 63 lines and high class (25-48%) 21 lines. The observed phenotypic ratio followed a 1:2:1 ratio (χ^2=2.63, P=0.25-0.5), thus supporting the hypothesis that two genes, acting in an additive manner, control oleic acid content in *B. juncea*. Two QTLs were found, explaining approximately 65.8-71.5% of the variation in oleic acid content (Table 4.1, Fig. 4.2). The QTLs showed strong epistasis, and this model explained up to 97% of the variation (Table 4.2).

4.3.4 Linoleic acid

Linoleic acid content of the HEP, LEP and F_1 were 15.10%, 33.71% and 22.12% respectively. The recombinant DH population segregated for linoleic acid into three classes: lower class (<18%), intermediate class (18% to 22%), higher class (>22%) with 22, 58 and 32 lines respectively. The three classes followed a 1:2:1 ratio (χ^2=1.93, P=0.25-0.5). This supported the hypothesis that the trait was under the control of two genes acting in an additive manner. Two QTLs were associated with linoleic acid content, explaining approximately 63.1-68.7% of the variation in different environments (Table 4.1, Fig. 4.2). Up to 85% of the phenotypic variation was explained by a model that included epistatsis between the QTLs (Table 4.2).

It is interesting to note that the QTLs associated with oleic and linoleic acids were in the same position as those associated with erucic acid. However, for oleic and linoleic acids, the QTLs acted in an opposite direction i.e. E_{1a} and E_{1b} decreased, while e_{1a} and e_{1b} increased levels of oleic and linoleic acids (Table 4.1, Fig. 4.2).

4.3.5 Linolenic acid

Linolenic acid content of the HEP, LEP and F_1 were 12.1%, 15.26% and 11.8% respectively. However, the recombinant DH population followed a near normal

distribution, and exhibited high transgressive segregation (data not shown). This indicated that linolenic acid was inherited as a quantitative trait. Five QTLs (E_{1a}, E_{1b}, LN_2, LN_3 and LN_4) significantly affected this acid. The proportion of the total phenotypic variation explained individually by these QTLs varied from 4.2 to 35.9%, and collectively from 66.4 to 76.4% in different environments (Table 4.1). Of five QTLs, two were in the same position as for oleic, linoleic and erucic acids, but with smaller effects (9.3-15.1%) (Table 4.1, Fig. 4.2). For linolenic acid, a major QTL (LN_2) was present that explained up to 35.9% of the variation in the population. This QTL was tightly linked with the RFLP locus wg4d7b (Table 4.1, Fig. 4.2). Epistasis existed between E_{1a} and E_{1b} and between E_{1a} and LN_2. The epistatic models explain more variation than that explained by a multilocus model (Tables 4.1 & 4.2).

4.3.6 Correlations

Oleic, linoleic and linolenic acids were positively and significantly correlated among themselves but negatively and significantly correlated with erucic acid (Table 4.4), thus confirming previous results in other *Brassica* species (Craig 1961; Stefanson and Houghen 1964; Stefansson and Storgaard 1969; Chen and Beversdorf 1990). Levels of erucic and eicosenoic acids had both positive and significant correlation. All fatty acids except eicosenoic acid followed the same pattern at different erucic acid content (Fig. 4.3). Eicosenoic acid, on the other hand, first increased with increasing erucic acid content, and then decreased at higher erucic content. Similar findings have been reported by Jonsson (1977) in *B. napus*.

4.3.7 Eicosenoic acid

Kondra and Stefansson (1965) reported that eicosenoic acid content was controlled by the same genes as for erucic acid, but the genes showed complete dominance for eicosenoic acid content (Table 4.5).

As explained earlier, eicosenoic acid followed a different pattern at various erucic acid contents. The distribution of eicosenoic acid at different erucic acid contents indicated that the eicosenoic acid values formed clusters/groups, rather than following a distribution pattern (Fig. 4.3). These four groups corresponded to genotypes, $E_{1a}E_{1a}E_{1b}E_{1b}$ (high erucic acid), $e_{1a}e_{1a}e_{1b}e_{1b}$ (low erucic acid)(parental group) $E_{1a}E_{1a}e_{1b}e_{1b}$, $e_{1a}e_{1a}E_{1b}E_{1b}$ (intermediate group). These groups had very small range and standard deviation, and significantly differed from each other (Table 4.6) (Fig. 4.3). Aside from the group associated with genotype $e_{1a}e_{1a}e_{1b}e_{1b}$, eicosenoic acid was negatively and significantly correlated with erucic acid ($r=-0.81$). In the biosynthetic pathway of fatty acids, oleic acid serves as the precursor and eicosenoic acid as the intermediate product for erucic acid. It has been shown that alleles controlling erucic acid content could differ significantly in their contribution to the total fatty acid content, and had very strong epistasis between them (Tables 4.2 & 4.3).

In the proposed model for eicosenoic acid inheritance, each allele controlling erucic acid content has more or less an equal potential of initiating the first step of chain elongation from 18:1 to 20:1. The difference in potential of producing erucic acid content is exhibited in the second elongation step i.e. from 20:1 to 22:1. Thus, alleles with high potential of producing erucic acid convert most of eicosenoic acid to erucic acid, and vice versa. The absence of effective alleles would never initiate the elongation step from 18:1

to 20:1. This explains why zero erucic acid cultivars always have traces of eicosenoic acid. The model explains why the group with genotype $e_{1a}e_{1a}E_{1b}E_{1b}$ had higher eicosenoic acid contents than that of the group associated with genotype $E_{1a}E_{1a}e_{1b}e_{1b}$ because E_{1b} contributed less as compared to E_{1a} towards total erucic acid content. The proposed model explains why low erucic parents in our cross and Kondra and Stefansson's (1965) crosses had similar amounts of erucic acid, and why high erucic parents in both the studies had different eicosenoic acid content. In the present study, it was possible to clearly differentiate medium (i.e. low medium and high medium) and high classes both for erucic and eicosenoic acids using marker genotypes at E loci. In the earlier studies, (Kondra and Stefansson 1965), medium and high classes for eicosenoicacid were mixed, and taken as a single class (Table 4.5).

One QTL was found explaining about 14% of the variation for eicosenoic acid content in the population. Ignoring the values associated with group $e_{1a}e_{1a}e_{1b}e_{1b}$, two QTLs (same as for erucic acid but acting in an opposite direction) were found, explaining about 77.2-85.1% of the variation in different environments (Table 4.1, Fig. 4.2). Using one-way ANOVA, none of the QTLs showed significant association with eicosenoic acid content (data not shown). However, strong epistasis between the QTLs was present, and this model explained approximately 88-92% of the variation in the data (Table 4.2).

4.3.8 QTL x environment interactions

QTL x environment interactions were determined by the stability of the QTLs in different environments. QTL x environment interactions were observed for the QTLs associated with linolenic acid. Of the QTLs mapped for linolenic acid, the QTL LN4 could not be identified in Ellerslie 2000 and Kelsey 2000. Also, the QTL could not be

detected in a fixed interval on linkage group 3 (Table 4.1, Fig 4.2). The QTL LN3 could be detected in all environments, but its position on linkage group 6 changed in different environments (Table 4.1). All other QTLs for all fatty acids were consistent in all environments. However, the proportion of the total phenotypic variation explained individually by these QTLs varied from one environment to another (Table 4.1).

4.3.9 G X E interactions

The study was conducted in different years and locations to determine the effects of environment on the fatty acid content. The strength of genotype x environment interactions was determined by calculating the correlations of the rankings of the lines in the different environments. Genotype x location interactions were non-significant for all the acids except for linolenic acid. However, genotype x year interactions were significant for all the acids (Tables 4.7 & 4.8). The genotype x environment interactions had little impact on the rankings of DH lines in different environments, since the correlations among the ranking of the lines in any two environments for any fatty acid was always positive and significant ($r>.85$). Heritability was high for all fatty acids; however, comparatively, linolenic acid had the lowest heritability of all fatty acids (Tables 4.7 & 4.8).

4.4 Discussion

In this paper, the first comprehensive and detailed study on the genetics of the fatty acid profile of *B. juncea* has been reported. The results of this study confirmed previous results, and provided further insight into mode of inheritance for levels of different fatty acids in *B. juncea*. For example, our results confirmed the previous findings of a 2-gene model for the inheritance of erucic acid content with additive gene

action (Kirk and Hurlstone 1983). Similar results have been reported for *B. napus* (Chen and Beversdorf 1990; Kondra and Thomas 1975) and *B. carinata* (Getinet et al.1997). In a DH population segregating for erucic acid in *B. napus*, Chen and Beversdorf (1990) found that the two genes for erucic acid controlled the accumulation of oleic acid. This is in agreement with the results in the present study. There are no published reports in the *Brassicas* of the inheritance of linoleic acid content in populations segregating for erucic acid. We propose that the same two genes controlling erucic acid content also control linoleic acid content. This is supported by our report that the same two QTLs were associated with these two acids. However, as far as inheritance of content of erucic acid and linoleic and oleic acids is concerned, the genes acted differently. This is further confirmed by a very strong and statistically negative correlation (r=90-.96) between erucic acid and oleic and linoleic acids, and a strong and positive correlation between oleic and linoleic acids (r=.86). In the biosynthesis of these acids (Fig. 4.1), oleic acid serves as the precursor for erucic and linoleic acids. When the erucic acid pathway is active, most of the oleic acid is converted into erucic acid and a small percentage desaturated to linoleic acid, thus resulting in a strong negative correlation between erucic, and oleic and linoleic acids. Linolenic acid behaves as a quantitative trait. Five QTLs for linolenic acid were found, two of which were the same as for erucic acid. It appears that there are at least three genes controlling linolenic acid content in *B. juncea*. Similar results have been reported in *B. napus* (Chen and Beversdorf 1990).

In the present study, no maternal effects were observed for any fatty acid in *B. juncea*. Maternal effects have been reported in *B. napus* for oleic, linoleic and linolenic

acids (Thomas and Kondra 1973; Diepenbrock and Wilson 1987; Pleines and Friedt 1989). This suggests that the maternal effects in the *Brassicas* may be species specific.

QTLs associated with erucic acid content in *B. napus* were reported to explain approximately 89-95% of the phenotypic variation in the population; the remainder of the variation was attributed to environmental and other factors (Thorman et al.1996; Jourdren et al. 1996b). Our multi-locus model explains 79-88% of the variation in the population. However, when epistatic interactions are considered, approximately 97-98% of the total variation in the population could be explained. Strong epistasis was observed between QTLs controlling levels of erucic, oleic, linoleic, eicosenoic and linolenic acids. Epistatic relationships beween the genes controlling linoleic acid content have been reported in *B. napus* (Rajcan et al. 1999). The present study appears to be the first to report such epistatic relationships between genes controlling other fatty acids in the *Brassicas*.

This study is also the first to report unequal contribution of genes controlling erucic acid content in *B. juncea*. However, both equal and unequal contribution of genes controlling erucic acid has been published for *B. napus* (Jourdren et al. 1996b; Krzymanski and Downey 1969). Krzymanski and Downey (1969) identified five alleles in *B. napus* namely e, E^a, E^b, E^c and E^d acting in an additive manner and contributing erucic acid content of <1, 10, 15, 30 and 3.5% respectively.

Environmental factors, especially temperature and day length markedly influence fatty acid content particularly levels of linolenic acid and erucic acids. The G x E interactions in the present study were most commonly observed for genotypes falling into the medium to high and high range (Craig 1961; Harvey and Downey 1963; Diepenbrock and Wilson 1987; Pleines and Friedt 1989). Genotype x environment interactions were

observed especially for linolenic acid, without any significant change in the rankings of the DH lines in different environments.

The mutilocus model (including epistasis) explained nearly all of the variation in major fatty acids of *B. juncea* except linolenic acid. Linolenic acid behaved in a quantitative manner, indicating that there must be other minor genes affecting this trait. These minor genes might have exhibited effects, too small to be picked up at high LOD values. Moreover, this was the only fatty acid influenced by environment as indicated by its lowest heritability and genotype x environment interactions (Tables 4.6 & 4.7).

All linkage groups except LK11, containing QTLs for different fatty acids belonged to the A genome of *B. juncea,* as identified earlier (Chapter II). LK14 was aligned with the linkage groups containing the QTLs for erucic acid in *B.* napus (Thorman et al. 1996) (Fig. 4.2). It is interesting to note that the RFLP locus wg4d7b associated with the major QTL for linolenic acid content in the present study is the same as that found in *B. napus* (Thorman et al. 1996) (Fig. 4.2).

4.5 Summary

To our knowledge, there are no reports of stability of QTLs controlling fatty acids in the *Brassicas* in different environments. In the present study, QTLs controlling levels of erucic, oleic, eicosenoic and linoleic acids were stable in different years and locations. QTLs controlling linolenic acid content were inconsistent in different environments. Some of the QTLs found in one environment were not significant in others, however, major QTLs were significant in all environments. In the present study, five QTLs significantly affected fatty acid content in *B. juncea*. Three of the QTLs (two for erucic, oleic, linoleic, eicosenoic and linoleic acids, and one for linolenic acid) were highly

consistent across different environments, and could be very effectively deployed in marker assisted selection for fatty acids in this species. The three genes are probably the same as have been cloned in *B. napus* (Roscoe et al. NCBI Accesssion U50771) and *Arabidopsis* (Arondel et al. 1992).

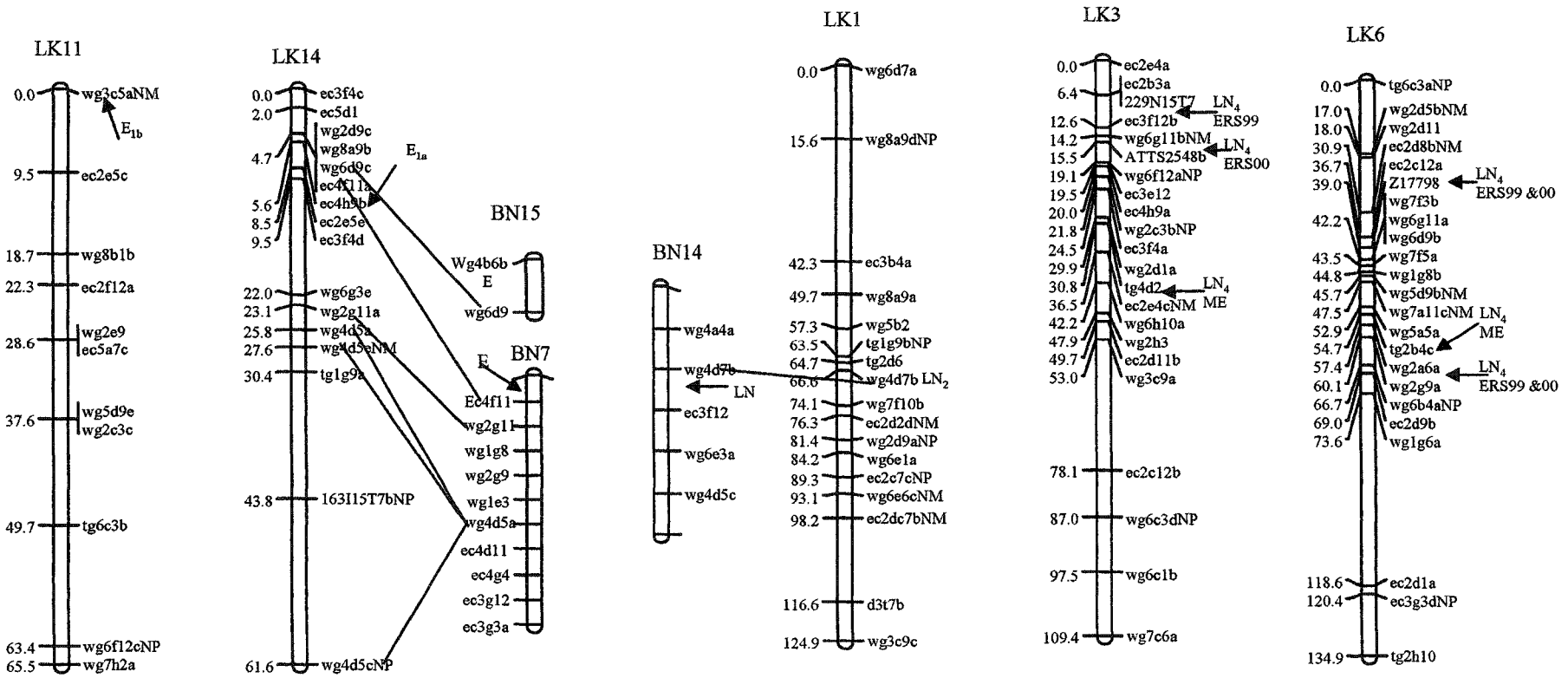

Fig 4. Homology and distribution of the QTLs associated with fatty acids in the *Brassicas*.
E = QTL associated with erucic acid in *B. juncea* and *B. napus* (Thorman et al. 1996),
LK = linkage group of *B. juncea*, BN = linkage group of *B. napus*. Only loci, detected by common probes in the two maps, are shown for *B. napus* linkage groups.

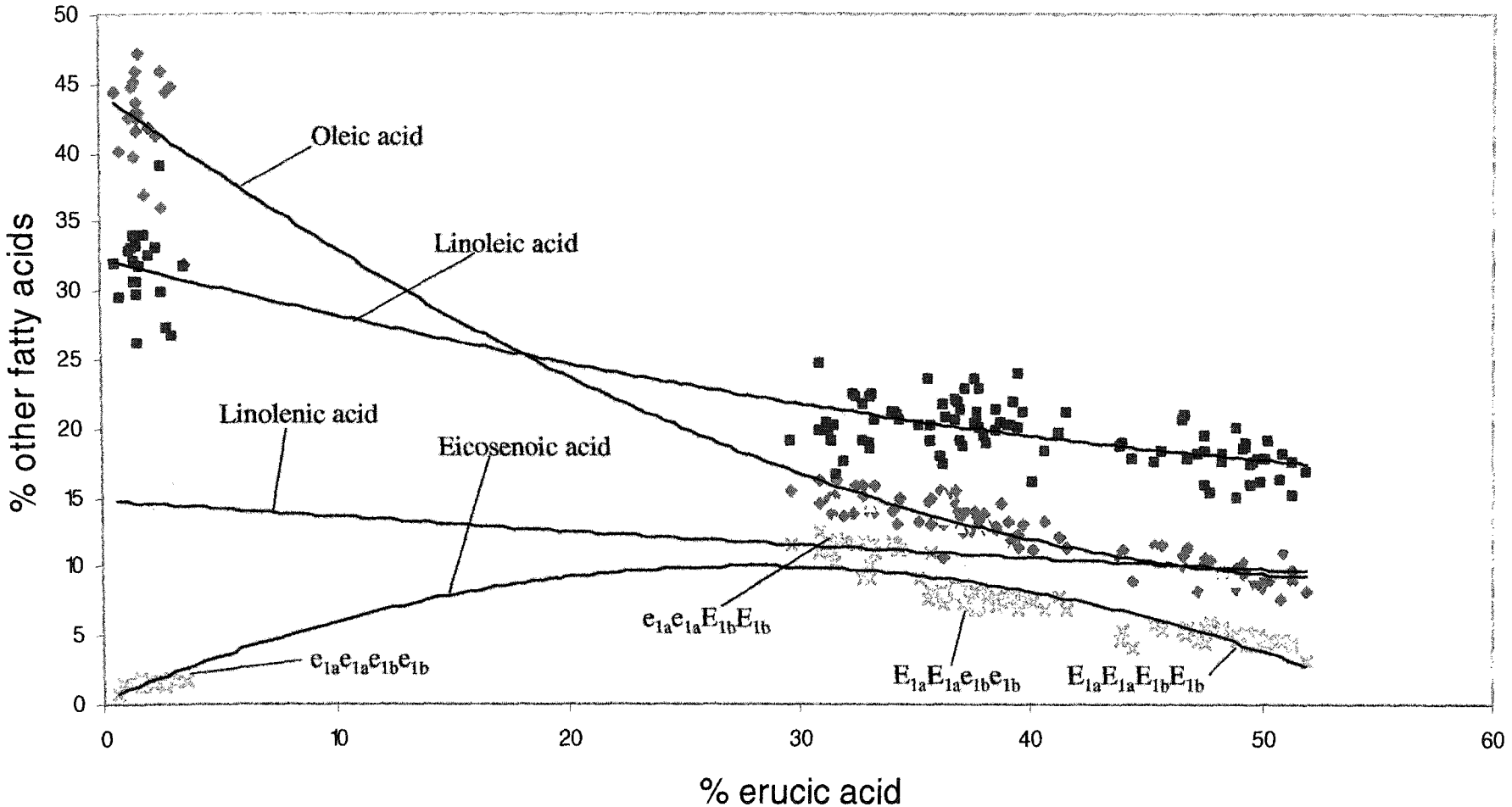

Fig. 4.3. Relationships between erucic and other fatty acids at varying erucic acid contents in a *B. juncea* DH populations segregating for erucic acid. Different lines show the variations in different fatty acids at various erucic acid contents. $E_{1a}E_{1a}E_{1b}E_{1b}$, $e_{1a}e_{1a}E_{1b}E_{1b}$ & $E_{1a}E_{1a}e_{1b}e_{1b}$ and $e_{1a}e_{1a}e_{1b}e_{1b}$ shows the eicosenoic acid content associated with high, medium and low classes of erucic acid respectively.

Table 4.1. Genetics of QTLs for fatty acids in *B. juncea* in different environments.

Acid	Env	QTL	LK	Dis (cM)		LOD	σ_P^2	Totσ_P^2	Add
Erucic	ERS99	E_{1a}	14	0	ec4h9b-ec2e5e	39.21	55	88.6	-12.37
		E_{1b}	11	5	wg3c5aNM-ec2e5c	28.68	33.6		-9.2
	ERS00	E_{1a}	14	0	ec4h9b-ec2e5e	36.54	53.9	85.4	-11.95
		E_{1b}	11	5	wg3c5aNM-ec2e5c	26.69	31.5		-8.79
	EL00	E_{1a}	14	0	ec4h9b-ec2e5e	33.92	51.6	83.6	-12.09
		E_{1b}	11	5	wg3c5aNM-ec2e5c	25.27	32		-9.15
	KE00	E_{1a}	14	0	ec4h9b-ec2e5e	32.49	48.9	79.8	-12.02
		E_{1b}	11	5	wg3c5aNM-ec2e5c	24.30	30.9		-9.23
	ME	E_{1a}	14	0	ec4h9b-ec2e5e	38.26	53.7	85.8	-12.15
		E_{1b}	11	5	wg3c5aNM-ec2e5c	27.16	32.1		-9.25
Oleic	ERS99	E_{1a}	14	0	ec4h9b-ec2e5e	22.73	43.1	71.5	8.03
		E_{1b}	11	0	wg3c5aNM-ec2e5c	17.20	28.4		6.37
	ERS00	E_{1a}	14	0	ec4h9b-ec2e5e	21.42	43.8	69.3	8.01
		E_{1b}	11	0	wg3c5aNM-ec2e5c	14.83	25.5		5.90
	EL00	E_{1a}	14	0	ec4h9b-ec2e5e	20.15	42.6	69	8.13
		E_{1b}	11	0	wg3c5aNM-ec2e5c	26.4	26.4		6.14
	KE00	E_{1a}	14	0	ec4h9b-ec2e5e	18.55	39.7	65.8	8.02
		E_{1b}	11	0	wg3c5aNM-ec2e5c	13.97	26.1		6.17
	ME	E_{1a}	14	0	ec4h9b-ec2e5e	22.17	42.9	70.9	8.04
		E_{1b}	11	0	wg3c5aNM-ec2e5c	16.65	28		6.32
Linoleic	ERS99	E_{1a}	14	0	ec4h9b-ec2e5e	18.08	35.4	68.1	3.3
		E_{1b}	11	5	wg3c5aNM-ec2e5c	15.96	32.7		3.11
	ERS00	E_{1a}	14	0	ec4h9b-ec2e5e	17.76	35.3	68.7	3.27
		E_{1b}	11	5	wg3c5aNM-ec2e5c	17.04	33.4		3.07
	EL00	E_{1a}	14	0	ec4h9b-ec2e5e	15.72	35.9	64.4	3.28
		E_{1b}	11	5	wg3c5aNM-ec2e5c	13.33	28.5		2.80
	KE00	E_{1a}	14	0	ec4h9b-ec2e5e	15.28	34.3	63.1	3.14
		E_{1b}	11	5	wg3c5aNM-ec2e5c	13.39	28.8		2.87
	ME	E_{1a}	14	0	ec4h9b-ec2e5e	18.60	36.4	68.3	3.25
		E_{1b}	11	5	wg3c5aNM-ec2e5c	16.02	31.9		2.98
Eicosenoic	ERS99	E_{1a}	14	0	ec4h9b-ec2e5e	31.64	84.2	84.4	3.24
		E_{1b}	11	5	wg3c5aNM-ec2e5c	18.74	26.9		1.5
	ERS00	E_{1a}	14	0	ec4h9b-ec2e5e	29.02	81.3	82.1	2.91
		E_{1b}	11	5	wg3c5aNM-ec2e5c	15.52	29.9		1.39
	EL00	E_{1a}	14	0	ec4h9b-ec2e5e	23.2	74	77.2	2.83
		E_{1b}	11	5	wg3c5aNM-ec2e5c	12.38	33.8		1.46
	KE00	E_{1a}	14	0	ec4h9b-ec2e5e	22.41	75.8	78.7	2.91
		E_{1b}	11	5	wg3c5aNM-ec2e5c	11.48	32.3		1.46
	ME	E_{1a}	14	0	ec4h9b-ec2e5e	32.87	84.4	85.1	2.98
		E_{1b}	11	5	wg3c5aNM-ec2e5c	18.87	30.2		1.44
Linolenic	ERS99	E_{1a}	14	0	ec4h9b-ec2e5e	5.18	9.3	66.6	0.95
		E_{1b}	11	5	wg3c5aNM-ec2e5c	6.79	14.1		1.12
		LN_2	1	5	wg4d7b-wg7f10b	11.99	30.2		-1.79
		LN_3	6	0	Z17798-wg7f3b	4.44	8.0		0.86
		LN_4	3	0	229N15T7-ec3f12b	2.80	5		0.67
	ERS00	E_{1a}	14	0	ec4h9b-ec2e5e	7.14	11.2	70	0.88
		E_{1b}	11	5	wg3c5aNM-ec2e5c	5.85	10.1		0.81
		LN_2	1	5	wg4d7b-wg7f10b	17.18	35.9		-1.65
		LN_3	6	0	Z17798-wg7f3b	5.65	8.6		.77

	LN_4	3	0	wg6g11bNM-ATTS2548b	2.94	4.2		-.54
EL00	E_{1a}	14	0	ec4h9b-ec2e5e	6.71	15.1	66.4	1.21
	E_{1b}	11	5	wg3c5aNM-ec2e5c	6.33	14.7		1.16
	LN_2	1	5	wg4d7b-wg7f10b	9.84	30.1		-1.8
	LN_3	6	0	wg2a6a-wg2g9a	3.13	6.5		.77
KE00	E_{1a}	14	0	ec4h9b-ec2e5e	6.16	13.9	66.7	1.19
	E_{1b}	11	5	wg3c5aNM-ec2e5c	6.28	14.8		1.21
	LN_2	1	5	wg4d7b-wg7f10b	9.94	32.4		-1.93
	LN_3	6	0	wg2a6a-wg2g9a	2.71	5.6		0.75
ME	E_{1a}	14	0	ec4h9b-ec2e5e	8.60	15.1	76.4	1.11
	E_{1b}	11	5	wg3c5aNM-ec2e5c	7.43	14.2		1.05
	LN_2	1	5	wg4d7b-wg7f10b	14.02	35.4		-1.70
	LN_3	6	0	tg2b4c-wg2a6a	4.48	7.5		.76
	LN_4	3	0	tg4d2-ec2e4cNM	2.52	4.2		-.55

σ_P^2 = %age of total phenotypic variance explained by individual loci, Totσ_P^2 = %age of total phenotypic variance explained by all QTLs detected in an environment, LOD =Lod value associated with detected QTL, Add = additive effect associated with detected QTL, ERS99 = Edmonton Research Station 1999, ERS00 = Edmonton Research Station 2000, EL00 = Ellerslie 2000, KE00 = Kelsey 2000, ME = mean environment, Env = environment, Dis = distance of the QTL from the first flanking marker, ♦ = positive additive effect showed that LEP alleles contributed to parameter at associated QTLs, • = negative additive effect showed that contributing alleles were coming from HEP

Table 4.2. Epistatic interactions among QTLs for fatty acids in *B. juncea*.

Acid			ME		ERS99		ERS00		EL00		KE00	
	SOV	DF	MS	R^2	MS	R^2	MS	R^2	MS	R^2	MS	R^2
Erucic	$E_{1a} \times E_{1b}$	1	***	0.98	***	0.97	***	0.97	***	0.97	***	0.97
Oleic	$E_{1a} \times E_{1b}$	1	***	0.97	***	0.96	***	0.96	***	0.96	***	0.96
Linoleic	$E_{1a} \times E_{1b}$	1	***	0.85	***	0.83	***	0.84	***	0.81	***	0.82
Linolenic	$E_{1a} \times E_{1b}$	1	**	0.38	**	0.36	**	0.36	*	0.32	*	0.33
	$E_{1a} \times LN_2$	1	**	0.60	NS	-	*	0.58	*	0.55	**	0.58
Eicosenoic	$E_{1a} \times E_{1b}$	1	***	0.92	***	0.91	***	0.91	***	0.87	***	0.88

ME = mean environment, ERS99 = Edmonton Research Station 1999,
ERS00 = Edmonton Research Station 2000, EL00 = Ellerslie 2000, KE00 =Kelsey 2000.
*, **, *** = significance level at 5%; 1%, .01% respectively, R^2 = total variation explained by the model.

Table 4.3. Contribution of genes controlling erucic acid content in *B. juncea*.

		ME		ERS99		ERS00		EL00		KE00	
Population	Genotype	Means	F Test	Means	F Test	Means	F Test	Means	F Test	Means	F Test
C	$E_{1a}E_{1a}e_{1b}e_{1b}$	37.08	***	36.05	***	36.97	***	37.58	***	37.59	***
	$e_{1a}e_{1a}E_{ib}E_{1b}$	32.54		30.89		31.97		33.82		33.74	
S	$E_{1a}E_{1a}e_{1b}e_{1b}$	42.05	***	41.42	***	41.71	***	42.41	***	43.17	***
	$e_{1a}e_{1a}E_{ib}E_{1b}$	32.09		29.90		31.99		33.42		33.47	
R	$E_{1a}E_{1a}e_{1b}e_{1b}$	37.31	***	36.13	***	37.13	***	37.96	***	38.06	***
	$e_{1a}e_{1a}E_{ib}E_{1b}$	32.36		31.34		31.72		33.89		33.81	

ME = mean environment, ERS99 = Edmonton Research Station 1999, ERS00 = Edmonton Research station 2000, EL00 = Ellerslie 2000, KE00 = Kelsey 2000, *, **, *** = significance level at 5%; 1%, .01%, NS = non-significant, S population = population derived from the orignal cross, R population = population derived from the reciprocal cross, C population = combined population (R and S together).

Table 4.4 Correlations among fatty acids in a *B. juncea* DH population segregating for erucic acid.

	Oleic Acid	Linoleic Acid	Linolenic Acid	Eicosenoic Acid
Erucic Acid	-0.96	-090	-0.51	0.31
Oleic Acid		0.86	0.43	-0.5
Linoleic Acid			0.26	-0.42
Linolenic Acid				-0.27

All correlations were highly significant.

Table 4.5. Summary inheritance of eicosenoic acid content in *B. napus*.

Back cross	Genotypes	Eicosenoic acid (%)		Observed	Expected	χ^2 value	p
		Mean	Range				
(Low x F_1) Liho-Z x (Liho-Z x Nug-E)	$e_1e_1e_2e_2$	0.5	0-2	13	1	1.85	0.1-0.25
	{$E_1e_1e_2e_2$ $e_1e_1E_2e_2$ $E_1e_1E_2e_2$}	11.6	7-15	59	3		
(High x F_1) Nug-E x (Liho-Z x Nug-E)	$E_1e_1E_2e_2$ $E_1E_1E_2e_2$ $E_1e_1E_2E_2$ $E_1E_1E_2E_2$	15.3	11-22	79			

E, e = dominant and recessive alleles respectively controlling eicosenoic acid content, χ^2 value = Chi-square value, p = probability, Nug-E, Liho-Z, Liho-Z x Nug-E = parents and F_1 used in the study.
Nug-E ($E_1E_1E_2E_2$), Liho-Z ($e_1e_1e_2e_2$), and F_1 ($E_1e_1E_2e_2$) (i.e. Liho-Z x Nug-E) had erucic acid content of 36.1%, 0% and 22.4% respectively, and eicosenoic acid content of 13.3%, 1.8% and 15.4% respectively (after Kondra and Steffansson 1965).

Table 4.6. Eicosenoic acid content associated with different genotypes (for erucic acid content) in *B. juncea*.

	ME			ERS99			ERS00			EL00			KE00		
Genotype	Means (%)	Range	SD	Means (%)	Range	SD	Means (%)	Range	SD	Means (%)	Range	SD	Means (%)	Range	SD
$e_{1a}e_{1a}e_{1b}e_{1b}$	1.50	0.71-1.94	.27	1.45	0.71-3.85	0.69	1.55	1.28-1.86	0.16	1.61	1.27-1.99	0.20	1.51	1.19-1.85	0.24
$e_{1a}e_{1a}E_{ib}E_{1b}$	11.19	9.11-11.9	.71	11.95	10.57-13.0	0.70	11.16	9.33-12.6	0.86	10.67	7.74-12.8	1.24	10.72	7.94-11.93	0.98
$E_{1a}E_{1a}e_{1b}e_{1b}$	7.80	6.85-6.19	.60	8.03	6.28-10.24	0.81	7.78	5.78-9.08	0.72	7.78	6.23-11.06	0.97	7.63	6.0-11.02	1.06
$E_{1a}E_{1a}E_{ib}E_{1b}$	5.29	3.25-7.78	1.1	5.58	3.25-9.23	1.31	5.38	3.37-7.94	1.14	5.09	3.10-7.54	1.15	5.0	3.02-7.45	1.14

All genotype averages are statistically significant (P<.0001) from each other in all environments. All the abbreviations are the same as for the previous tables.

Table 4.7 Genotype x location interactions for fatty acids in a *B. juncea* population segregating for erucic acid content at the Edmonton Research Station, Ellerslie and Kelsey during 2000.

		Erucic			Oleic			Linoleic			Linolenic			Tonic		
SOV	DF	MS	R^2	h^2	MS	R^2	h^2	MS	R^2	h^2	MS	R^2	h^2	MS	R^2	h^2
Loc	2	***	0.99	.92	NS	0.99	0.91	NS	0.96	0.85	*	0.91	0.72	***	.98	0.93
Rep(Loc)	4	NS			***			NS			NS			*		
Set	3	***			***			***			***			***		
Set X Loc	6	NS			NS			NS			NS			NS		
Lines(Set)	110	***			***			***			***			***		
Loc X Lines(Set)	204	NS			NS			NS			*			NS		

h^2 = heritability, rest of the abbreviations are the same as for the previous tables.

Table 4.8 Genotype x year interactions for fatty acids in a *B. juncea* population segregating for erucic acid content at the Edmonton Research Station during 1999 and 2000.

		Erucic			Oleic			Linoleic			Linolenic			Tonic		
SOV	DF	MS	R^2	h^2	MS	R^2	h^2	MS	R^2	h^2	MS	R^2	h^2	MS	R^2	h^2
Year	1	***	0.99	0.93	NS	0.99	0.94	*	0.96	0.88	***	0.89	0.87	***	0.97	0.82
Rep(Year)	4	*			***			NS			NS			NS		
Set	3	***			***			***			***			***		
Set X Year	3	***			*			***			NS			***		
Lines(Set)	112	***			***			***			***			***		
Year X Lines(Set)	110	***			***			***			*			***		

h^2 = heritability, rest of abbreviations are the same as for the previous tables.

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Chapter 5

Mapping QTLs for seed aliphatic glucosinolates in *Brassica juncea*.

5.1 Introduction

The *Brassica* oilseeds, *Brassica napus*, *B. rapa* and *B. juncea*, are one of the most important sources of edible vegetable oils in the world, and are commonly known as rapeseed and mustard species respectively. Canada, China, Northern Europe and the Indian subcontinent are the major producing areas for the *Brassicas* (Downey 1990). Erucic acid in the oil and glucosinolates in the residual meal are two important characteristic components associated with the *Brassicas*. High concentrations of erucic acid in oil may be associated with health risks (Beare et al. 1959) and high glucosinolate contents cause severe nutritional problems in non-ruminant livestock (Fenwick et al. 1983). These two components form the basis for the traditional definition of canola in North America (i.e. erucic acid <2% and glucosinolate <30μmole/g of oil-free meal).

In North America, *B. napus* and *B. rapa* are the only commercial canola species. Because of its superiority to existing canola species for agronomic traits, *B. juncea* has become a primary focus of plant breeders to convert it into a canola type (Downey 1990; Woods et al. 1991). This conversion requires lowering of glucosinolate and erucic acid contents in *B. juncea* to safer limits, as has occurred in *B. napus* and *B. rapa* (Downey 1990).

Glucosinolates are sulphur-containing glycosides, and commonly found in the families Capparaceae, Brassicaceae, Koerbiliniaceae, Moringaceae, Resedaceae and Tovariaceae of the order Capparales (see review by Fenwick et al. 1983.). Their common

formula is given in Fig. 5.1, and consists of a common glycone moiety and a variable aglycone side chain (-R). Glucosinolates with aliphatic side chains are called aliphatic glucosinolates, and most commonly found in the *Brassicas*. Following tissue disruption and hydrolation, glucosinolates are hydrolyzed by myrosinase into different products, depending upon the nature of side chain in the glucosinolate molecule. These hydrolytic products may cause cytotoxity (Horakava 1966), and digestive problems in non-ruminant livestock (Fenwick et al. 1983.).

In the *Brassicas*, the aliphatic glucosinolate profile is species specific. *B. napus* usually contains 3-butenyl, 4-pentenyl and their hydroxyl forms. The most common aliphatic glucosinolates in *B. rapa* and *B. carinata* are 3-butenyl and 2-propenyl respectively. The aliphatic glucosinolate profile of *B. juncea* comprises mainly 3-butenyl and 2-propenyl (Fenwick et al. 1983.).

Glucosinolate biosynthesis in the *Brassicas* is not completely understood, and various models have been proposed (Underhill et al. 1973; Magrath et al. 1994; Mithen et al.1995; Giamoustaris and Mithen 1996). A simplified biosynthesis model is given in Fig. 5.2. The genetics of different aliphatic glucosinolates in the *Brassicas* are complex and have been shown to be under maternal control, and affected by cytoplasmic and epistatic interactions (Magrath et al. 1993; Magrath et al. 1994). The glucosinolates are quantitative in nature, and controlled by a number of linked and unlinked loci in *B. napus* (Kondra and Stefansson 1970; Magrath et al. 1993; Magrath et al. 1994). Artificially resynthesized *B. napus* has been crossed with naturally occurring *B. napus* to study the genetic control of biosynthesis of aliphatic glucosinolate (Magrath et al. 1993; Magrath et al. 1994; Parkin at al. 1994; Mithen 1995). It has been shown that seven loci are

responsible for glucosinolate synthesis, one locus for the presence of propyl (GSL-pro), one for pentyl (GSL-elong-A) and one for butyl (GSL-elong-C). Two loci regulate the synthesis of alkenyl glucosinolates, regardless of the length of alkyl chain (GSL-alk-A and GSL-alk-C) (Magrath et al. 1993; Magrath et al. 1994). The conversion of 3-butenyl and 4-pentenyl to their hydroxyl forms is governed by two genes (GSL-oh-A and GSL-oh-C) (Magrath et al. 1994; Parkin et al. 1994). Loci GSL-elong-A and GSL-oh-A, and GSL-elong-C and GSL-oh-C have been mapped on the homoeologous linkage groups 3 and 13 (Magrath et al. 1994; Parkin et al. 1994). In *Arabidopsis thaliana*, GSL-elong-Ar regulates the side chain elongation, and is believed to be homologous to GSL-elong-A and GSL-elong-C (Magrath et al. 1994). Toroser et al. (1995) and Uzunova et al. (1995) found four major QTLs each, associated with seed glucosinolates in *B. napus*. The QTLs together accounted for 61-71% of the total phenotypic variation.

Love et al. (1990) crossed two *B. juncea* lines, one high in 2-propenyl glucosinolate and the other in 3-butenyl glucosinolate, and found that the glucosinolates were controlled by multiple alleles at the same loci in a complex manner. Stringam and Thiagarajah (1995) also reported complexity in glucosinolate inheritance. They crossed a high glucosinolate *B. juncea* line (high in both 2-propenyl and 3-butenyl) with a low glucosinolate *B. juncea* line, and proposed that 5-9 recessive alleles controlled low values of aliphatic glucosinolates.

The present study is an extension of the work earlier reported by Stringam and Thajarajah (1995). The objective was to elaborate the complex inheritance of aliphatic glucosinolates in *B. juncea* and identify QTLs associated with the glucosinolates. The

RFLP markers which co-segregate with these QTLs, can be used in marker assisted breeding in the development of low aliphatic glucosinolate *B. juncea* cultivars.

5.2 Materials and methods

The plant material used in this study has been described earlier (chapter 2). Experimental layout is the same as for the fatty acid study (chapter 4). Description for QTL analysis has been described earlier (chapter 3).

One sample, taken from the harvested seed, for each line in each replication was analyzed for glucosinolate contents by the method of Daun and McGregor (1983). However, Propyl, butyl and pentenyl contents were computed by using the formula:

Propyl (%) = (2-propenyl / total aliphatic glucosinolate) x 100

Butyl (%) = ((3-butenyl + 2-hydroxybut-3-enyl) / total aliphatic glucosinolate) x 100

Pentyl (%) =((4-pentenyl + 2-hydroxypent-4-enyl) / total aliphatic glucosinolate) x 100

5.3 Results

5.3.1 Aliphatic glucosinolate profile

One hundred and twelve DH lines were used to construct the linkage map for *B. juncea*. However, two lines showed an abnormal aliphatic glucosinolate profile in all environments, and were excluded from the final analysis. The aliphatic glucosinolate profile for the F_1, LEP (low-gluc parent) and HEP (high-gluc parent) is shown in Table 5.1 (based on the average of four environments, the Edmonton Research Station 1999 & 2000, Ellerslie 2000 and Kelsey 2000). The F_1, LEP, HEP, and DH lines differed significantly for 2-propenyl, 3-butenyl, propyl, butyl and total aliphatic glucosinolates (Data not shown). Transgressive segregants, which exceeded the HEP, were observed for 2-propenyl (81% of DH lines), 3-butenyl (4% of DH lines) and total aliphatic

glucosinolate (11% of DH lines) (Figs. 5.3, 5.4 & 5.5). DH lines 88 and 72 segregated transgressively for total aliphatic glucosinolate but had contrasting 2-propenyl and 3-butenyl glucosinolate profiles; the 2-propenyl and 3-butenyl contents of DH lines 88 and 72 were 2.02 μmole/g, 166.48μmole/g and 175.04μmole/g, 5.18μmole/g respectively (Fig. 5.6).

5.3.2 RFLP and QTL analysis

5.3.2.1 Total aliphatic glucosinolate

The mean and range of the segregating population for total aliphatic glucosinolate were 124.76 μmole/g and 4.56-193-31 μmole/g respectively. Five QTLs, GSL10, GSL11, GSL12, GSL16 and GSL22, affected total aliphatic glucosinolate (TA) in different environments (Table 5.2). The proportion of the total phenotypic variation explained collectively by these QTLs varied from 29.5 to 45.1% in different environments. Individually, these QTLs explained about 6.7-15.5% of the total phenotypic variation in different environments. In the mean environment, a new QTL GSL12 appeared, which was not detected in other environments. QTLs GSL16 and GSL22 were common in all environments, GSL11 in three environments respectively. All QTLs appearing in any environment were also found to be significantly affecting total glucosinolate. For all QTLs, alleles from HEP contributed to total aliphatic glucosinolate (Table 5.2).

5.3.2.2 Individual glucosinolates

5.3.2.2.1 2-Propenyl glucosinolate

The mean and range of the segregating population for 2-propenyl glucosinolate were 72.1 μmole/g and 1.5-178.2 μmole/g respectively. Five QTLs significantly influenced 2-propenyl glucosinolate (PE) in different environments (Table 5.2). QTL

GSL10 could only be identified in the mean environment. QTLs GSL6a and GSL16 were present in all environments. However, GSL16 could not be identified in the mean environment. QTL GSL22 (LK22 = unlinked segment D in the genomic map of *B. juncea*) was found in three locations in 2000 i.e. Edmonton Research Station, Ellerslie and Kelsey but could not be identified in the mean environment. These QTLs collectively explained approximately 57.9-78.2% of the total phenotypic variation in different environments. The proportion of the total variation explained individually by these QTLs varied from 5.8 to 49.8%. At QTL GSL6a, the HEP alleles caused a reduction in 2-propenyl glucosinolate. However, at other QTLs, HEP increased 2-propenyl glucosinolate (Table 5.2).

5.3.2.2.2 3-Butenyl glucosinolate

For 3-butenyl glucosinolate, the mean and range for the segregating DH population were 50.9 μmole/g and 0.9-166.5μmole/g respectively. Three QTLs siginificantly affected 3-butenyl glucosinolate in different environments. Two QTLs GSL6a and GSL6b were consistent in all environments and explained approximately 35.3-41.6% and 19.7-33.1% of the total phenotypic variation respectively in different environments (Table 5.2). QTL GSL15 could be detected only in Ellerslie 2000 and the mean environment, and explained approximately 6% of the total phenotypic variation in these environments. For all these QTLs, the HEP alleles increased 3-butenyl glucosinolate. Interestingly, the QTL GSL6a also affected 2-propenyl glucosinolate, however, the HEP alleles decreased 2-propenyl glucosinolate at the QTL (Table 5.2).

5.3.2.2.3 Propyl and butyl glucosinolates

For the segregating DH population, the means for propyl and butyl glucosinolates were 57.8% and 41.2% respectively and ranges were 1.2-98.3% and 0.7-98.1% respectively. Three QTLs, GSL6a, GSL6b and GSL16, affected propyl and butyl glucosinolates in different environments (Table 5.2). QTL GSL6b could not be identified in the mean environment for both propyl and butyl glucosinolates. All other QTLs were common in all environments. The proportion of the total phenotypic variation explained collectively and individually by these QTLs was essentially the same for propyl and butyl in different environments. HEP alleles at GSL6a and GSL6b increased butyl and decreased propyl content; however, HEP alleles at GSL16 contributed to propyl but reduced butyl content (Table 5.2).

5.4 Discussion

5.4.1 Compexity in alkenyl glucosinolate inheritance

Nearly all *B. juncea* aliphatic glucosinolates (97-100%) in the present study consisted of propyl and butyl. All propyl was converted into 2-propenyl, and almost all of butyl (97-100%), into 3-butenyl (Table 5.2). Under these circumstances, it is difficult to distinguish between chain-modifying and chain-elongating genes. Analysis of QTLs for propyl and butyl showed that at least three QTLs were responsible for chain elongation (Table 5.2). The proportion of the total phenotypic variation explained by these QTLs varied from 70.9% to 86.6%, suggesting that other minor genes and environmental factors might also be involved in the presence/absence of propyl and butyl glucosinolates. QTLs GSL6a and GSL6b were also found affecting 3-butenyl glucosinolate, and the HEP alleles at these QTLs contributed to 3-butenyl glucosinolate (Table 5.2). This suggests

that these two QTLs were responsible for presence/absence of butyl, and could be regarded as GSL-buts (homologous to GSL-elong-C (Magrath et al.1994). By similar reasoning, QTL GSL16 appeared to regulate the presence or absence of propyl, and thus could be regarded as GSL-pro (Magrath et al. 1994). However, the continuous distribution of the DH population for propyl and butyl glucosinolates, instead of forming clusters (Figs. 5.6 & 5.7), does not support this model. This complex picture can be simplified by examining the common biosynthetic pathways of propyl and butyl glucosinolates i.e. the products are in competition with one another (Fig 5.1). In the presence of effective alleles for propyl or butyl, only propyl or butyl glucosinolates are detected respectively. When the effective alleles for both propyl and butyl were present, the recombinant lines had both glucosinolates. Very strong negative correlations between propyl and butyl glucosinolates (r=-.999**), and between 2-propenyl and 3-butenyl (r=-.75**) (Figs. 5.6 & 5.7) favored this model. This is further supported by contrasting additive effects of QTLs GSL16, GSL6a and GSL6b for propyl and butyl glucosinolates (Table 5.2).

Three QTLs significantly affected 2-propenyl in four environments and explained up to 78.2% of the phenotypic variation (Table 5.2). This confirms previous results (Stringam and Thiajarajah 1995) where a three-recessive-gene model has been proposed for the absence of 2-propenyl.

Two linked QTLs, GSL6a and GSL6b, were associated with 3-butenyl in all environments (Table 5.2). This was confirmed by a large proportion of DH lines having very low (0.88-7.74μmole/g) and very high (74.29-166.28μmole/g) 3-butenyl (parental type) and a small proportion having intermediate 3-butenyl (recombinant type). This

explains why a two-recessive-gene model could not fit the 3-butenyl glucosinolate segregation in the DH and BC progeny (Stringam and Thiajarajah 1995).

5.4.2 Breeding strategies for low aliphatic glucosinolates

Toroser et al. (1995) and Uzunova et al. (1995) mapped four QTLs each explaining about 71% and 61% of the total phenotypic variation respectively for total seed aliphatic glucosinolates in *B. napus*. These QTLs were similar in their additive effects, with alleles from the high-glucosinolate parent contributing to total glucosinolate. However, in the present study, QTLs for total seed aliphatic glucosinolate explained approximately 29%-45% of the total variaton (Table 5.2). Some of these QTLs such as GSL12 and GSL10 were highly inconsistent in different environments. QTLs for individual glucosinlates explained a very high proportion of the total phenotypic variation i.e. 57.9-78.2%, 57-74.7%, 70.9-86.6% and 72.1-85.6% for 2-propenyl, 3-butenyl, propyl and butyl glucosinolates respectively, and were consistent in different environments (Table 5.2). This discrepancy could be due to the specific aliphatic glucosinolate profile, and correlations between the individual glucosinlates of *B. juncea* in the present study. Major QTLs GSL6a and GSL6b had the opposite additive effects for the individual glucosinolates. These QTLs completely disappeared when QTL analysis was done for total aliphatic glucosinolates (Table 5.2). Therefore, the best strategy to breed for low aliphatic glucosinolate in *B. juncea* would be to follow the QTLs associated with individual glucosinolates. Of the six QTLs associated with the individual glucosinolates, two (GSL15 and GSL10) were inconsistent across environments, and explained only a minor proportion of the total phenotypic variation. The remaining four QTLs (GSL6a, GSL6b, GSL22, GSL16) could be successfully employed in a MAS breeding program to

alter the glucosinolate profile of non-canola *B. juncea* to one similar to that of canola species grown in Canada.

5.4.3 Homology of aliphatic glucosinolate QTLs in *Brassicas*

QTL GSL6b was flanked by wg2d11 and ec2d8bNP. Locus wg2d11 has been associated with aliphatic seed glucosinolate in *B. napus* (Toroser et al. 1995) (Fig. 5.8) Wg2d11 is 1 cM away from one of the flanking markers of QTL GSL6a. Locus wg2d11 is 11 cM (16.4cM away from the QTL position) away from one of the flanking markers (Linkage group 18) of a QTL associated with seed glucosinolate in *B. napus* (Uzunova et al. 1995). Wg6c1c was the closest flanking marker for the QTL GSL22, and only 5.4 cM away from wg3f7. This locus has been reported to have a significant effect on seed glucosinolate content in *B. napus* (Uzunova et al. 1995; Toroser et al. 1995) (Fig. 5.8).

5.5 Conclusions

QTL analysis using RFLP markers confirmed that the inheritance of seed aliphatic glucosinolates was complex, and controlled by a number of major and minor linked, and unlinked genes in *B. juncea* (Stringam and Thiagarajah 1995). When using MAS for the development of low gluconinolate cultivars, an unique breeding strategy should be adopted for *B. juncea*, as compared to that for *B. napus*. Rather than using RFLP markers associated with QTLs for total seed aliphatic glucosinolates, as proposed for *B. napus* (Toroser et al. 1995 and Uzunova et al. 1995), more consistent results can be achieved for *B. juncea* by using molecular markers associated with QTLs for individual glucosinolates.

Fig. 5.1 Structural formula for glucosinolate (Fenwick et al. 1983).

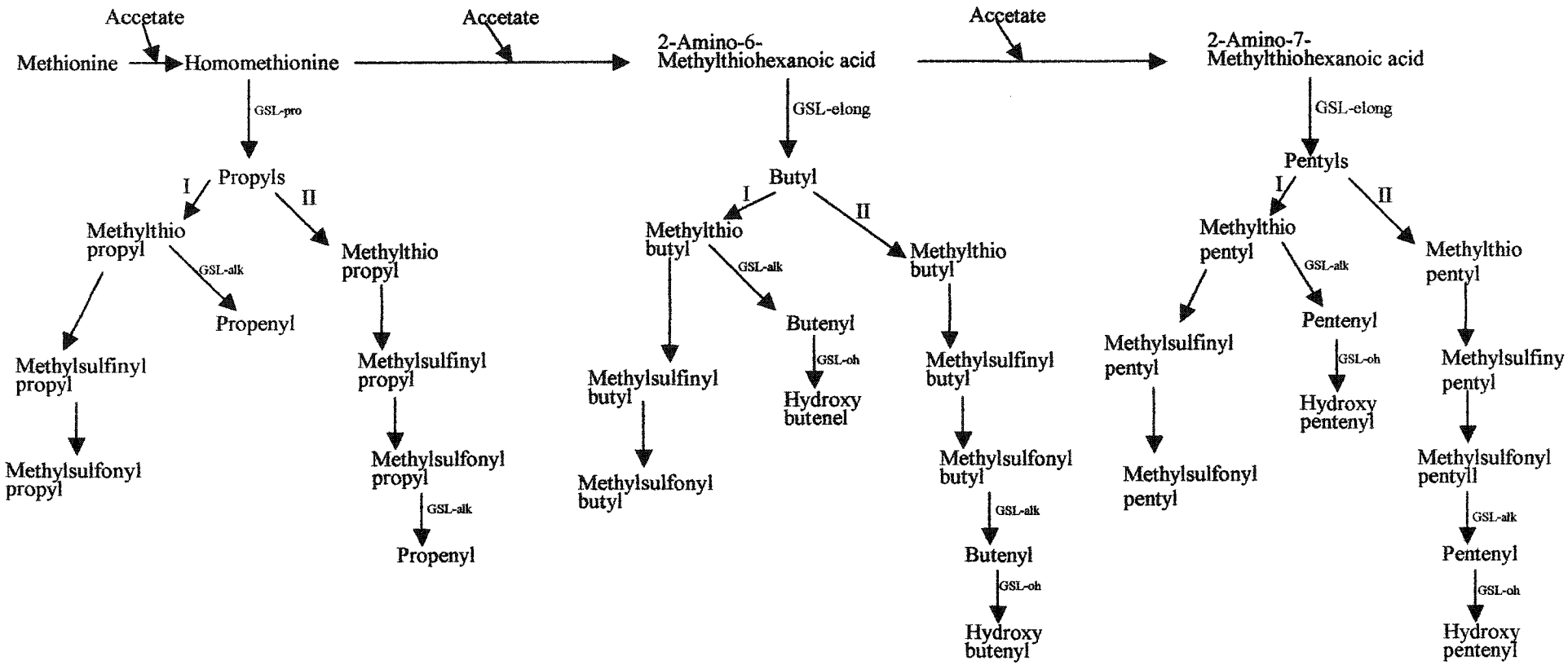

Fig. 5.2. A simpified pathway for synthesis of aliphatic glucosinoaltes in the *Brassicas.*
I = path suggested by Magrath et al. (1993), II = path suggested by Magarath et al. (1994).

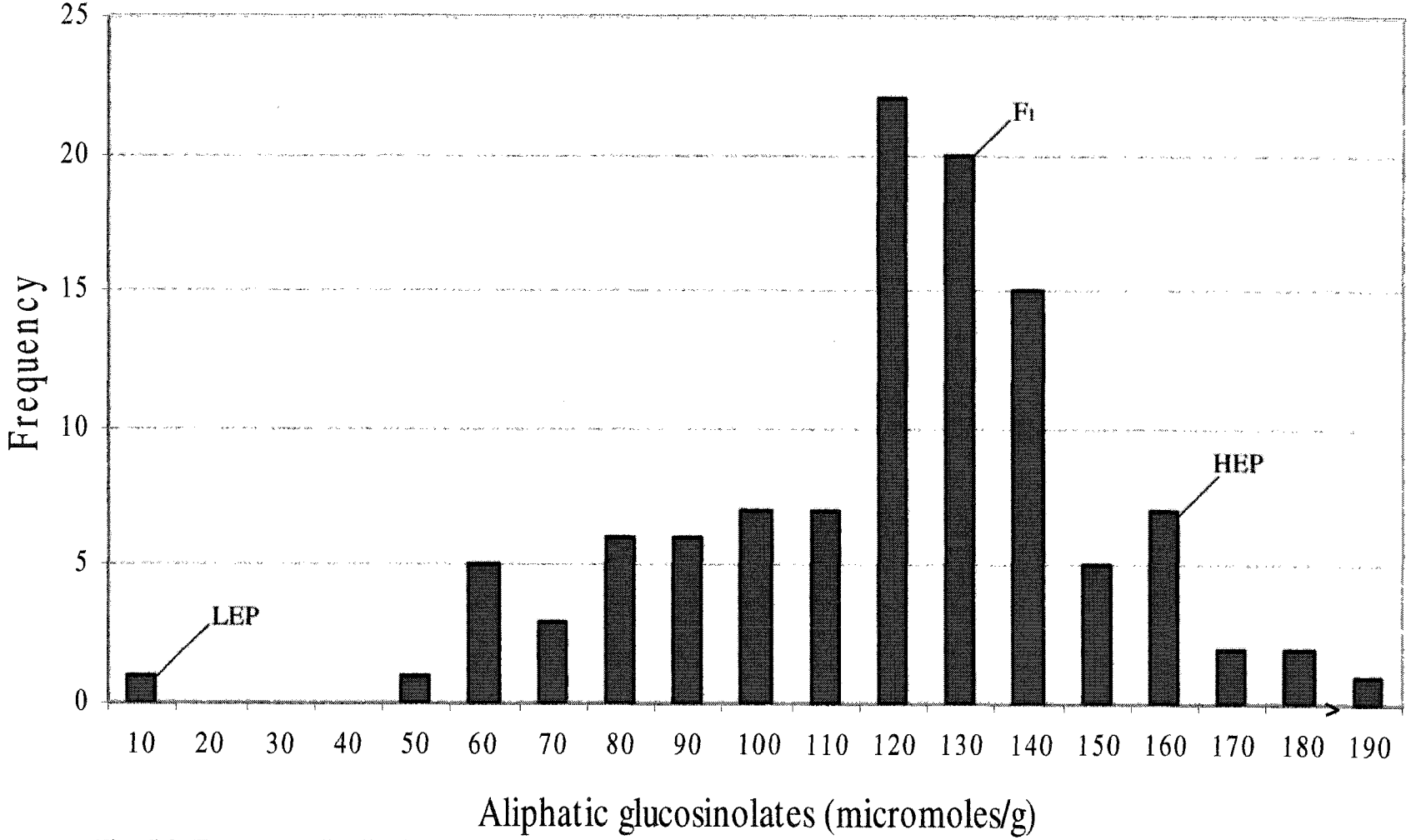

Fig. 5.3. Frequency distribution of recombinant DH lines of *B. juncea* for total seed aliphatic glucosinolates. The relative positions of parents and the F_1 are indicated in the distribution.
HEP = high-glucosinolate parent, LEP = low-glucosinolate parent.

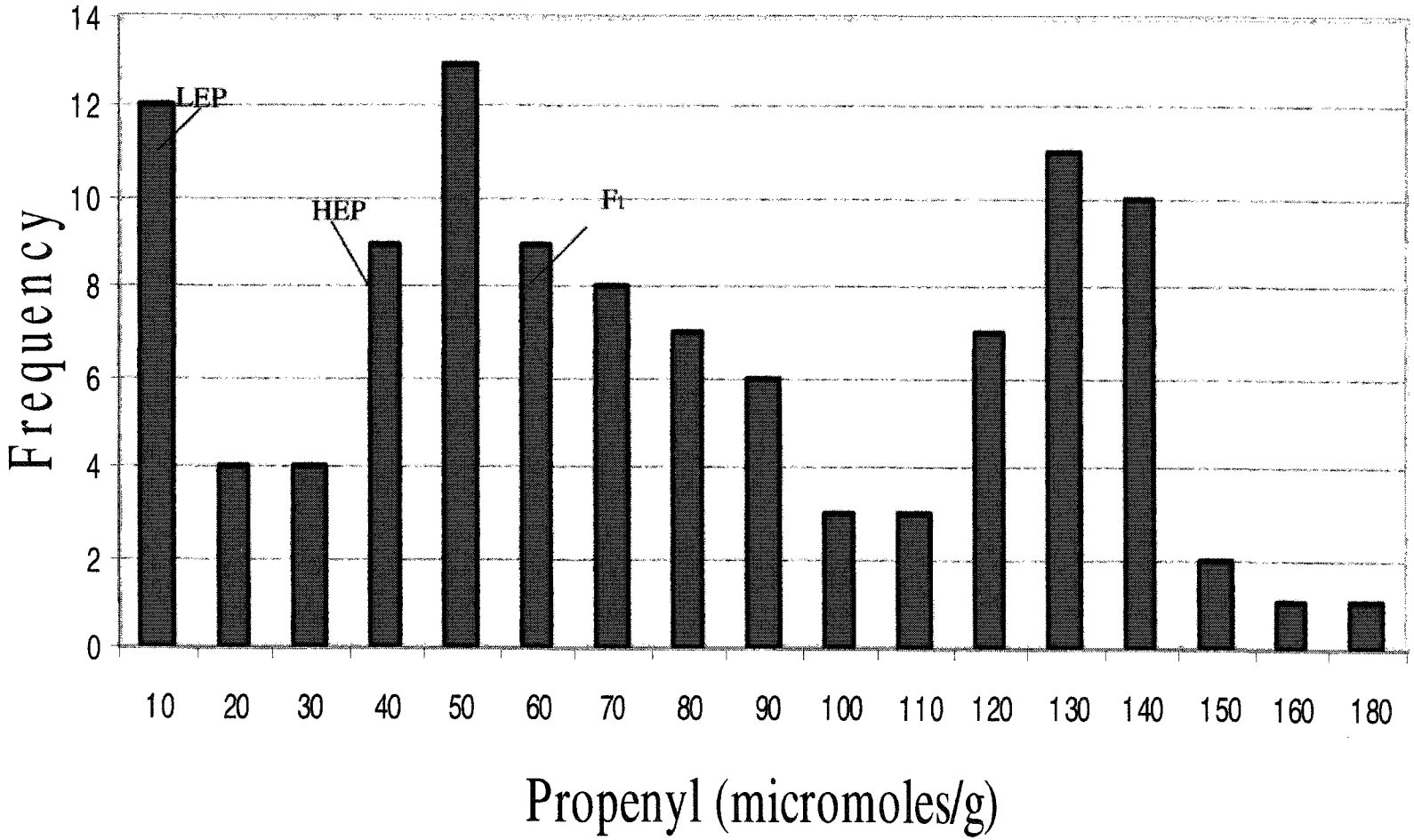

Fig. 5.4. Frequency distribution for recombinant DH lines of *B. juncea* for 2-propenyl glucosinolate. The relative positions of parents and the F_1 are indicated in the distribution.

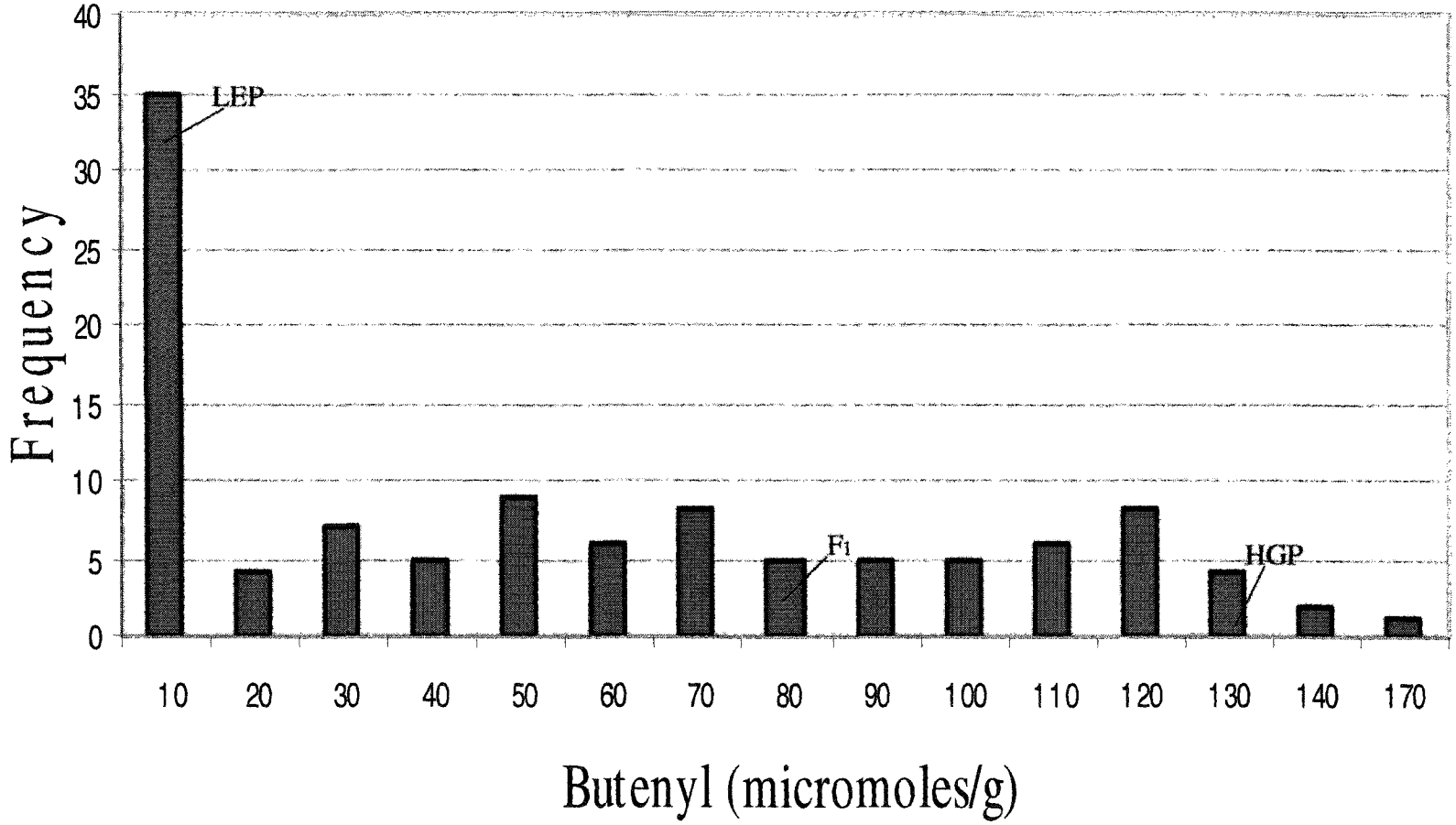

Fig. 5.5. Frequency distribution of recombinant DH lines of *B. juncea* for 3-butenyl glucosinolate. The relative positions of parents and the F_1 are indicated in the distribution.

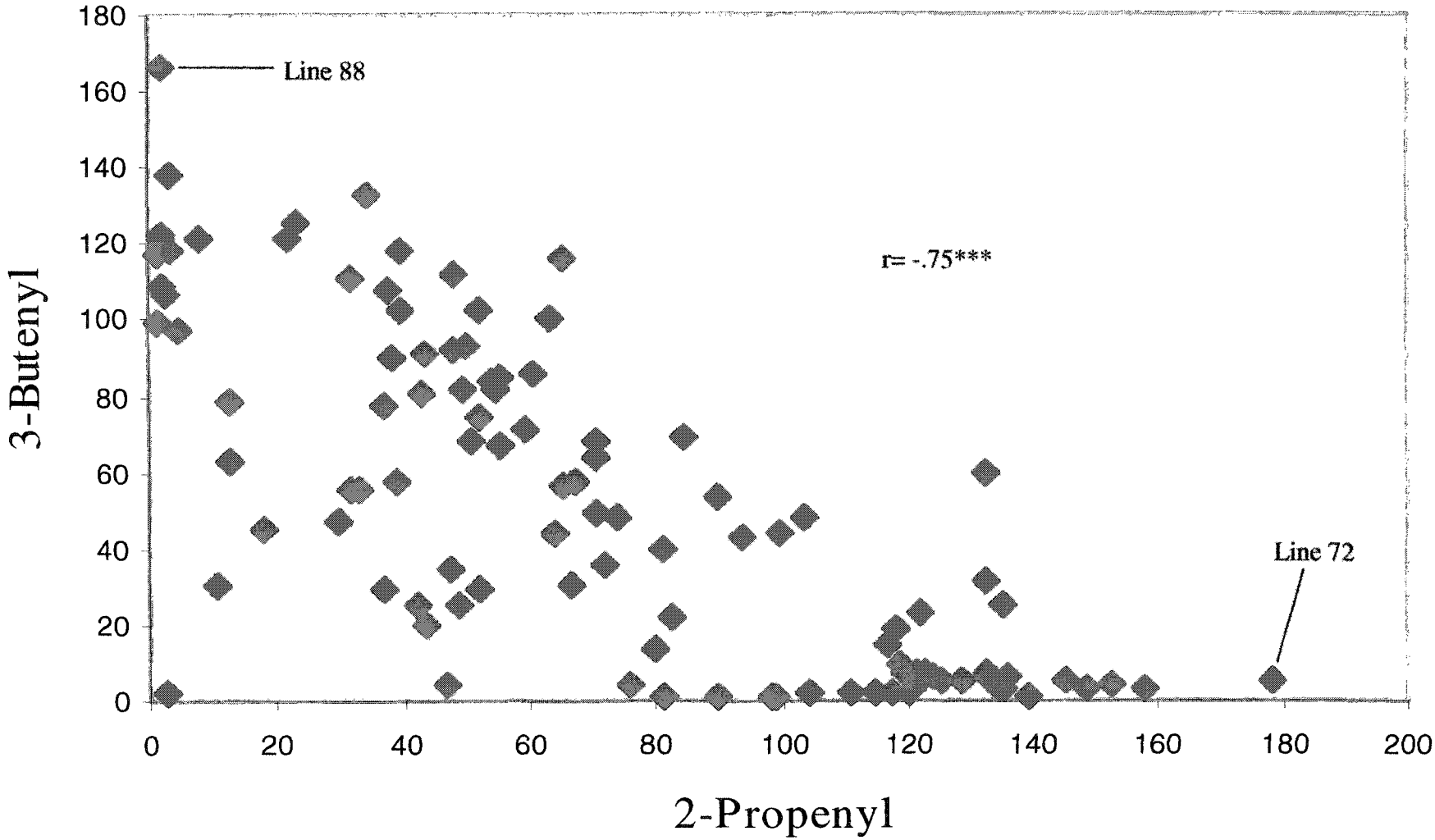

Fig. 5.6. Distribution of 3-butenyl glucosinolate (μmoles/g) at various levels of 2-propenyl glucosinolate (μmoles/g) in recombinant DH lines of *B. juncea*.

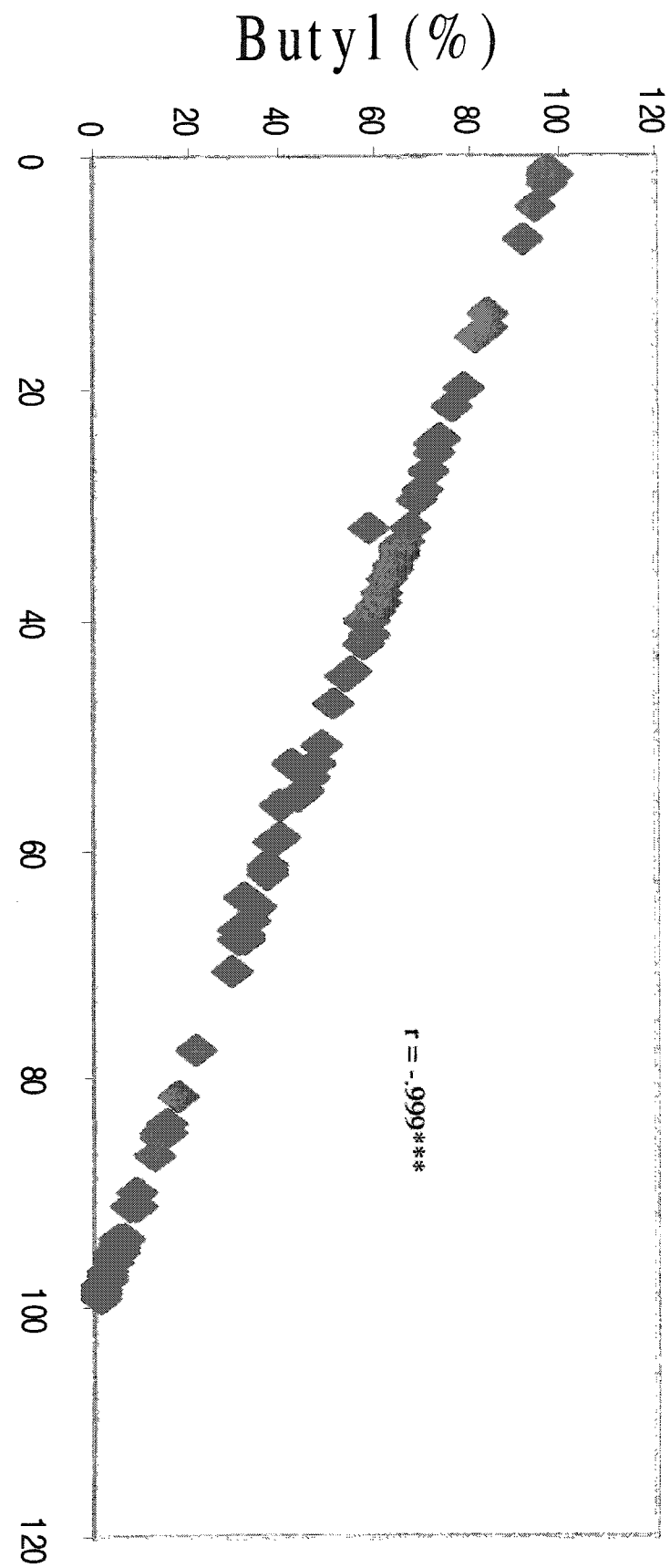

Fig. 5.7. Distribution of butyl at various levels of propyl in recombinant DH lines of *B. juncea*.

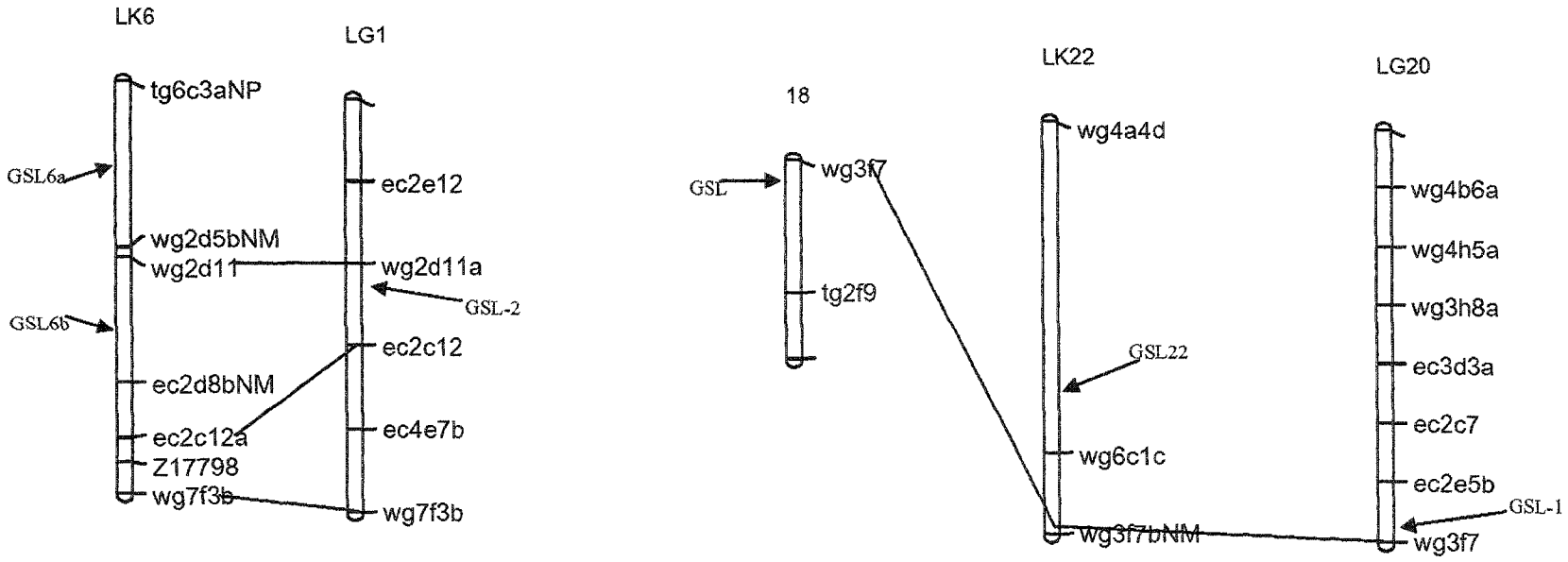

Fig. 5.8. Homology of QTLs associated with glucosinolates in the *Brassicas*.
LK = linkage group, LK22= E segment in the present study, GSL = QTLs associated with glucosinolates, LG1 = linkage group of *B. napus* (Troser et al. 1995), 18= linkage group of *B. napus* (Uzunova et al. 1995), Only loci detected by common probes are shown for the *B. napus* map.

Table 4.1. Aliphatic glucosinolate composition of seeds of parental and F1 plants (μmole/g of oil-free seed)

	Total Alkenyl	2-Propenyl	3-Butenyl	2-Hydroxy butenyl	4-Pentenyl	3-Hydroxy pentenyl	Pentyl (%)	Butyl (%)	Pentyl (%)
HEP	159.80	34.01	123.77	1.42	0.47	0.13	21.28	78.34	0.38
LEP	4.11	0.76	2.05	1.19	0.09	0.03	18.49	78.64	2.91
F_1	128.56	52.31	71.74	3.06	1.22	0.22	40.69	58.18	1.12

HEP = high-gluc parent, LEP = low-gluc parent.

Table 5.2. Genetics of QTLs for various glucosinolates in different environments in *B. juncea*.

Trait	Env	QTL	LK	Dis (cM)	Loci	LOD	σ_P^2	Tot σ_P^2	Add
TA	ERS99	GSL16	16	5	wg6h10a-ec2h2a	3.69	14.0	29.5	!-11.5
		GSL22	22	25	wg4a4d-wg6c1c	4.31	15.5		-11.62
	ERS00	GSL10	10	2.9	ec2d8cNM-wg2h1b	3.06	8.8	43.6	-12.03
		GSL11	11	5	wg3c5aNM-ec2e5c	3.95	12.1		-12.72
		GSL16	16	5	wg6h10a-ec2h2a	4.23	13.7		-14.09
		GSL22	22	20	wg4a4d-wg6c1c	3.02	9.0		-11.25
	EL00	GSL11	11	5	wg3c5aNM-ec2e5c	3.31	11.7	38.2	-11.78
		GSL16	16	5	wg6h10a-ec2h2a	3.21	11.5		-12.11
		GSL22	22	15	wg4a4d-wg6c1c	3.96	15		-13.41
	KE00	GSL11	11	0	wg3c5aNM-ec2e5e	2.55	8.2	42.3	-9.54
		GSL16	16	5	wg6h10a-ec2h2a	5.64	20.9		-15.86
		GSL22	22	20	wg4a4d-wg6c1c	3.81	13.2		-12.17
	ME	GSL12	12	5	ec3f12cNP-wg5d9d	2.62	6.7	45.1	-8.11
		GSL10	10	0	ec2d8cNM-wg2h1b	2.67	6.7		-8.61
		GSL11	11	5	wg3c5aNM-ec2e5c	3.81	10.3		-10.09
		GSL16	16	5	wg6h10a-ec2h2a	3.98	11.1		-10.99
		GSL22	22	20	wg4a4d-wg6c1c	3.74	10.3		-10.28
PE	ERS99	GSL6a	6	5	tg6c3a-tg2d5bNM	8.05	40.9	57.9	Δ26.8
		GSL16	16	8.6	wg6h10a-ec2h2a	5.60	16.9		-18.07
	ERS00	GSL6a	6	5	tg6c3a-wg2d5bNM	7.85	39.3	70.1	30.55
		GSL16	16	8.6	wg6h10a-ec2h2a	6.06	24.4		-24.06
		GSL22	22	25	wg4a4d-wg6c1c	2.46	6.4		-13.08
	EL00	GSL6a	6	5	tg6c3a-wg2d5bNM	9.31	46.3	77.8	32.99
		GSL16	16	8.6	wg6h10a-ec2h2a	6.02	22.8		-22.55
		GSL22	22	20	wg4a4d-wg6c1c	2.75	8.7		-14.12
	KE00	GSL6a	6	5	tg6c3a-wg2d5bNM	11.6	49.8	78.2	33.75

	GSL16	16	8.6	wg6h10a-ec2h2a	7.53	21.2					-23.42
	GSL22	22	20	wg4a4d-wg6c1c	2.63	7.2					-13.01
ME	GSL6a	6	5	tg6c3a-wg2d5bNM	10.1	40.7	64.7	29.11			
	GSL10	10	8.4	ec3f12cNP-ec2d8cNM	2.49	5.8		-11.93			
	GSL6b	6	23	wg2d1-ec2d8bNP	3.0	19.7		-39.63			
	GSL16	16	0	wg6h10a-ec2h2a	6.99	18.2		-20.41			
BE ERS99	GSL6a	6	10	tg6c3a-wg2d5bNM	9.20	37.3	57	-34.55			
	GSL6b	6	23	wg2d1-ec2d8bNP	4.90	30.8		-45.01			
ERS00	GSL6a	6	5	tg6c3a-wg2d5bNM	8.06	38.3	69.1	-39.17			
	GSL6b	6	23	wg2d1-ec2d8bNP	3.26	25.3		-43.96			
	GSL15	15	4.2	wg2g11cNP-wg3c5b	2.52	6		-12.06			
EL00	GSL6a	6	5	tg6c3a-wg2d5bNM	7.93	40.9	72.2	-38.48			
	GSL6b	6	23	wg2d1-ec2d8bNP	3.26	25.3		-43.96			
	GSL15	15	4.2	wg2g11cNP-wg3c5b	2.52	6		-12.06			
KE00	GSL6a	6	10	tg6c3a-wg2d5bNM	11	41.6	74.7	-42.91			
	GSL6b	6	23	wg2d1-ec2d8bNP	7.64	33.1		-46.51			
ME	GSL6a	6	5	tg6c3a-wg2d5bNM	7.64	35.3	63.4	-34.88			
	GSL6b	6	23	wg2d1-ec2d8bNP	2.98	22.2		-39.69			
	GSL15	15	4.2	wg2g11cNP-wg3c5b	2.69	5.9		-11.12			
PY ERS99	GSL6a	6	10	tg6c3a-wg2d5bNM	10.8	43	70.9	27.1			
	GSL6b	6	5	wg2d1-ec2d8bNP	2.84	20.6		27.69			
	GSL16	16	8.6	wg6h10a-ec2h2a	3.21	7.3		-9.14			
ERS00	GSL6a	6	10	tg6c3a-wg2d5bNM	8.86	43.5	86.6	28.71			
	GSL6b	6	5	wg2d1-ec2d8bNP	3.38	34.6		31.19			
	GSL16	16	8.6	wg6h10a-ec2h2a	3.27	8.5		-10.47			
EL00	GSL6a	6	10	tg6c3a-wg2d5bNM	9.50	43.9	83.5	28.70			
	GSL6b	6	5	wg2d1-ec2d8bNP	3.49	30.1		31.23			
	GSL16	16	8.6	wg6h10a-ec2h2a	3.87	9.3		-11.05			
KE00	GSL6a	6	10	tg6c3a-wg2d5bNM	9.68	34.7	72.9	27.9			
	GSL6b	6	5	wg2d1-ec2d8bNP	6.19	27.8		31.84			
	GSL16	16	8.6	Wg6h10a-Ec2h2a	4.56	10.4		-11.64			
ME	GSL6a	6	10	tg6c3a-wg2d5bNM	17	74	84.2	28.17			

		GSL16	16	8.6	wg6h10a-ec2h2a	3.12	7.2		-9.45
BY	ERS99	GSL6a	6	10	tg6c3a-wg2d5bNM	11.3	42.9	72.1	-26.92
		GSL6b	6	5	wg2d1-ec2d8bNP	3.27	21.8		-27.63
		GSL16	16	8.6	Wg6h10a-Ec2h2a	3.35	7.4		9.39
	ERS00	GSL6a	6	10	tg6c3a-wg2d5bNM	8.62	42.5	85.6	-27.97
		GSL6b	6	5	wg2d1-ec2d8bNP	3.30	34.2		-30.61
		GSL16	16	8.6	wg6h10a-ec2h2a	3.45	8.9		10.54
	EL00	GSL6a	6	10	tg6c3a-wg2d5bNM	9.83	44	80.7	-28.56
		GSL6b	6	5	wg2d1-ec2d8bNP	3.74	30.4		-31.05
		GSL16	16	8.6	wg6h10a-ec2h2a	3.88	6.3		10.93
	KE00	GSL6a	6	10	tg6c3a-wg2d5bNM	9.85	34.6	80.7	-28
		GSL6b	6	5	wg2d1-ec2d8bNP	6.46	27.8		-31.58
		GSL16	16	8.6	wg6h10a-ec2h2a	4.62	10.5		11.58
	ME	GSL6a	6	10	tg6c3a-wg2d5bNM	17.4	73.7	81.1	-27.82
		GSL16	16	8.6	wg6h10a-ec2h2a	3.24	7.4		9.46

TA =total aliphatic glucosinolate, PE = 2-propenyl, BE = 3-butenyl, PY = Propyl, BY= Butyl, ERS99 = Edmonton research station 1999, ERS00 = Edmonton Research Station 200, EL00 = Ellersile = 2000, KE00= Kelsey 2000, Env = environment, σ_P^2 = phenotypic variance explained by QTL, Tot = Total, Add = additive effect,! = negative additive effect means that HEP alleles increased glucosinolate at a certain QTL , Δ = positive additive effect means that LGP alleles increased glucosinolate at a certain QTL, Dis= distance of the locus from the first flanking marker, LK22= unlinkedsegment D in the *B. juncea* RFLP map.

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Chapter 6

Summary

Condiment mustard, *B. juncea*, is a non-canola quality crop, high in erucic acid and glucosinolate contents. Because of this species' superior characteristics i.e. blackleg and shattering resistance, drought tolerance and higher yield potential than either canola species (*B. napus* and *B. napus*), it is of interest to plant breeders to convert it into a canola quality crop (Downey 1990; Woods et al. 1991). The present project was initiated to develop breeding strategies using molecular markers to hasten this conversion. The project had three main components:

1) Construction of an RFLP genomic map of *B. juncea.*
2) To study the genetics of qualitative and quantitative traits, and to associate them with molecular markers.
3) To study the stability of the identified QTLs for the traits across years and locations.

6.1 Construction of an RFLP genomic map in *B. juncea*

Two RFLP maps constructed from the S and R populations (DH populations developed from the cross and its reciprocal) were homogenous, indicating that the recombination rates were independent of sex-based differences in *B. juncea*. This finding could have very important implications in developing breeding strategies for *B. juncea* (Young and Tanksley 1989). The combined map contained 18 linkage groups (probably corresponding to the n number of chromosomes in *B. juncea*), seven small segments and

16 unlinked loci. The average locus interval was approximately 5.21cM, and could be very effectively used for marker assisted selection.

It was found that the *Brassica* genome was highly duplicated, in agreement with previous studies on related species (Lagercrantz and Lydiate 1996; Song et al 1991; Slocum et al. 1990). Comparisons of the *B. juncea* map with those of *B. napus*, *B. oleracea*, and *B. rapa* revealed numerous rearrangements in approximately 50% of the cases. Rearrangements have been observed in nearly all of the *Brassica* maps published so far. The canola quality parent in the present study had a complex pedigree with introgression from *B. rapa* and other races of *B. juncea*. The non-canola parent was an introduction from India, and distantly related to the LEP. Therefore, the complex genetic makeup of the parents might have contributed to the numerous rearrangements observed in *B. juncea*.

6.2 Yield and yield contributing parameters

Sixty-five QTLs significantly affected yield and yield contributing parameters across environments. Forty percent of the QTLs could be identified in the mean environment. Inconsistencies in QTL expressions across environments have been documented (Paterson et al. 1991; Bubeck et al. 1993; Lu et al. 1996; Lee et al. 1996), however, 69% of the total QTLs associated with parameters such as pod length, 1000-grain weight, days to first flowering and number of pods per main raceme, appeared in the mean environment, thus confirming previous results that QTL x environment interactions were trait dependent (Lu et al. 1996; Lee et al. 1996). The consistent QTLs associated with these traits have been included in the selection index for indirect selection for yield.

6.3 Fatty acid profile

Five QTLs were associated with the fatty acid profile of *B. juncea* in the present study. Two of the QTLs were identical for erucic, oleic, linoleic, linolenic and eicosenoic acids, and acted in the opposite direction. The alleles from the HEP and LEP increased and decreased erucic acid content respectively. The same alleles originating from HEP (E_{1a}, E_{1b}) and LEP (e_{1a}, e_{1b}) decreased and increased respectively contents of oleic, linoleic, linolenic and eicosenoic acids. Of the additional three alleles associated with linolenic acid contents (LN_2, LN_3 LN_4), LN_3 explained up to 35% of the variation in the population, and were very stable across environments. The other two QTLs had smaller effects, and were inconsistent in different environments. The three major and consistent QTLs have been recommended for use in marker assisted selection to align the fatty profile of *B. juncea* with those of existing canola species.

6.4 Aliphatic glucosinolate profile

Species-specific aliphatic glucosinolate profiles in the *Brassicas* are well documented (Fenwick et al.1983). *B. juncea* has predominantly 2-propenyl and 3-butenyl glucosinolates. Major QTLs associated with these glucosinolates were the same (GSL6) but acted in the opposite direction. At QTL GSL6a, alleles from HEP increased and decreased 2-butenyl and 3-butenyl glucosinolates respectively, however, LEP alleles increased 3-propenyl but decreased 2-butenyl. This was further confirmed by their additive effects with opposite signs, and very strong negative correlation between 2-butenyl and 3-propenyl. The QTLs were highly consistent across environments. Because of their specific effects, these QTLs disappeared when considering total glucosinolates. Instead, minor and inconsistent QTLs appeared for total glucosinolates. Therefore, a

different breeding strategy was recommended for *B. juncea*; marker assisted selection should be based on QTLs associated with individual glucosinolates, rather than on QTLs associated with total glucosinolate, as was recommended for *B. napus* (Toroser et al. 1995; Uzunova et al. 1995).

6.5 Conclusions and future directions

Quantitative trait loci are simply statistical inferences, and have no physical basis. As demonstrated in the present study, their proper use could assist plant breeders in designing breeding strategies for marker assisted selection for simply inherited and multigenic traits. Questions remained unanswered in the present study, especially regarding differential expression of QTLs in different environments. Differential environmental environmental effectsdo not affect the expression of the QTL but only change the direction of expression, and could have serious implications in marker assisted selection. QTLs detected in the mean environment have been recommended for marker assisted selection (Veldoom and Lee 1996). The number of QTLs for any trait and their associated additive effect could be seriously affected by differential environmental effects. QTL FP12 (for flowering period) was identified across years and locations but not in the mean environment because of its differential expression in different environments. The additive effect and phenotypic variance explained by GW12 was minimal in the mean environment. At GW12, alleles from the HEP increased grain weight in the Edmonton Research Station 1999 & 2000 and Ellerslie, however, in Kelsey 200, alleles from the LEP (for GW12) increased grain weight. The genetic basis of differential environmental effects of a QTL in different environments is unclear. An

integration of physiological and biochemical approaches with QTL analysis needs to occur to exploit the potential of molecular markers for marker assisted selection.

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Appendix I. Epistatic relationships among different loci associated with yield and yield-associated traits in *B. juncea* in different environments.

ERS99		ERS00		EL00		KE00		ME	
FF	p	**FF**	p	**FF**	p	**FF**	p	**FF**	p
FF8a x PH16	0.05	FF8b X FF4	0.02			FF8a x LF8b	0.03	FF8b x SN6	0.02
FF18 x FP11	0.03	FF8b xMT8b	0.03			FF8a x FP8	0.03	FF8b x PH6	0.01
		FF8b x MT5b	0.04			FF1 x FP1	0.05	FF8a x PH6	0.04
		FF4 x LF12	0					FF2 x PL12	0.04
		FF4 x FP12	0.03					FF2 x NP12	0.04
		FF4 x GW12	0.02						
		FF4 x SN2	0						
LF		**LF**		**LF**		**LF**		**LF**	
		LF8b x FF4	0.03	LF5a x FF8a	0.04	LF8b x LF5b	0.04	LF3 x NP15	0.04
		LF12 x PL11	0.01	LF5A x FF18	0.05	LF8b xFP5b	0.04		
		LF18 x NP8	0.04	LF5a x FP18	0.05	LF5b x FP8	0.04		
				LF5a x MT18	0.05	LF5b x PL8	0.04		
				LF5a x PH6	0.01	LF5b x SN8	0.02		
				LF5a x PH18	0.05	LF18 x PL14	0.01		
				LF2 x FF8a	0.04				
				LF2 x PH6	0.03				
FP		**FP**		**FP**		**FP**			
FP12 x PL2	0.03			FP5a x NP4	0.04	FP5b x FF1	0.03		
FP12 x NP15	0.05			FP5a x PH16	0.02	FP5b x MT8a	0.04		
FP12 x PH16	0.01			FP18 x PH6	0.02	FP5b x SN3	0.04		

MT		**MT**		**MT**		**MT**		**MT**	
MT2 x FF1	0.02	MT8b x PL11	0.03	MT8a x LF5a	0.03	MT8a x MT12	0.02	MT8b x PL12	0.01
MT2 x SN8	0.02	MT5b x LF12	0.01	MT8a x NP10	0.01	MT8a x LF12	0.02	MT8b x SN6	0.03
		MT5b x FP12	0.05	MT18 x FF8a	0.01	MT8a x FP12	0.02	MT8b x NP3	0.05
				MT18 x NP3	0.04	MT8a x SN12b	0.02	MT8b x NP12	0.01
						MT8a x NP12	0.02	MT8b x GW12	0.01
						MT8a x GW12	0.02		
						MT8a x YL12	0.02		
PL		**PL**		**PL**		**PL**		**PL**	
PL9 x FF1	0.02	PL9 x PL12	0.02	PL15 x NP3	0.05	PL8 x FF1	0.01	PL9 x FF1	0.01
PL12 x NP3	0.03	PL9 x MT1	0.03			PL12 x YL13	0.01	PL9 x FP3	0.02
		PL11 x FF8b	0			PL15 x LF8b	0.02	PL12 x PL4	0.03
		PL11 x MT8b	0.01			PL15 x FP8	0.02	PL12 x NP2	0.03
		PL11 x SN8	0.01			PL15 x SN8	0.03	PL12 x NP4	0.04
		PL11 x NP8	0.03			PL14 x FF1	0.04	PL2 x NP4	0.02
		PL11 x LF8b	0.03					PL4 x NP12	0.03
SN		**SN**		**SN**		**SN**		**SN**	
SN8 x SN6	0.04	SN8 x FF8b	0.02	SN5 x LF2	0.05	SN12a x NP17	0.05	SN8 x PH4	0.02
		SN2 x MT5b	0.04	SN5 x LF12	0.04	SN12b Xnp4	0		
				SN5 x FP12	0.04				
				SN5 x PL12	0.04				
				SN5 x GW12	0.04				
				SN12a x FF18	0.02				
				SN12 x MT18	0.02				
				SN12a x FP18	0.02				

Appendix I continued

				SN12a x PH18	0.02				
NP		**NP**		**NP**		**NP**		**NP**	
NP3 x FP11	0.03	NP8 x FF18	0.01	NP10 x NP4	0.01	NP12 x NP4	0.05	NP3 x GW12	0.03
		NP8 x LF18	0.01			NP4 x FF8a	0.03	NP2 x NP4	0.01
		NP8 x PH18	0.01			NP4 x LF12	0.05	NP4 x FF2	0
		NP8 x MT1	0.04			NP4 x PL12	0.05	NP4 x PL2	0.05
		NP8 x PL9	0.04			NP4 x MT12	0.05	NP15 x PL9	0.04
						NP4 x SN12b	0.05		
						NP4 x FP12	0.05		
						NP4 x GW12	0.05		
						NP4 x YL12	0.05		
PH		**PH**		**PH**		**PH**		**PH**	
PH16 x SN16	0.04	PH6 x SN8	0.04	PH6 x PH15	0			PH6 x PL15	0.01
		PH6 x NP8	0.02	PH5 x PH18	0.03			PH6 x GW12	0.05
		PH6 x NP15	0.03	PH5 x FF18	0.03			PH6 x PL12	0.02
		PH18 x FP3	0.01	PH5 x LF18	0.03			PH4 x NP12	0.02
				PH5 x MT18	0.03			PH18 x LF3	0.04
				PH5 x PL12	0.02				
				PH5 x SN12a	0.03				
				PH4 x SN12a	0.02				
				PH4 x NP10	0.02				
				PH18 x PL15	0.03				
GW		**GW**		**GW**		**GW**			
GW12 x NP15	0.03	GW12 x FF4	0.04			GW12 x NP17	0.05		
YL		**YL**		**YL**		**YL**			

YL13 x PL8	0.01
YL13 x SN8	0.04
YL13 x NP8	0.02
YL11 x SN8	0.04

p=proability.

Appendix I continued