Nurhasanah

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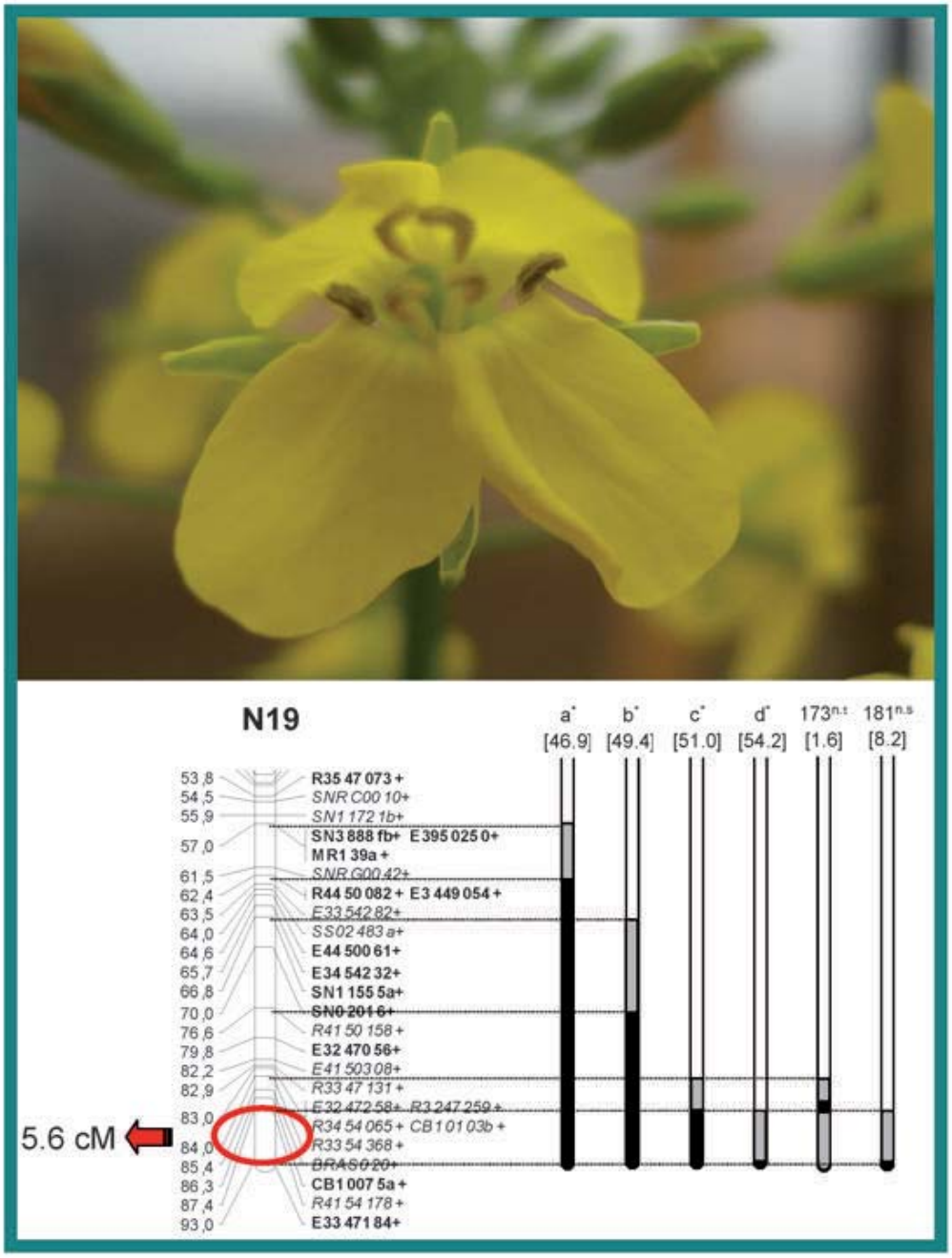

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QTL Mapping using Intervarietal Substitution Lines in Rapeseed (*Brassica napus* L.)

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presented by

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Dedicated to:

My beloved father Baharuddin and mother Sri Salmiah

Contents

1. Introduction

1.1. The importance of rapeseed

Rapeseed, *Brassica napus* L., is an amphidiploid species that belongs to the family *Brassicaceae*. *B. napus* (2n=38, AACC) has been formed by a spontaneous hybridization between *B. rapa* (2*n*=20, AA) and *B. oleracea* (2*n*=18, CC) (Prakash and Hinata 1980). It consists of spring and winter forms that are distinguished by their vernalization requirement. The winter type *B. napus* is the main oilseed crop in most of Europe and parts of China. The spring type *B. napus* is mainly grown in Canada, northern Europe, and China (Raymer 2002).

Rapeseed is utilized to produce vegetable oil for human consumption, biodiesel and for industrial purposes; the meal is used as animal feed. Rapeseed is the second largest oilseed crop world wide after soybean (USDA 2010). With a production of 60.4 million tons, it supplies about 15% of world oilseed. In Europe, rapeseed is the most important oilseed crop followed by sunflower and soybean. Germany is currently the leading producer of rapeseed in Europe, and the fourth in the world after Canada, China and India (FAO, 2008).

In the last four decades the improvement of the rapeseed quality has led to canola quality cultivars containing zero erucic acid content in the oil and low glucosinolate content in the meal, also known as double zero cultivar. The quality improvement of double zero rapeseed cultivars convert rapeseed into high quality source for human consumption's oil and livestock feed.

Rapeseed oil for human consumption with canola quality contains less than 2% erucic acid (22:1) whereas natural rapeseed oil contains about 50%. In addition, commodity canola oil contains 5% to 8% saturated fatty acids, 60% to 65% monounsaturated fatty acids, and 30% to 35% polyunsaturated fatty acids (Raymer 2002).

For industrial purposes, oilseed rape as renewable raw material is used in the plastic film manufacture, in the synthesis of nylon and in the lubricant and emollient industries (Leonard 1994; Murphy 1996). The term "industrial rapeseed" traditionally referred to any rapeseed variety producing oil with erucic acid content higher than about 45% (Piazza and Foglia 2001).

Rapeseed is the world's second source of protein meal after soybean (FAO 2008; USDA 2010). After the processing of its oil, the by-product, the meal is used as animal feed due to its high protein content. For livestock feed, rapeseed should contain low levels of glucosinolates with less than 30 μmol/g in its meal.

Its significant economic value and broad utilization makes rapeseed as one of the most important crops in the world. Therefore, many plant breeding research projects are aimed to improve the understanding of the genetics and inheritance of desired characters in rapeseed and to identify the genes contributing significantly to the variation of the traits.

1.2. The genetic background of important traits in rapeseed

Glucosinolate and erucic acid are two important traits in rapeseed for the characterization of its seed as canola quality. Therefore many investigations have been focused on these characters. A number of studies have been carried out with different crosses of *B*. *napus* to identify QTL for seed glucosinolate content. Three to four major QTL were described involved in the genetic control of total seed glucosinolate accumulation (Uzunova et al. 1995; Toroser et al. 1995; Gül 2002; Howell et al. 2003; Quijada et al. 2006).

It is well studied that erucic acid content in *B*. *napus* is controlled by two genes acting in additive manner with no dominance involved (Harvey and Downey 1964). The inheritance of this two-gene-pair systems fall into segregation of zero, intermediate and high erucic acid content (Kondra and Stefansson 1965). In molecular markers studies, it was verified that the two genes are located on linkage groups N8 and N13 (Ecke et al. 1996; Thormann et al. 1996; Jourdren et al. 1996a; Fourmann et al. 1998; Peleman et al. 2005; Qiu et al. 2006) corresponding to the two *FAE1* genes encoding the enzyme responsible for fatty acid elongation from oleic acid (C18:1) to erucic acid (C22:1).

High seed oil and meal protein content are the most important quality objectives in *Brassica* oilseed breeding programs. As complex quantitative traits, many genes are involved in the variation of these traits with mainly additive and epistatic gene action (Zhao et al. 2006). Oil is known to be negatively correlated with protein content and their QTL exhibit pleiotropic effect (Gül et al. 2002; Mahmood et al. 2006; Zhao et al. 2006). It is known that oil and protein share basic resources in the metabolic pathways (Röbbelen and Thies 1980).

Fatty acids composition is the important character determining quality of canola oil. The increasing level of monounsaturated oleic acid (C18:1) content in rapeseed oil is of interest correlated to higher oxidative stability. On the other hand, low level of polyunsaturated fatty acids such as linoleic (C18:2) and linolenic acid (C18:3) are desirable because they are easily oxidized to cause off-flavour and rancidity to oil resulting in a shortened shelf life. The genetic loci controlling these traits correspond to desaturase genes. It has been well known that the *fatty acid desaturase 2* (*fad2*) gene encodes the enzyme responsible for the desaturation of oleic to linoleic acid, (Tanhuanpää et al. 1998; Schierholt et al. 2000) and the *fatty acid desaturase 3* (*fad3*) gene is responsible for the desaturation of linoleic into linolenic content (Jourdren et al. 1996b; Tanhuanpää and Schulman 2002; Hu et al. 2006).

Recently, interest is grown on phytosterol and sinapate ester content. After corn oil, rapeseed oil is the second highest natural source of phytosterols providing up to 1% of the crude rapeseed oil (Piironen et al. 2000). Phytosterols are very important for human consumptions because of their benefit in reducing cholesterol absorption (Best et al. 1954; Nissinen et al. 2002; Trautwein et al. 2003). Contrary to phytosterol content, the reduction of sinapate esters content is a substantial requirement for establishing rapeseed as a protein crop, since sinapate esters are described as principal antinutritive factor in rape seeds contributing to the bitter taste, dark colour and low nutritive value of the meal (Shahidi and Naczk 1992). Studies on the genetic variability showed a large natural genetic variation in sinapate ester content (Velasco and Möllers 1998; Zum Felde et al. 2006). However not many investigations about QTL mapping for phytosterol and sinapate esters content in rapeseed has been conducted. Amar et al. (2008) identified three QTL for phytosterol content and four QTL for sinapate esters content. Two QTL with the strongest additive effects for phytosterol and sinapate esters were mapped within the confidence intervals of the two erucic acid genes. It is suggested that there is a pleiotropic effect of the two erucic acid genes on phytosterol and sinapate ester content.

The preference of using hybrid varieties is increasing due to the superior performance of F_1 hybrids than its parents based on heterosis. Plant height gain is one of the hybrid characters due to the hybrid vigour which leads to high risk of lodging at late stages of rapeseed development. Susceptibility to lodging is a serious problem in hybrid varieties which can contribute to yield loss (Islam and Evans 1994). The development of semi-dwarf rapeseed hybrids marks a further advance in improving rapeseed (Foisset et al. 1995;

Barret et al. 1998; Muangproom et al. 2006). Therefore the identification of QTL contributing in plant height reduction is a specific interesting in breeding programs correlated to the lodging problem.

1.3. QTL mapping

Most agronomically important characters of crops are inherited quantitatively. They exhibit continuous variation, because they are typically controlled by many genes and usually termed quantitative trait loci (QTL). Quantitative characters have been a major area of genetic study for over a century because they are a common feature of natural variation in populations and typically of commercially important traits in crop plants.

An important development during the last decades in quantitative genetics was the ability to identify genome regions responsible for variation of a trait with molecular markers (Paterson et al. 1988). The development of molecular markers has allowed the construction of dense genetic maps and lead to the development of new approaches in QTL mapping (Lander and Botstein 1989).

QTL mapping involves the development of mapping population, genetic marker assays, evaluation of traits of interest and making inferences of the QTL based on associations between genetic markers and traits (Collard et al. 2005). The QTL mapping is affected by the heritability of a trait, the total number of QTL affecting the trait, the distribution of QTL in the genome, interaction between QTL, variation due to environment, type and size of the population used for mapping, genome size and marker density (Liu 1998).

Common types of populations used in QTL mapping are segregating populations such as F_2, backcross (BC), F_1-doubled haploid (DH) and recombinant inbred lines (RILs) populations. QTL mapping using segregating populations have several limitations in the accuracy of identifying and localization a QTL. The major limitation is its low-resolution power. QTL mapping in segregating populations usually gives a rough estimate or even a bias of QTL positions and QTL effects (Melchinger 1998; Utz et al. 2000; Monforte et al. 2001; Burns et al. 2003; Collard et al., 2005), depending on the size of the segregating population, the total variance of the character analysed and the QTL effect. Confidence intervals for QTL positions have been estimated to be in the range of several ten cM (Van Ooijen 1992; Darvasi et al. 1993; Hyne et al. 1995; Kearsey and Farquhar 1998). These populations are also unsuitable for a fine mapping of QTL, because it is very difficult to reduce the confidence intervals to much less than 10 cM even for a QTL with large effects.

1.4. Intervarietal substitution lines

Intervarietal substitution lines having one or a few defined segments of a donor genome in a common genetic background of a recurrent parent can be used to search the genome for donor alleles affecting traits. A complementary set of substitution lines ideally represents the whole donor genome divided into a limited number of distinct segments, each carried by a different line.

In general, the development scheme of a set of intervarietal substitution lines can be illustrated as in Figure 1. A set of intervarietal substitution lines is developed via several generations of backcrossing with selection using marker assisted selection for the final isolation of a single segment of donor genome in the genetic background of a recurrent parent (Eshed and Zamir 1994; 1995; Howell et al. 1996; Burns et al. 2003).

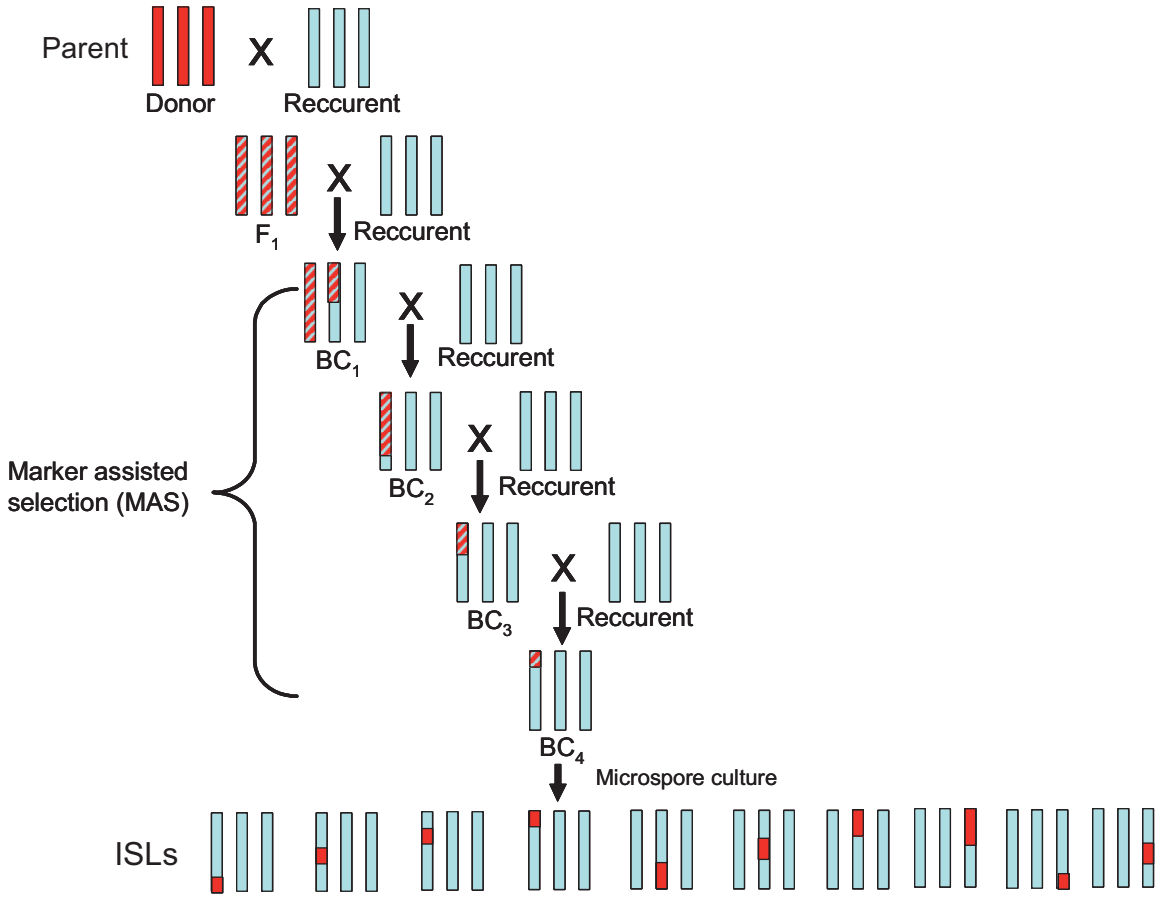

Figure 1 An illustration of the development scheme of intervarietal substitution lines (ISLs). ISLs are developed through several backcross generations. Using marker assisted selection, lines carrying a complementary set of donor segments are selected. Donor: donor parent; Reccurent: recurrent parent; F_1: the first filial; BC: backcrossing.

An ideal intervarietal substitution lines has been successfully developed in tomato by Eshed and Zamir (1994; 1995). They called the population as “introgression lines (ILs)”. The ILs contain a single homozygous chromosome segment of a wild species of tomato *Lycopersicon pennelii* in the genetic background of an elite cultivar *L. esculentum*. The set of the ILs represent a complete of the wild species genome as donor parent.

The term “introgression” refers to the introgressed of the wild species genome segments in the lines which are nearly isogenic to the recipient genotype. The same term was also used by Tian et al. (2006) in rice. They constructed introgression lines carrying wild rice (*Oryza rufipogon* Griff.) segments in the genetic background of cultivated rice (*O. sativa* L.). The ILs contained donor segments ranging from 0-8 and represented 67.5% of *O. rufipogon* genome.

Other populations which are similar to intervarietal substitution lines have been reported earlier. An advanced backcross (AB) population was performed to simultaneously identify and transfer valuable alleles from donor genotype into the genomes of elite cultivars (Tanksley and Nelson 1996 and Tanksley et al. 1996). By several backcrosses, the valuable wild QTL are isolated and introduced to an adapted parent eliminating much of the wild genetic background. From an AB population, near isogenic lines (NILs) can be isolated for the QTL of interest by further backcrossing and selfing. Through an advanced backcross QTL analysis (AB-QTL analysis) method from a cross between an unadapted germplasm and an elite cultivar, a near isogenic lines (NILs) population carrying small introgressions of donor parent segments is generated for the discovery and mapping of valuable donor QTL alleles in some crops such as tomato (Tanksley et al. 1996; Monforte et al. 2001; Brouwer and Clair 2004), maize (Szalma et al 2007), and rice (Shen et al. 2001, Xie et al. 2006).

The difference between NILs and intervarietal substitution lines is that intervarietal substitution lines should cover the whole donor genome without selection for favourable genes or specific genome region, while NILs do not necessarily cover the whole donor genome and could be constructed for specific regions of the genome declared contain a QTL, to introgress gene of interest to produce a better cultivar.

Recombinant chromosome substitution lines (RCSLs) in barley (Matus et al. 2003), chromosome segment substitution lines (CSSLs) (Ebitani et al. 2005; Hao et al. 2009) and single-segment substitution lines (SSSLs) (Xi et al. 2006) in rice are also identical populations to substitution lines, containing defined chromosome segment of a donor

genotype in the genetic background of recurrent line. These populations allowed detailed and reliable QTL analyses to be performed.

1.5. QTL mapping using intervarietal substitution lines

In the study of complex traits, substitution lines were suggested as efficient materials to estimate QTL effects more precisely (Eshed and Zamir 1994 and 1995; Hyne et al. 1995; Howell et al. 1996; Ramsay et al. 1996; Monforte and Tanksley 2000). Interactions between donor alleles in substitution lines are limited to those between genes on the same homozygous substituted segment, simplifying calculations of the significance and magnitude of the mean effects of each segment (Eshed and Zamir 1995; Howell et al. 1996).

In the ISLs the entire donor genome can be represented by relatively few substitution lines, therefore this population offers the opportunity for large scale replication, increasing the power of detection for QTL. In addition, individual lines can be analysed independently from the whole set. Furthermore, with overlapping donor segments in different substitution lines and their respective phenotypic values, QTL position can be narrowed down to a few centimorgan, allowing a higher precision of QTL localization for fine mapping (Eshed and Zamir 1995; Rae et al.1999; Burns et al. 2003).

The use of intervarietal substitution lines that has successfully mapped QTL in relatively small intervals have been reported earlier. Eshed and Zamir (1995), using an introgression line population of *Lycopersicon pennellii* segments in the genetic background of cultivated tomato mapped QTL for fruit mass. The QTL region carrying a single QTL that is overlapped by two introgressions, can be resolved into three linked QTL and mapped to the interval of 3.2 cM, 3.7 cM and 14.1 cM. Rae et al. (1999) mapped QTL for flowering time in *Brassica oleracea* using substitution lines in range of interval from 8 to 43 cM which were considerably smaller compared to using a segregating population resulting in confidence intervals of 18 to 52 cM. Burns et al. (2003) revealed potential QTL with interval values less than 10 cM in substitution lines of *Brassica napus* for seed oil and fatty acid composition.

1.6. General Objective

The main objectives of this study were:

1. To develop a set of intervarietal substitution lines (ISL) from the cross of 'Express' x resynthesized line 'RS239'.
2. To map QTL for some agronomically important traits in two sets of ISL populations developed from the cross of 'Mansholt' x 'Samourai' and 'Express' x resynthesized line 'RS239'.
3. To compare QTL results mapped in the ISL population with existing QTL that had been mapped in F_1DH population developed from the cross 'Mansholt' x 'Samourai'.

The first set of ISLs was developed from a cross between two winter rapeseed varieties 'Mansholt' and 'Samourai' ('MxS'). It was considered that a cross between two winter rapeseed varieties is especially suitable for the identification of QTL for agronomically important traits that still show allelic diversity. A segregating population (F_1DH) from the cross 'MxS' had been used to map QTL for a number of agronomically important traits. The investigation of these QTL in an advanced generation such an ISL population will allow a much better characterization of the QTL and their effect.

A cross between 'Express', a current canola quality cultivar, and a resynthesized line 'RS239' ('ExRS239'), was used to develop the second set of ISL. *B. napus* lines resynthesized from its diploid ancestors, *B. rapa* and *B. oleracea*, show a high degree of polymorphism to conventional rapeseed phenotypically and in marker analyses (Becker et al. 1995), indicating a high degree of allelic differences. By transferring traits from an exotic genotype to an elite cultivar in which has a wide genetic distance is considered could detect valuable alleles for specific traits carried by the resynthesized line which are useful for rapeseed breeding.

2. Materials and Methods

2.1. Plant materials

2.1.1. Mapping population

Ninety-three doubled-haploid lines were developed from F_1 plants (F_1DH) generated from a cross between the winter rapeseed variety 'Express' and the spring type resynthesized rapeseed line 'RS239' ('ExRS239'). The doubled-haploid lines were developed by the breeding company NPZ-Lembke®, Germany. These lines will be used as a population to develop a new genetic linkage map in the cross 'ExRS239'.

2.1.2. ISL populations

2.1.2.1. A cross between 'Express' and the resynthesized line 'RS239' ('ExRS239')

The plant material consisted of 51 BC_4 (backcross fourth generation) plants developed by the previous researchers in Dr. Ecke's research group. The plants have been used to develop a set of intervarietal substitution lines (ISL) by microspore culture and cholchicine treatment/re-treatment.

In the development of the ISLs, 'RS239' was used as donor parent and 'Express 617' as recurrent parent. 'RS239' was developed through an interspecific hybridization of *B. rapa* (Yellow Sarson) and *B. oleracea* (cauliflower). 'Express 617' is an inbred line of the winter line cultivar 'Express' known as a high yielding variety of canola quality, with good *phoma* tolerance and high seed oil content that was released by NPZ-Lembke® in 1993.

The development scheme of the ISLs is shown in Figure 2. Brief information about the development of the ISL (Kebede 2007, Ecke pers. communications) is explained below:
An F_1 line was crossed to the recurrent parent to produce BC_1 plants. Ninety BC_1 plants were genotyped using 23 AFLP primer combinations producing a genetic map of 1327.1 cM with 220 markers distributed on 22 linkage groups. This genetic map was used for marker assisted selection.

***Selection in BC_1*:** Ten BC_1 plants were selected containing donor segments covering the whole genome of the donor parent. The selected lines then were crossed to the recurrent parent to generate the BC_2 generation.

***Selection in BC_2*:** Twenty BC_2 plants were selected with donor segments covering between 161.5 and 370.8 cM of the donor genome in individual plants. These selected plants contained 6-14 donor segments with a mean of 10 donor segments.

Selection in BC_3: Eighteen BC_3 plants carrying less or equal to four donor segments were selected. The segments in these plants together cover a minimum of 60% of the mapped rapeseed genome.

Selection in BC_4: A total of 27 BC_4 genotypes were selected having less than 4 donor segments. In addition, 24 BC_4 genotypes were also selected as reserve carrying more targeted donor segments than in the 27 originally selected plants.

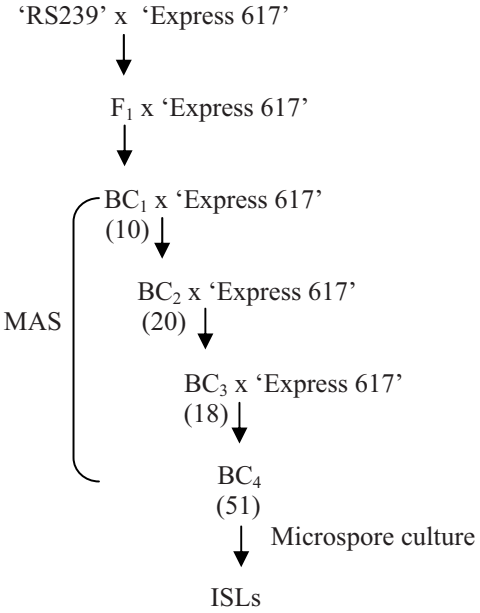

Figure 2 Breeding scheme of the development of ISL. Numbers in the parenthesis are number of plants selected in every backcross generation.

2.1.2.2. A cross between 'Mansholt' x 'Samourai' ('MxS')

A set of ISL consisted of 270 lines was used as plant material. The ISLs were developed by the previous researchers in Dr. Ecke's researchs group. The ISLs were generated from a cross between doubled haploid lines of the two winter rapeseed varieties, 'Mansholt' and 'Samourai'. 'Mansholt's Hamburger Raps', an old Dutch cultivar characterized by high seed glucosinolate and erucic acid content, was chosen as donor parent. The recurrent parent is the French cultivar 'Samourai', a canola quality variety with low seed glucosinolate and erucic acid content.

The development scheme of the ISLs is shown in Figure 3. A segregating population (F_1DH) of 151 lines had been used to develop the genetic map and to localize QTL for seed quality traits (Uzunova et al. 1994; Ecke et al. 1995; Gül 2002; Amar et al. 2008). The genetic map covers 1800 cM of the rapeseed genome using RFLP, AFLP, SSR and RAPD markers.

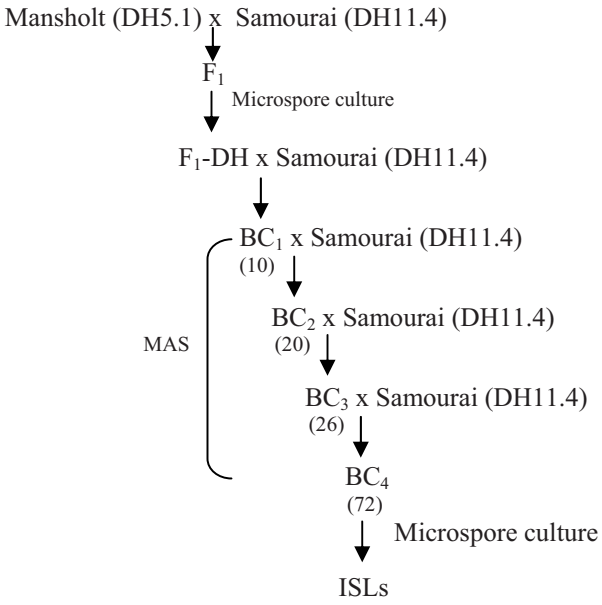

Figure 3 Breeding scheme of the development of ISL. Numbers in the parenthesis are the number of plants selected in every backcross generation.

According to Marschalek (2003), Kebede (2007) and personal communication with Dr. Ecke, the development of the ISLs is briefly explained below:

***Selection in BC_1*:** Ten F_1DH lines were selected with donor segments that together represented the whole genome of the donor parent. Those plants were crossed with the recurrent parent to generate plants equivalent to BC_1. The BC_1 plants then were crossed to the recurrent parent to generate the BC_2 generation.

***Selection in BC_2*:** Twenty out of three hundred BC_2 plants were selected using 161 AFLP, 2 SSR and 1 RAPD markers. These selected plants contained 7-18 donor segments, with a mean of 11 donor segments. The donor segments collectively covered 1.325 cM (130 loci) of the genetic map. Hereafter only the AFLP markers were used for marker assisted selection in the further development of the ISLs.

***Selection in BC_3*:** Twenty-six BC_3 plants carrying less or equal to four donor segments were selected. The donor segments in these plants cover a minimum of 658 cM of the donor genome.

***Selection in BC_4*:** A total of 72 BC_4 genotypes were selected carrying less or equal to three donor segments covering a minimum of 525 cM of the mapped rapeseed genome.

Development of ISLs: The selected BC_4 plants were the donors of microspores to generate double haploid ISLs. A total of 366 ISLs could be generated from 72 microspore donor plants, and only 270 ISLs producing enough seeds were used for further analysis.

2.2. Microspore culture and ploidy analysis

The microspore culture protocol was based on the procedure of Lichter (1982) and Möllers et al. (1994) with modifications, for details see Kebede (2007). A 0.01% colchicine solution (w/v) was applied in the first 24 h of microspore incubation. The ploidy status of the plants was analyzed after 3 weeks in the acclimatization stage using flow cytometry.

2.3. Acclimatization and vernalization

Plantlets that had already well-developed root system were transferred from in-vitro culture to a controlled-climate room to adapt to normal growth condition by removing agar medium and transferring the plantlets to multi-pot trays containing T-soil (Fruhstorfer Erde; pH 5.9). Temperature in the growth chamber for acclimatization was kept at 12^{o} C for 16 hours (h) day length, at 6^{o}C for 8 h dark for 1 month. The plants were then transferred to controlled-climate chamber at 4°C with 8 h light for vernalization for 2 months. After vernalization, the plants were re-potted to 11 cm diameter pots containing normal compost soil and grown in the greenhouse.

2.4. Colchicine re-treatment

2.4.1. Root immersion method

The root immersion method used was based on Fletcher et al. (1998). The roots of haploid plants were washed with water and immersed in a 0.34% (w/v) colchicine solution for 3 hours. Plants were placed under strong light during immersion. After treatment, the treated roots were rinsed with water and the plants were re-potted. The efficiency of the method was tested at three developmental stages of the plants: after acclimatization, after vernalization and with adult plants. For adult plants, 3 different durations of immersion were evaluated: 3 hours, 6 hours and overnight (more than 12 hours).

2.4.2. Cutting immersion method

Shoots of haploid plants with 2 leaves were obliquely cut, immersed in 0.34% (w/v) colchicine solution under strong light. Two shoots were cut from each haploid plant for two different immersion times, 3 hours and overnight. After treatment, cuttings were washed and then the wounded stem surface was treated with root hormone powder rhizopon (0.1%) containing 1-naphthaleneacetic acid (Rhizopon B. V, Rijndik, the

Netherlands) to initiate root development, and planted in soaked soil in multi-pot trays. Cuttings were not watered for 1-2 weeks and re-potted after the root system was well formed.

2.5. DNA extraction and AFLP analysis

DNA was extracted from young leaves of 2-3 weeks old plants using Nucleon PhytoPure plant extraction kits (GE Healthcare, Illustra™) according to manufactures instructions and quantified using Bio-Rad Fluorescent DNA Quantification Kit (Bio-Rad Laboratories CA, USA). For AFLP (Amplified Fragment Length Polymorphisms) analysis, DNA was digested with *Eco*RI and *Mse*I. Restriction, ligation, pre-amplification and selective amplification for AFLP analysis were performed following the multiplex protocol published by Ecke et al. 2010.

The following primer combinations were applied in AFLP analysis:

'ExRS239'

E32M47 (PET)	E32M49 (PET)	E37M50 (PET)	E32M54 (PET)	E33M54 (NED)
E33M47 (NED)	E33M49 (NED)	E39M50 (NED)	E34M54 (VIC)	E35M54 (FAM)
E34M47 (VIC)	E34M49 (VIC)	E41M50 (FAM)	E39M54 (NED)	E37M54 (PET)
E35M47 (FAM)	E45M49 (FAM)	E44M50 (VIC)	E41M54 (FAM)	E44M54 (VIC)

'MxS'

E32M47 (PET)	E32M49 (PET)	E32M59 (PET)	E40M60 (FAM)	E32M62 (PET)
E33M47 (NED)	E33M49 (NED)	E33M59 (NED)	E32M61 (PET)	E33M62 (NED)
E32M48 (PET)	E32M50 (PET)	E38M59 (FAM)	E33M61 (NED)	E35M62 (VIC)
E33M48 (NED)	E33M50 (NED)	E35M60 (VIC)	E38M61 (FAM)	E38M62 (FAM)

The *Eco*RI primers used in AFLP reactions were labelled with one of four different fluorescent dyes: (6.5) FAM (blue), VIC (green), NED (yellow) and PET (red) (Applied Biosystems, Darmstadt, Germany) (Appendix 1).

The amplification products were separated on an ABI PRISM 3100 genetic analyzer (Applied Biosystems) with GeneScan-500 LIZ size standard (Applied Biosystems). The raw data were collected using Genotyper software version 3.7 NT (Applied Biosystems). The markers were scored using Genemapper software version 3.7 (Applied Biosystems).

The AFLP markers nomenclature consisted of *Eco*RI and *Mse*I primer combination, size of amplification product, and the visible allele. As an example marker E32M47-177E scored in the cross 'ExRS239' means that the marker is originated by the primer combination between *Eco*R1 32 (E32) and *Mse*1 47 (M47), the size is 177 base pairs, and the visible allele comes from recurrent parent 'Express'.

2.6. Genetic linkage map construction

For the construction of a genetic linkage map, the F_1DH population consisting of 93 lines (see part 2.1.1) was analysed using the 20 AFLP primer combinations. The DNA was provided by the breeding company KWS. In addition, SSR marker analysis was also carried out in the population by the breeding company KWS. The AFLP markers and the SSR markers scored were applied in the mapping process for the construction of the genetic linkage map.

Linkage analyses were performed using MAPMAKER/EXP 3.0 (Lincoln et al. 1993). The linkage groups were presented using the MapChart program (Voorrips, 2002). The genetic linkage map was constructed using a Perl script that controls MAPMAKER and automates the mapping process (Ecke, personal communication) according to the principle of a high fidelity (HF) map. The HF map is a subset of markers in which all marker orders are supported by minimal log-likelihood difference of 3.0 with a maximal distance to the previous marker of 30 cM. All other markers were then placed individually relative to the markers of the HF map at their most likely position without changing the distances between the HF markers.

The fit of marker segregation ratios to the 1:1 segregation ratio expected in a doubled haploid population was tested for each marker locus by a χ_2 test ($P = 0.05$). Markers significantly deviated to 1:1 segregation ratio were marked as skewed segregation markers. Markers with strongly skewed segregation (not significantly different to 3:1 segregation ratio or even stronger) were initially excluded from the HF map construction. After the basic HF map was constructed, the strongly skewed segregation markers were then tested and mapped into the HF map, if the following criteria were fulfilled: marker was unambiguously linked to a specific linkage group and the distance to the neighbouring marker was not more than 20 cM. The marker was permanently discarded, if it was linked to more than one linkage group.

To optimize the map, each linkage group was constructed 120 times starting with the random subset of 5 highly informative according to MAPMARKER's 'order' command-markers to calculate possible variants of the HF map. The optimal variant was chosen with the following criteria: the HF map should comprise as many markers as possible, the markers in the HF map should be as evenly distributed as possible, contain as few double crossover as possible and the first marker of the full map should preferably be a marker on the HF map.

2.7. Field trials and trait analysis

Field trials were conducted at different locations with different agro-ecological conditions in Germany. The ISLs of the cross 'ExRS239' were sown in the growing season 2008/2009 in Göttingen, Einbeck, Hadmersleben, Malchow and Thuele. Two years of field experiments were conducted with the ISLs of the cross 'MxS' in year 2006/2007 and 2007/2008. The first year test was carried out in Göttingen, Seligenstadt, Grundschwalheim, Hohenlieth and Thuele. The same locations were chosen for the second year, except that Seligenstad was replaced by Einbeck.

The field trials were carried out in small plots (2.0 - 5.0 m^2) as completely randomized designs without replication. The recurrent parents were used as check and grown after every nine plots of ISLs. Field management, herbicides and fertilizer applications were done according to local practices.

Phenological characters and plant height at different stages were evaluated on the field. After harvesting, the seed quality traits were estimated. Seed quality traits were analysed in bulked open pollinated seeds using Near-Infrared Reflectance Spectroscopy (NIRS) (Tillmann 2007). The bulked open pollinated seed were harvested from the main racemes of ten different plants from the same line. All agronomical characters, method of measurement and units are presented in Table 1.

Table 1 Traits evaluated in the ISLs

Trait	Method of measurement	Unit
Beginning of flowering (BOF)	Measured as days from 1st of April to BOF. BOF was scored when 70% of the plants within the plot had the first 3 opened flowers.	[days]
End of flowering (EOF)	Measured as days from 1st of May to EOF. EOF was scored when at least 70 % of the plants within the plot had ceased to flower.	[days]
Duration of flowering (DOF)	Measured as difference between EOF and BOF	[days]
Plant height (PH) at BOF	Measured at plot level from the soil to the top of the plants at begin of flowering.	[cm]
Plant height (PH) at maturation	Measured at plot level from the soil to the top of the plants at maturation time.	[cm]
Oil content (Oil)	Measured by NIRS[1] as a percentage of seed dry matter content at 9% of moisture	[%]
Protein content (Pro)	Measured by NIRS as a percentage of seed dry matter content at 9% of moisture.	[%]
Glucosinolate content (GLS)	Measured by NIRS in µmol/g seeds.	[µmol/g]
Oleic acid content (C18:1)	Measured by NIRS in % of the total seed fatty acid content.	[%]
Linolenic acid content (C18:3)	Measured by NIRS in % of the total seed fatty acid content.	[%]
Erucic acid content (C22:1)	Measured by NIRS in % of the total seed fatty acid content.	[%]
Sinapate Esters content (SE)	Measured by NIRS in mg/g seeds	[mg/g]
Total Phytosterol content (Phyto)	Measured by NIRS)in mg/kg seeds	[mg/kg]

[1]NIRS: near infrared reflectance spectroscopy

2.8. Statistical analysis

The statistical analysis of the collected data was performed using PLABSTAT software (Utz 2007) for analysis of variance and heritability. For the analysis of variance of the ISLs in the cross 'ExRS239', the following model was used:

$$Y_{ij} = \mu + g_i + l_j + gl_{ij}$$

where Y_{ij} is an observation of genotype i in location $_j$; μ is the general mean; g_i is the effect of genotype i; l_j is the effect of location j; gl_{ij} is the interaction between genotype i and location j. In our case gl_{ij} included ε_{ijr} (error of obervation Y_{ij}), since r=1 (no replicate per location). Genotype and location were treated as fixed effects.

For the analysis of variance of the ISLs in the cross 'MxS', the following model was used:

$$Y_{ijk} = \mu + g_i + y_k + l_j(y_k) + gy_{ik} + gly_{ijk}$$

where Y_{ijk} is an observation of genotype i in location j and in year k; μ is the general mean; g_i is the effect of genotype i; y_k is the effect of year k; $l_j(y_k)$ is the effect of location j nested in year k; gy_{ik} is the interaction effect between genotype i and year k; gly_{ijk} is the interaction effect between genotype i, location j and year k, and included ε_{ijkr} (error of obervation Y_{ijk}), since r=1 (no replicate per location). The genotype was treated as fixed effect, whereas location and year were treated as random effects.

The Anscombe and Tukey test was used for outliers detection (Utz 2007) based on the detection of extreme residuals. The data were checked for errors for the outliers with the highest standardized residual. The analyses were then repeated with the outliers considered as missing values.

Coefficients of correlation among the traits were calculated with Pearson correlation using PLABSTAT software (Utz 2007).

2.9. QTL localization

Significant phenotypic differences between ISLs and the recurrent parent were ascribed to the introgressed segment of the donor genome. The differences were attributed to QTL within the defined introgressed region (Ramsay et al. 1996; Burns et al. 2003). Each substitution line was compared to the check (recurrent parent) with Dunnet's multiple comparison (Dunnet 1955) using STATISTICA version 8 software (StatSoft). A QTL was detected if the line was significantly different from the check.

Ideally, ISLs carry a single define donor segment in the genetic background of recurrent parent. And a complementary set of ISL ideally represents the whole donor genome divided into a limited number of distinct segments, each carried by a different line. In this study, the ISLs cannot face this ideal situation, meaning that not all donor genome is represented by the lines and many of the lines carry more than one donor segment. Therefore, to eliminate the segments irrelevant for the traits, the segments and their effects carried by the ISLs were compared. For segments, which were also present in other lines but showed no effect of the trait, it was concluded that they do not carry a QTL for the respective trait.

The minimum length of donor segment was calculated from the first to the last marker contiguously scored for the donor parent alleles. The maximum donor segment length was calculated from a previous to next locus of the recurrent parent alleles closest to the donor segment. The medium donor segment length was estimated as the mean length between maximum and minimum length. The minimum length was defined as 0 cM if only one locus was detected for donor allele and the locus beside it has alleles from recurrent parent (Figure 4).

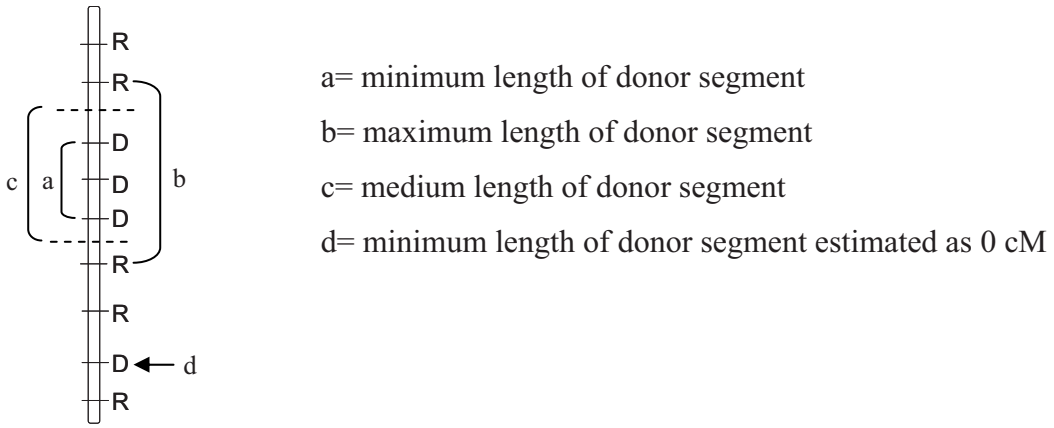

Figure 4 The estimation of donor segment length. D= marker scored for donor parent allele, R= marker scored for recurrent parent allele.

The effect of introgressed segment and additive effect was estimated using the following equation:

$$EIS = VISL - VC$$

$$AE = EIS/2$$

EIS is effect of introgressed segment, *VISL* is the mean of phenotypic value of intervarietal substitution line evaluated at all locations, *VC* is the mean value of all checks, and *AE* is additive effect. If the introgressed segment was carried by more than one ISL, then the *VISL* is the mean of phenotypic value of all the intervarietal substitution lines carrying the segment evaluated at all locations. When the donor allele increased the trait, the effect of introgressed segment and additive effect was marked with a positive sign. Positive values showed that the phenotypic value of the ISL is higher than of the check, and *vice versa*.

3. Results

3.1. Construction of a genetic linkage map in the cross of 'ExRS239'

Ninety-three doubled-haploid lines generated from F_1 plants (F_1DH) were analysed using 20 AFLP primer combinations. A total of 641 AFLP markers showing polymorphisms between the parents could be clearly scored in the 93 F_1DH lines. These were combined with data of 276 SSR markers produced by the breeding company KWS to develop a genetic linkage map. From a total of 917 markers, 724 markers comprising 484 AFLP and 240 SSR markers could be mapped and 568 genetic loci were defined on the map. The map covers 2003 cM of the rapeseed genome, organised in 23 linkage groups (Figure 5). In addition to 19 major linkage groups there were three triplets and one marker pair. There were 193 unmapped markers, consisted of 157 AFLP and 36 SSR markers. About 93% of the unmapped markers were markers with strongly skewed segregation.

The new map was aligned to an AFLP and SSR map developed in a cross between the resynthesized line 'R53' and 'Express 617' based on shared markers (Ecke et al. 2010). In the marker analysis the line 'R53' had been included. Therefore a direct comparison of the AFLP markers showing a polymorphism in the cross between 'RS239' and 'Express 617' and between 'R53' and 'Express 617' was possible. In addition, the SSR markers were aligned to established SSR linkage maps (Lowe et al. 2004; Piquemal et al. 2005; Radoev et al. 2008; Sharpe and Lydiate, unpublished data; Ecke, unpublished data).

After alignment of the map, the linkage groups could be assigned names according to the 'N' nomenclature of *B. napus* linkage groups (Parkin et al. 1995). The linkage groups represented N1 to N19 of the rapeseed genome with the exception of N16 which could not be identified (Figure 5). Linkage group 18 was split into two unlinked parts, N18a and N18b, indicating that this linkage group was not entirely covered by the markers. Four additional linkage groups namely LG20, LG21, LG22 and LG23 could not be aligned to the linkage groups of the reference maps. These four small linkage groups together covered 53.6 cM of the genome.

All of the linkage groups had markers on HF map (see part 2.6) as zero position or as the first marker (Figure 5). The linkage groups N06, N09, N13, N17 and N18b were observed where the first marker of the full map was not marker on the HF map. And the negative position of the marker refers to marker which was only placed individually relative beyond the first position (position zero) of a marker on HF map.

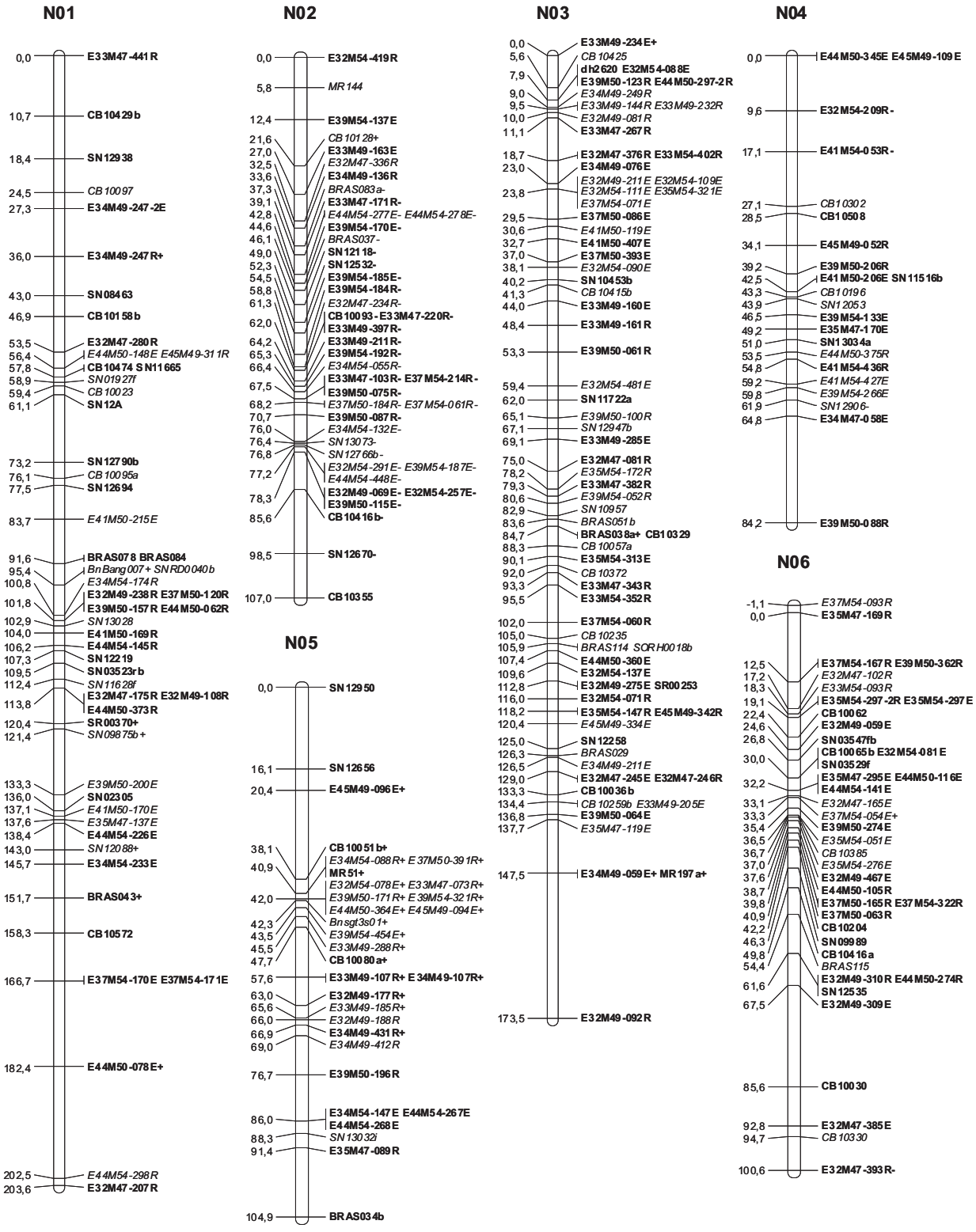

Figure 5 Genetic linkage map of *B. napus* from the cross of 'ExRS239'. Marker positions are presented in absolute positions from the beginning of the linkage groups in cM. The markers in bold are markers in the high fidelity (HF) map. Markers in italic are markers placed individually relative to the markers of the HF map at their most likely position without changing the distances between the HF markers. The markers with skewed segregations are designated with '+' if skewed towards 'Express' alleles, and '-' if the 'RS239' allele is the more frequent one. Continue to the next page.

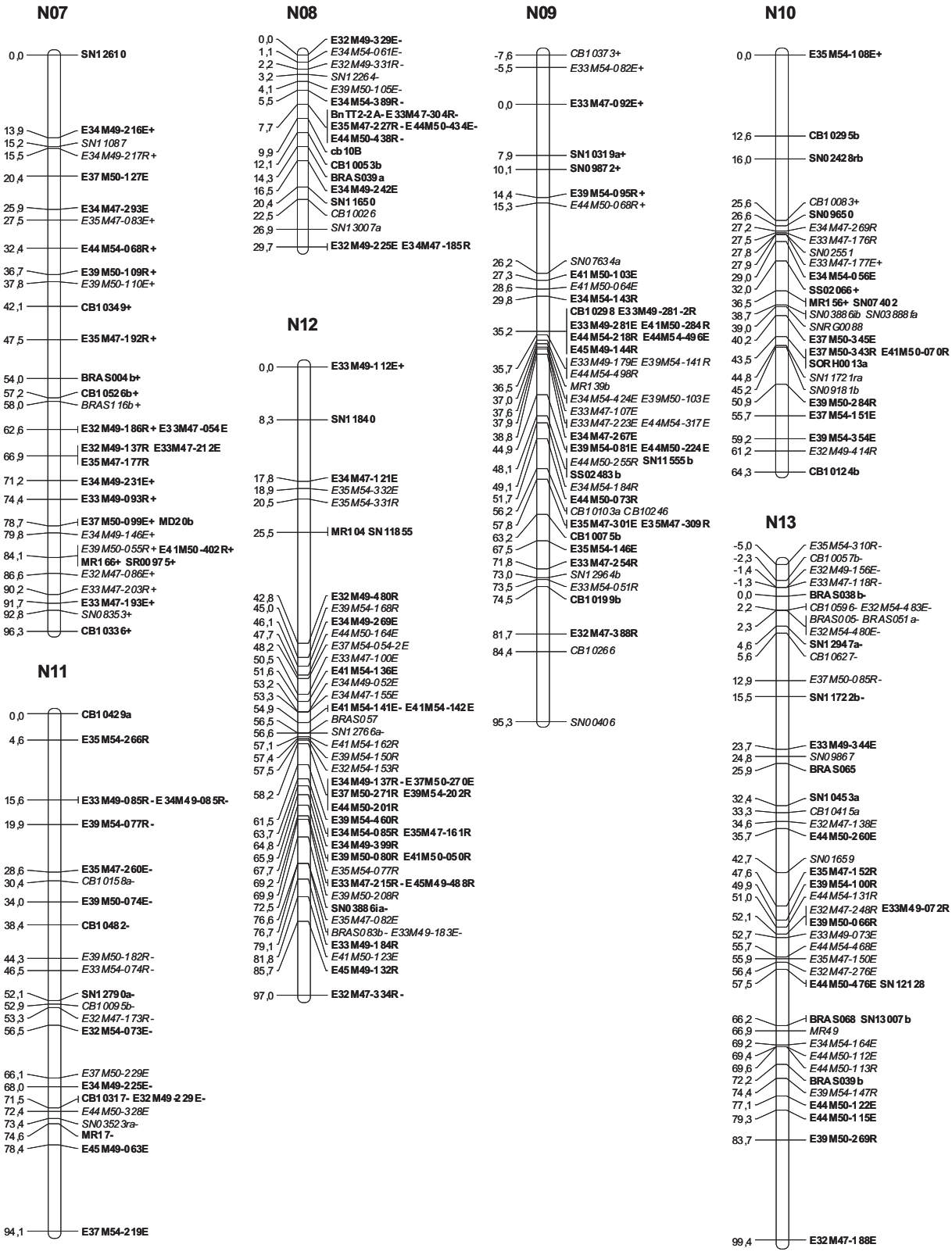

Figure 5 Continued from page 21.

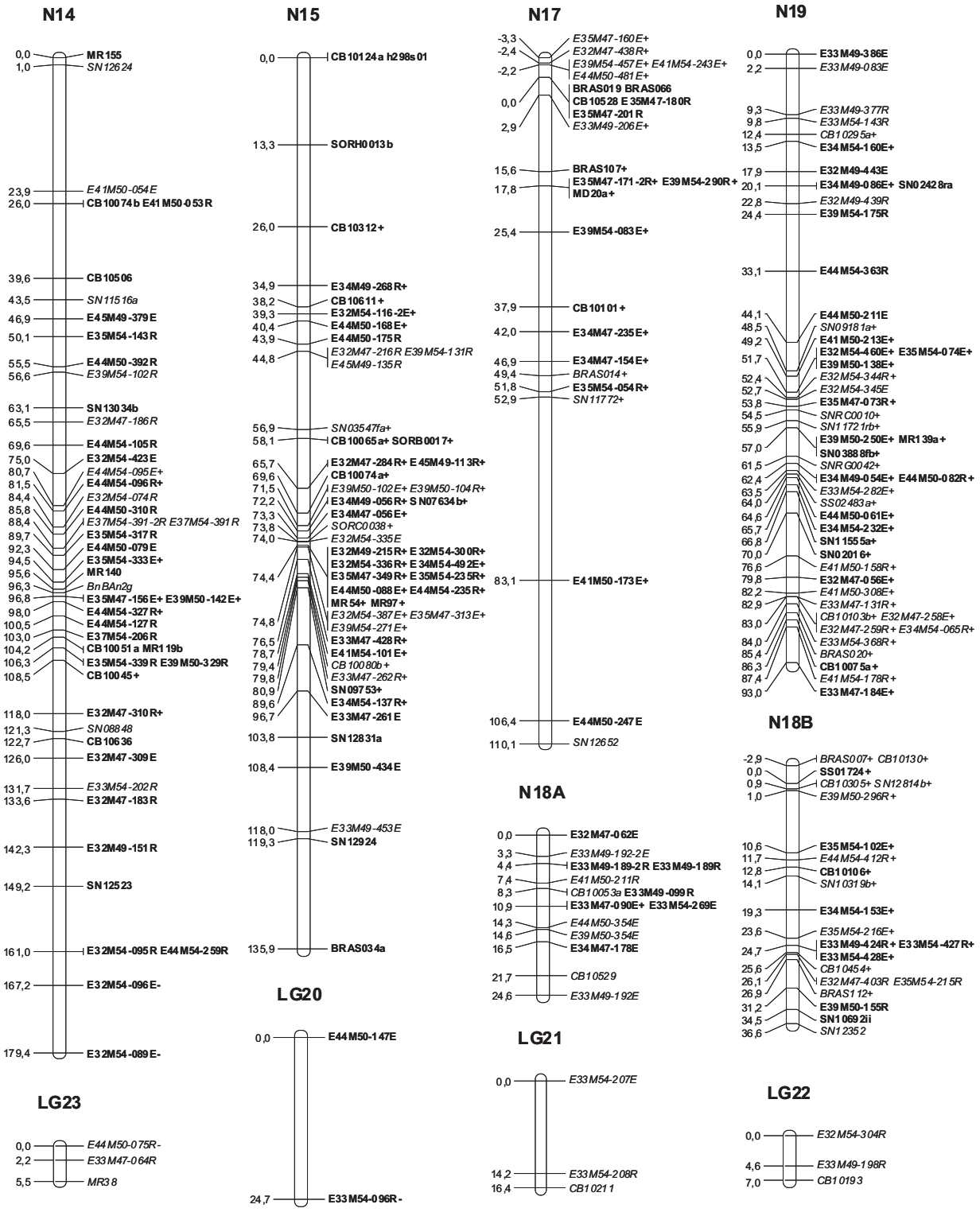

Figure 5 Continued from page 22.

About 38% of the mapped markers (281 markers) showed a significant (P=0.05) deviation from the expected 1:1 segregation ratio. The markers with skewed segregation were observed for both types of markers, the percentage of skewed AFLP markers (38.2%) was not significantly different to the SSR markers (40%). The markers with skewed segregation demonstrating an excess of 'RS239' alleles was observed in 93 markers (33.1%) while an excess of 'Express' alleles was observed in 188 markers (66.9%). The majority of the markers with distorted segregation were clustered on linkage groups N02, N05, N07, N08, N11, N15, N17, N18B, and N19.

The map has a high density of markers with an average marker density of one marker per 2.77 cM. The 19 major linkage groups had marker density ranging from 1.49 for N08 to 4.54 cM for N17. The size of the linkage group, number of markers and marker density per linkage group are presented in Table 2.

Table 2 Linkage group size, number of markers and marker density per linkage group

Linkage group	Size (cM)	Number of markers	Marker density (cM)
N01	203.6	54	3.77
N02	107.0	42	2.55
N03	173.5	72	2.41
N04	84.2	22	3.83
N05	104.9	31	3.38
N06	101.7	40	2.54
N07	96.3	34	2.83
N08	29.7	20	1.49
N09	102.9	48	2.14
N10	64.3	27	2.38
N11	94.1	24	3.92
N12	97.0	46	2.11
N13	104.4	46	2.27
N14	179.4	48	3.74
N15	135.9	50	2.72
N17	113.4	25	4.54
N18a	24.6	14	1.76
N18b	39.5	22	1.80
N19	93.0	48	1.94
LG20	24.7	2	24.7
LG21	16.4	3	5.47
LG22	7.0	3	2.33
LG23	5.5	3	1.83
Total	2003.0	724	2.77

3.2. Detection of duplicated regions in the linkage map

Using SSR primer pairs, which can amplify more than one locus, duplicated regions can be detected. A total of 40 primer pairs out of 240 (16.67%) amplified more than one polymorphic locus. These duplications showed a high level of similarity between the homoeologus A and C genomes. From 40 duplicated loci, 38 duplications were between the A and C genome that were possibly homoeologus regions in the genome. One duplication was within the A and one within the C genome. More testimony for homoeology was observed between N1 and N11, N3 and N13, N09 and N19 and between N10 and N19 (Figure 6). N1 and N11 shared 5 duplicated loci, which covered regions of about 99 and 73 cM. Seven duplicated loci were observed between N3 and N13 covering regions of about 48 and 34 cM, respectively. N19 shared four markers on the lower part of this linkage group with N09 which covered 20 and 55 cM, respectively. In addition N19 shared 5 markers in the middle region with N10, which covered 36 and 33 cM, respectively.

3.3. Development of the ISLs by doubled haploid plants production from BC_4 plants of the cross 'ExRS239'

3.3.1. Effect of colchicine treatment at microspore culture stage

There were 1200 microspore-derived plants regenerated from microspore of the 51 BC_4 donor plants. According to flow cytometry 32% of them were diploid, 49% haploid, 3% tetraploid, and 15% had an unknown status because their peaks differed from the checks with known ploidy status.

About 28% of the 1200 microspore derived plants produced seeds after colchicine treatment at the microspore stage. This number comprised 261 plants that were diploid, 20 that were tetraploid and 51 of unknown ploidy status according to flow cytometry. Since the number of doubled haploid plants producing seeds after colchicine treatment at the microspore stage was too small, haploid plants were re-treated to increase the number of fertile plants.

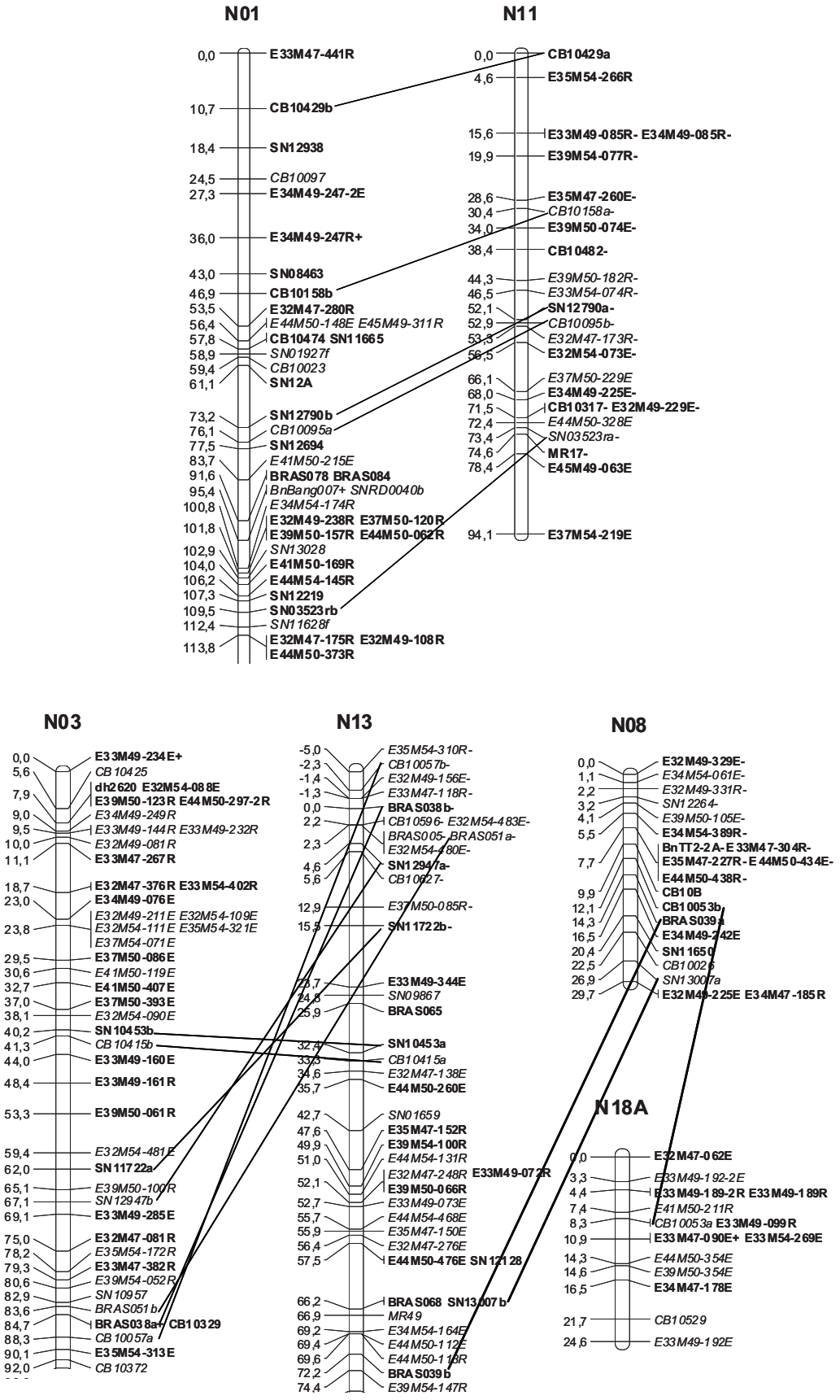

Figure 6 The duplicated regions observed in the genome of *Brassica napus*. Duplicated loci are connected by lines. Continue to the next page.

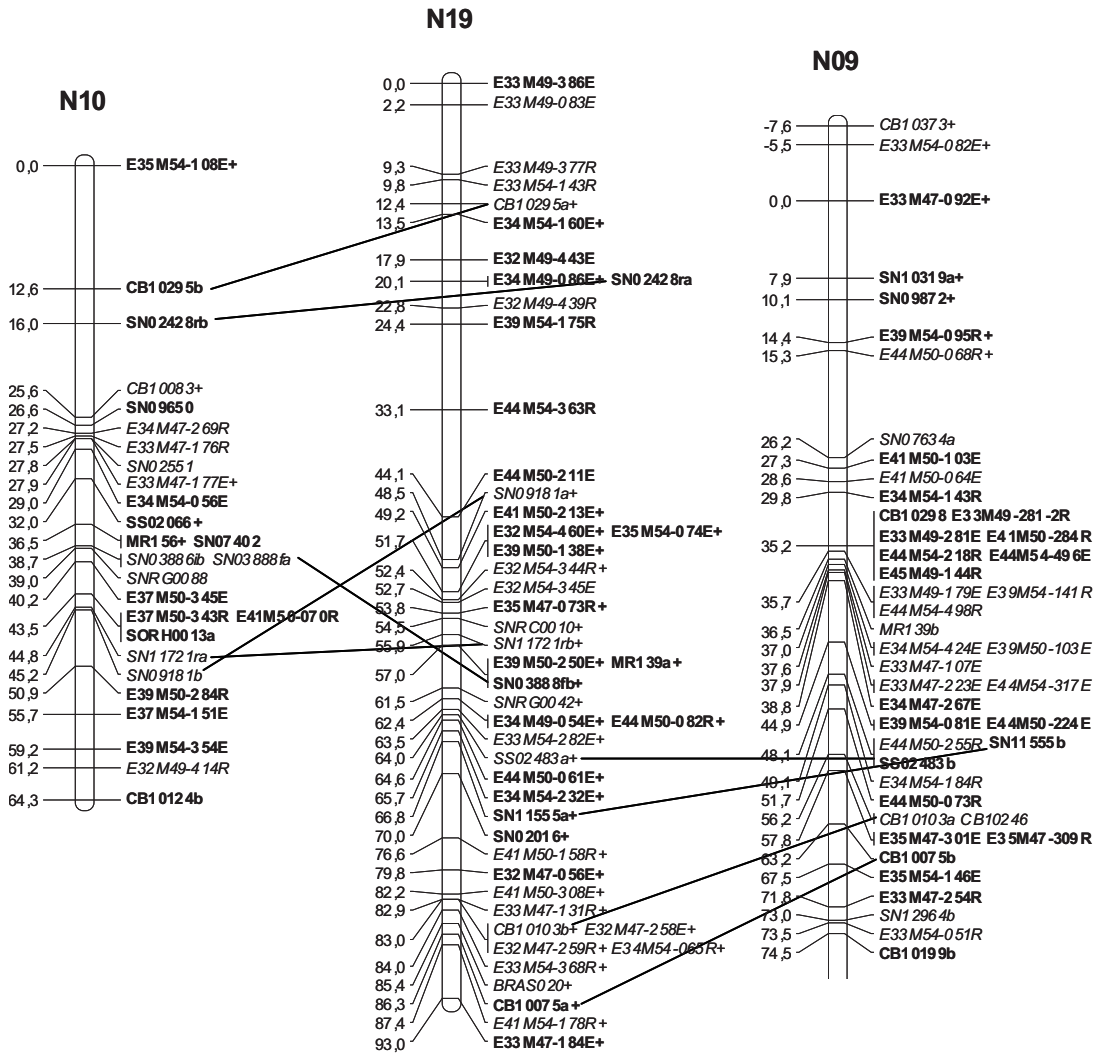

Figure 6 Continued from page 26

3.3.2. Effect of colchicine re-treatment

The success of diploidization due to colchicine re-treatment was measured as the ability of the plants treated to produce seeds. Colchicine re-treatment by root immersion of haploid plants at a young stage after determination of ploidy level at the end of the acclimatization period revealed a higher diploidization rate than after vernalization or with adult plants for the same method and duration of colchicine re-treatment (Table 3).

Table 3 Effect of different colchicine re-treatments at different developmental stages of the plants

Treatment	Developmental Stage	Duration of immersion	No. of plants treated	% of plants that produced seeds
Root immersion	After aclimatization	3 h	98	13.3
Root immersion	After vernalization	3 h	135	6.7
Root immersion	Adult plants	3 h	35	0.0
Root immersion	Adult plants	6 h	40	5.0
Root immersion	Adult plants	overnight	152	4.6
Cutting immersion	Adult plants	3 h	96	47.9
Cutting immersion	Adult plants	overnight	96	14.6

Different durations of treatment lead to different frequencies of doubled haploid plants. At the adult plant stage, 3 hours of root immersion was insufficient to induce diploidization. The extension of the immersion period to 6 hours produced 5% fertile plants, and the extension to overnight again decreased the percentage of fertile plants. Cutting immersion with 3 hours of immersion resulted in the highest diploidization rate. It decreased drastically when the immersion was extended from 3 hours to overnight.

The number of plants generated from microspore culture and the number of fertile plants obtained from the 51 microspore donor plants is shown in Figure 7. Fertile plants shown in the figure were those, which produced seeds due to colchicine treatment at microspore stage or colchicine re-treatment at other stages. The genotype played an important role in the number of doubled haploid plants obtained. In this experiment the rate of diploidization varied from 6.25% (microspore donor plants number 4.1.11) with only 1 fertile plant from 16 plants examined, to 93.33% (microspore donor plants number 4.12.13) with 42 fertile plants derived from 45 plants examined.

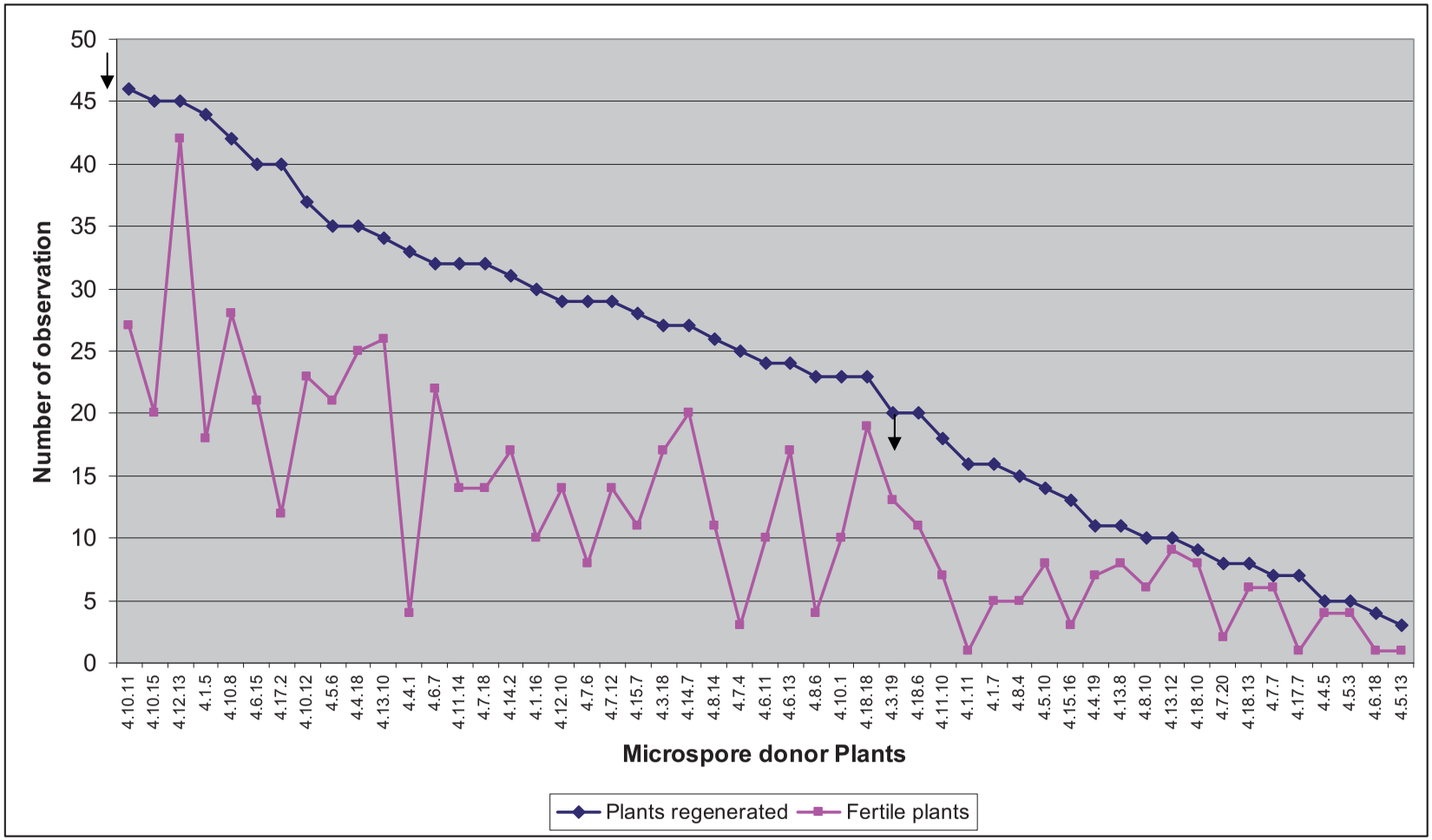

Figure 7 Variation of the numbers of regenerated and fertile plants obtained from 51 microspore donor plants. Frequency of fertile plants varied from 6.25% (line 4.1.11) to 93.33% (line 4.12.13) (arrows).

3.3.3. Number of ISLs generated

In total there were 618 ISLs generated from BC_4 plants. Three hundred fifty ISLs were selected for molecular marker analysis and field trials. In the selection process, the first concern was 15 ISLs generated from each 27 original selected BC_4 plants. If this number was not fulfilled, then ISLs generated from 24 BC_4 selected as reserve were also included.

3.4. Molecular analysis of the ISLs generated from the cross 'ExRS239'

Three hundred fifty ISLs were analysed using 20 AFLP marker combinations to identify donor segments carried by the lines. The 20 AFLP marker combinations were marker combinations used in the development of the genetic linkage map of the same cross. Five lines of 350 ISLs had unclear amplifications products and could not be scored unambiguously, the rest could be successfully analysed. The number of donor segments carried by the lines varied from 1 to 9 segments (Table 4). In twenty-two lines no donor segment was detected.

Table 4 Number of donor segments carried by the ISLs of the cross 'ExRS239'

Number of ISLs	Number of donor segment carried
22	0
56	1
87	2
61	3
37	4
46	5
20	6
10	7
4	8
2	9
Mean	2.99

The minimum, maximum and medium coverage of linkage groups by donor segments are presented in Table 5. The donor segments carried by the ISLs cover a minimum of 950.89 cM and a maximum of 1587 cM, corresponding to 52.8% and 88.1% of the mapped rapeseed genome. A lower minimum coverage of donor genome (below 30%) was observed on linkage groups N03 (20.9%), N06 (19.6%), N08 (18.5%), N17 (23.7%), N18 (3.7%) and LG20 (0%). On LG20, the donor segment was detected only on one locus (see part 2.9, Figure 4).

Table 5 Minimum, maximum and medium coverage of linkage groups (LG) by donor segments in the ISLs of the cross 'ExRS239'

LG	Length of LG in AFLP map[1] in cM	Minimum coverage[2] in cM (%)	Maximum coverage[2] in cM (%)	Medium coverage[2] in cM (%)
N01	203.6	112.1 (55.1)	182.4 (89.6)	161.0 (79.1)
N02	78.3	58.8 (75.1)	78.3 (100)	68.6 (87.6)
N03	173.5	36.4 (20.9)	110.3 (63.6)	75.0 (43.2)
N04	84.2	80.2 (95.2)	84.2 (100)	84.2 (100)
N05	71.0	33.8 (47.6)	66.4 (93.5)	50.1 (70.6)
N06	101.7	19.9 (19.6)	66.3 (65.2)	34.2 (33.6)
N07	77.8	38.4 (49.3)	72.4 (93.1)	57.9 (74.4)
N08	29.7	5.5 (18.5)	16.5 (55.6)	11.0 (37.0)
N09	87.2	32.9 (37.7)	74.8 (85.8)	53.9 (61.8)
N10	61.2	22.1 (36.1)	60.9 (99.5)	47.1 (77.0)
N11	89.5	43.0 (48.0)	78.4 (87.6)	65.9 (73.6)
N12	97.0	86.2 (88.9)	97.0 (100)	91.7 (94.5)
N13	104.4	93.1 (89.2)	99.6 (95.4)	98.2 (94.0)
N14	155.5	120.3 (77.4)	134.3 (86.4)	129.2 (83.1)
N15	83.1	40.8 (49.1)	76.2 (91.7)	59.4 (71.5)
N17	109.7	26.0 (23.7)	109.7 (100)	98.1 (89.4)
N18	24.6	0.9 (3.7)	10.9 (44.3)	9.6 (39.0)
N18	30.2	30.2 (100)	30.2 (100)	30.2 (100)
N19	93.0	49.3 (53.0)	92.5 (99.5)	87.1 (93.7)
LG20	24.7	0[*] (0)	24.7 (100)	12.4 (50.0)
LG21	14.2	14.2 (100)	14.2 (100)	14.2 (100)
LG22	4.6	4.6 (100)	4.6 (100)	4.6 (100)
LG23	2.2	2.2 (100)	2.2 (100)	2.2 (100)
Sum	1800.90	950.89 (52.8)	1587 (88.1)	1345.5 (74.7)

[1]Map without SSR markers, since the ISLs were only characterized by the AFLP markers
[2]Minimum, maximum and medium coverage of donor genome were calculated based on estimation of minimum, maximum and medium length of donor segments, respectively, see part 2.9 and Figure 4
[*]See part 2.9 and Figure 4

3.5. Phenotypic analysis of the ISLs generated from the cross 'ExRS239'

Three hundred fifty ISLs and the parental, 'Express' and resynthesized line 'RS239', were grown on the field for phenotypic data collection. The phenotypic data of donor parent 'RS239' (a spring type rapeseed) could not be collected because it was not survive through winter.

3.5.1. Seed quality trait

In the seed quality traits, most ISLs were similar to the recurrent parent, only a small number of lines deviated significantly. This could be clearly observed in the distribution of the traits in the population (Figure 8).

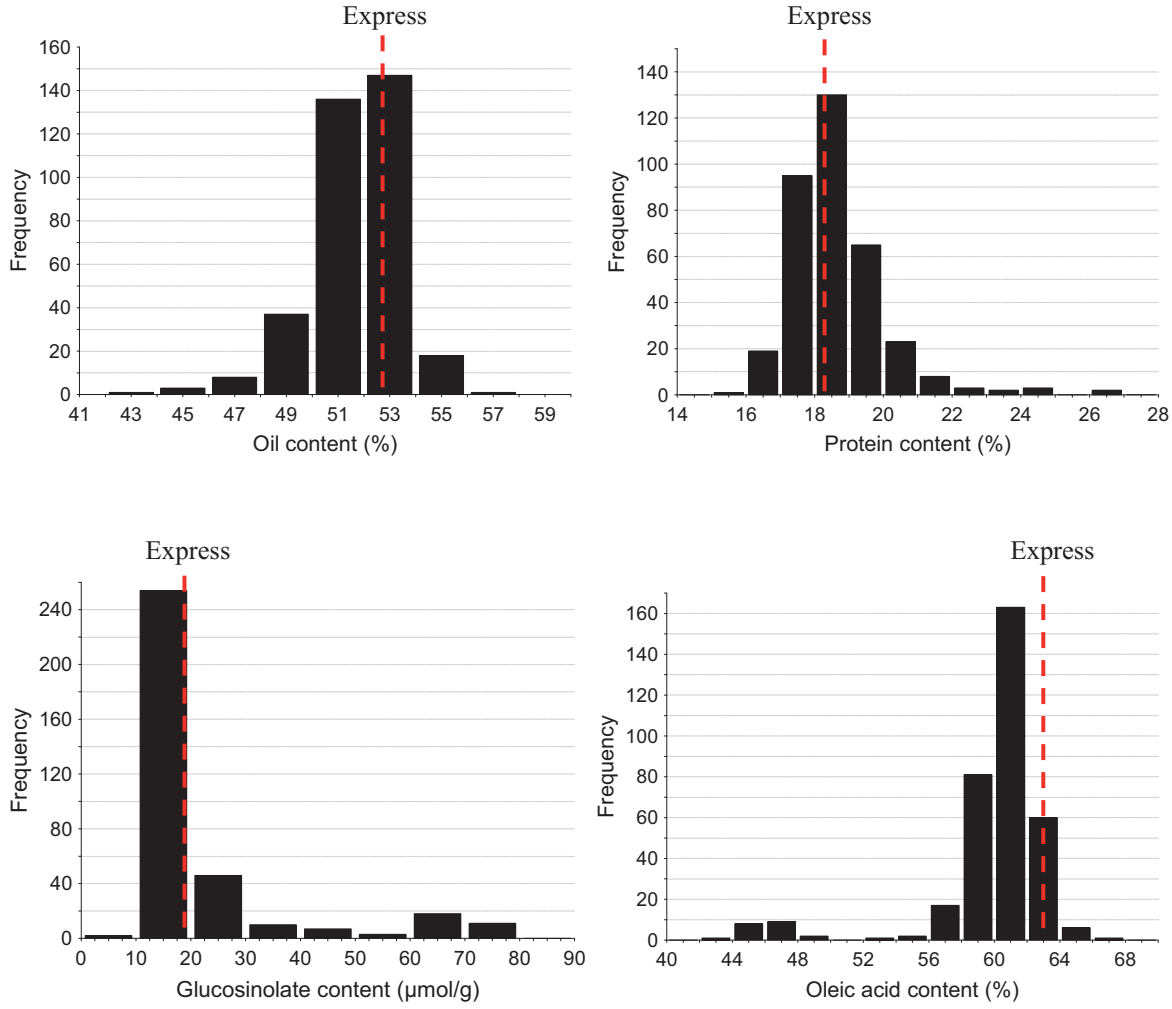

Figure 8 Distribution of oil, protein, glucosinolate, oleic acid, linolenic acid, erucic acid, sinapate esters and phytosterol content in the seeds of the ISLs estimated by NIRS (n=350). Data represent mean values from 4 locations: Einbeck, Hadmersleben, Malchow and Thuele in year 2009. Continue to the next page.

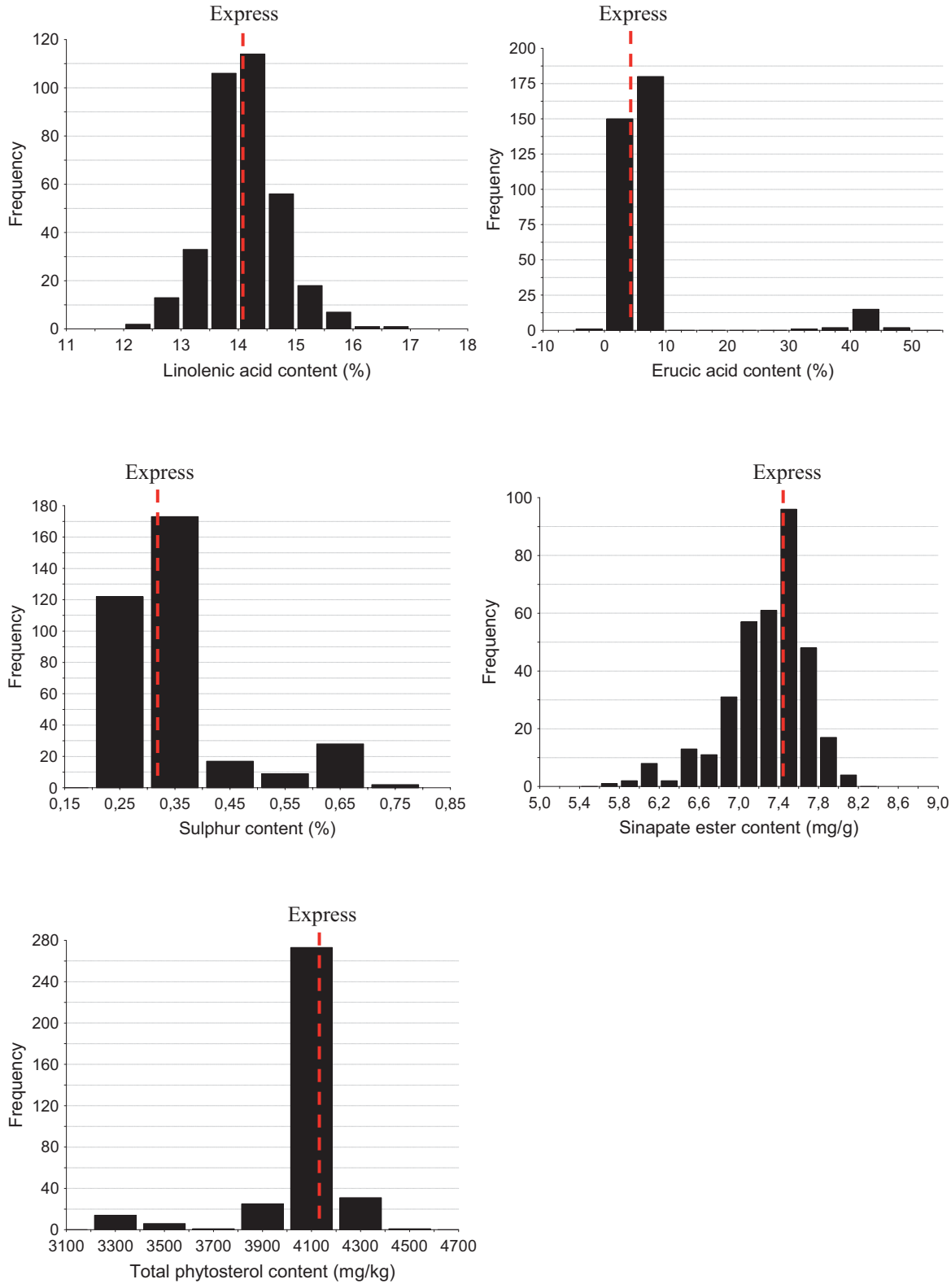

Figure 8 Continued from page 32

Seed quality traits data collected from Goettingen were discarded from analysis. Because erucic acid content of the seeds were high even for the recurrent parent, Express, which is known to have almost zero erucic acid content. All seeds quality traits were estimated in open pollinated seeds. The high erucic acid content in the ISLs seeds harvested in Goettingen was suspected due to cross pollination by pollen from high erucic acid population grown in the neighbour plot. Since seed erucic acid content is known to be correlated with other seeds quality traits, such as oil, oleic acid, phytosterol and sinapate esters content, therefore it would influence the accuracy of seeds quality traits data analysis.

Mean values of ISLs of all evaluated traits were almost similar to the recurrent 'Express' (Table 6). The donor parent 'RS239' has low oil, protein, oleic acid, linolenic acid, sinapate ester and phytosterol content, and has high glucosinolate, sulphur and erucic acid content compared to the recurrent parent estimated in selfed seed produced in greenhouse.

Analysis of variance and heritability of seed quality traits are summarized in Table 6. The effect of genotype and location were highly significant (P=0.01) for all seed quality traits. A high heritability of more than 90% was observed for glucosinolate, oleic acid, erucic acid, sulphur and phytosterol content of the seed.

Correlations among seed quality traits in the ISLs are presented in Table 7. A significant negative correlation was observed between oil and protein content ($r = -0.79^{**}$). Sulphur was positively correlated with glucosinolate ($r=0.97^{**}$) and Protein ($r=0.52^{**}$) content. A negative correlation was detected for erucic acid content and phytosterol ($r=-0.93^{**}$), sinapate esters ($r=-0.58^{**}$), and oleic acid ($r=-0.89^{**}$) content. Phytosterol was positively correlated with oleic acid ($r=0.77^{**}$) and sinapate esters ($r=0.57^{**}$) content. A positive correlation was also observed between oleic acid and sinapate esters ($r=0.53^{**}$) content.

Table 6 Analysis of variance and heritability of seed quality traits in the ISLs of the cross 'ExRS239'

Traits[a]	Min[b]	Max[c]	Mean[d]	Express[e]	'RS239'[#]	σ^2_g	σ^2_l	h^2
Oil	40.8	60.3	51.7	52.6	33.4	2.4**	3.1**	78.6
Protein	13.8	28.8	18.6	17.9	28.9	1.4**	3.7**	71.2
Glucosinolate	1.4	96.5	22.3	17.2	148.7	242.8**	21.1**	98.2
Oleic acid	41.8	76.5	59.9	62.3	35.1	13.4**	2.8**	94.3
Linolenic acid	11.2	19.6	14.1	13.9	10.5	0.3**	0.15**	81.3
Erucic acid	-12.7	48.7	7.1	3.8	47.7	71.9**	4.5**	98.8
Sulphur	0.1	0.9	0.4	0.3	1.3	0.01**	0.002**	95.0
Sinapate esters	5.3	10.1	7.3	7.4	8.0	0.13**	0.03**	80.0
Phytosterol	2991.5	4741.5	4066.0	4130.7	2431.4	34585.4**	16739.7**	95.9

Data observed at four locations: Einbeck, Hadmersleben, Malchow and Thuele in year 2009
**Significant at p=0.01
[a]For units see Table 1.
[b,c,d]Minimum, maximum and mean value of all ISLs, respectively
[e]Mean value of the recurrent parent 'Express'
[#]Analysed in self pollinated seeds produced in the greenhouse
σ^2_g, σ^2_l, h^2: Variance of genotype, variance of location and heritability, respectively

Table 7 Correlations among different seed quality traits in the ISLs of the cross 'ExRS239'

	Oil	Protein	GSL	C18:1	C18:3	C22:1	Sulphur	SE
Protein	-0.79**							
GSL	-0.28**	0.37**						
C18:1	-0.22**	-0.09	-0.38**					
C18:3	-0.48**	0.29**	-0.02	0.05				
C22:1	0.32**	0.10	0.27**	-0.89**	-0.32**			
Sulphur	-0.44**	0.52**	0.97**	-0.26**	0.06	0.16**		
SE	-0.22**	-0.11*	-0.23**	0.53**	0.27**	-0.58**	-0.17**	
Phyto	-0.09	-0.33**	-0.36**	0.77**	0.35**	-0.93**	-0.31**	0.57**

*, **Significant at p=0.05 and p=0.01, respectively. Abbreviations: see Table 1.

3.5.2. Plant height and phenological traits

Plant height at different developmental stages and phenological traits, namely begin of flowering, end of flowering and duration of flowering were evaluated in the field trials. The distribution of these traits is shown in Figure 9.

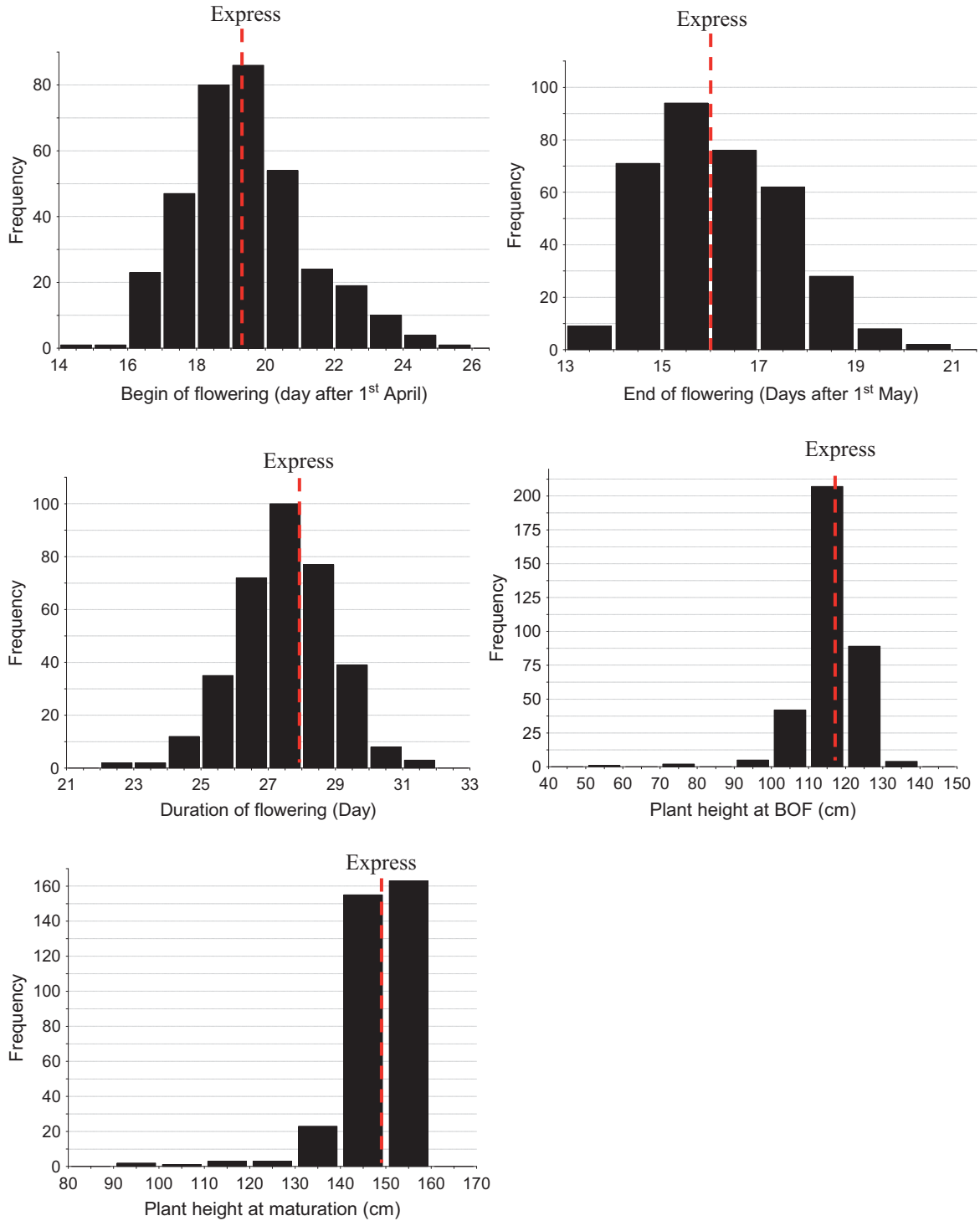

Figure 9 Distribution of begin of flowering, end of flowering, duration of flowering, plant height at begin of flowering and plant height at maturation in the ISLs (n=350). Data represent mean values of the ISLs from all locations observed in year 2009. Locations of observation see Table 8.

The analysis of variance and heritability of plant height and the phenological traits is summarized in Table 8. The effects of genotype and location were highly significant for all of traits evaluated. High heritability with more than 80% was observed for begin of flowering and plant height at maturation.

Table 8 Analysis of variance and heritability of plant height and phenological traits in the ISL of the cross 'ExRS239'

Traits[a]	Min[b]	Max[c]	Mean[d]	Express[e]	σ^2_g	σ^2_l	h^2
BOF[G,H,M]	9.5	25.0	19.5	19.0	2.8**	4.6**	86.0
EOF[G,M]	10.0	24.0	16.4	15.5	1.2**	20.5**	60.9
DOF[G,M]	16.0	39.5	27.6	27.8	0.8**	49.5**	37.1
PH at BOF[G,M]	51.5	165.0	115.7	118.5	38.7**	1220.7**	64.1
PH at maturation[G,H,E,M]	60.0	170.0	148.6	149.9	45.9**	50.9**	81.4

**Significant at p=0.01
[G,E,H,M]Locations of observation: Goettingen, Einbeck, Hadmersleben and Malchow, respectively, in year 2009
[a]For units see Table 1.
[b,c,d]Minimum, maximum and mean value of all ISLs, respectively
[e]Mean value of the recurrent parent 'Express'
σ^2_g, σ^2_l , h^2: Variance of genotype, variance of location and heritability, respectively

3.6. QTL analysis in the ISLs generated from the cross 'ExRS239'

3.6.1. Seed quality trait

The mapping process of QTL is shown in detail for seed glucosinolate and erucic acid content as major quality traits. QTL results for the other seed quality traits evaluated in the ISLs are summarised in Table 9.

Glucosinolate content: Forty seven ISLs were observed with significantly differences in seed glucosinolate content compared to the recurrent parent 'Express' (Table 10). Four linkage group with donor segments relevant to the trait were detected indicating that at least four QTL were present in the ISLs from the cross 'ExRS239'. The four QTL were located on linkage groups N01, N10, N12 and N19. Together they explained 64.7 µmol/g of seed glucosinolate content additive effect.

Thirty out of forty seven of the ISLs that were significantly different to the recurrent parent carried a relevant donor segment for seed glucosinolate content on linkage group N19 (Table 10). All of these lines carried a different length of donor segment in the lower part of the linkage group, ending on terminal marker E33M47-184E (Figure 10). Two substitution lines (DH lines 105 and 205) were not significantly different from the recurrent parent, also carried donor segments at markers E33M47-184E and E41M54-178R, indicating that the QTL for seed glucosinolate content in this linkage group is not located on or close to these markers. From the comparison of segments and effects carried

by the lines it was concluded that the QTL on linkage group N19 is located between but not at the markers E33M47-184E and E41M54-178R, in an interval of maximally 5.6 cM. It is also possible that the QTL is located downward from the terminal marker E3347-184E, in a region that is not covered by the linkage map. The additive effect of the QTL on this linkage group is 25.2 µmol/g of seed glucosinolate content showing that the donor allele increases the trait.

By the comparison of segments and its effects as on linkage group N19, the QTL on linkage group N01, N10 and N12 were localized in maximal intervals of 15.7, 11.2 and 22.3 cM, respectively (Figure 10). The additive effect of these QTL are 10.5 (N12), 9.6 (N10) and 19.5 µmol/g (N01) showing alleles increasing the seed glucosinolate content contributed by the donor parent. The QTL on linkage groups N01, N10 and N12 were mapped in the region of the possible extension of donor segments. They were localized with an assumption that the lines with significant differences in seed glucosinolate content carried an extension of donor segments that were not carried by the lines having no significant effect for the trait.

Concerning to the QTL on linkage group N01 and N10, there are three possible explanations proposed. First, the QTL on linkage group N01 was carried by lines in group ‘j’, and lines in group ‘h’ and ‘i’ carried the QTL on linkage group N10 (Figure 10). Considering the fact that the ‘j’ lines have a large different of effect of introgressed segment to ‘h’ and ‘i’ lines, consequently it is not possible that they carry the same QTL. Second, lines grouped into ‘h’ and ‘i’ might carry both of the QTL on linkage group N01 and N10, but the QTL on N10 is a QTL where the donor allele reduces the glucosinolate content and the interaction between the two QTL (on N01 and N10) cause reduction of the effect in the lines carrying both QTL. Third, lines in group ‘h’, ‘i’ and ‘j’ actually carry QTL only on linkage group N01 but not on N10, with the assumption that there are two linked QTL carried by lines in group ‘j’ causing the large effect carried by the lines, while the lines in group ‘h’ and ‘i’ carry only one of the QTL.

The large effect carried by the lines in group ‘g’ that is almost two times the effect carried by the lines in group ‘f’, indicate that the ‘g’ lines might carry another QTL besides the QTL on N12, which is undetected in this analysis. An alternative explanation is a QTL with donor allele increasing the trait present in ‘g’ region, on the other hand there were two QTL in ‘f’ region with donor alleles increasing and decreasing glucosinolate content that is not covered in ‘g’ region.

We assumed that lines in group ‘d’, carried only a QTL on N19 and not the QTL on N12. It means that the lines do not carry an extension of donor segment on N12 where the QTL on this linkage group was placed. Since the effect of introgressed segment carried by these lines were almost similar to the lines in group ‘a’, ‘b’, ‘c’ and ‘e’ which carried only a QTL on N19.

All the QTL found in the ISLs showed loci where the donor alleles increased seed glucosinolate content. The donor parent, ‘RS239’, has high glucosinolate content in its seeds (148.7µmol/gr) as estimated using NIRS in selfed seeds produced in the greenhouse. The presence of additional QTL in this population may be possible since there are four other substitution lines having effects of introgressed segments of 12.2-26.9 µmol/g glucosinolate content likely carrying donor segments undetected in this analysis.

Erucic acid content: Nineteen ISLs were identified with significant differences in erucic acid content compared to the recurrent parent ‘Express’ (Table 11.). Eleven lines were detected with donor segments relevant to the erucic acid content on linkage group N13 and a QTL could be mapped on this linkage group. The eleven lines carried the donor segment from marker E44M50-115E to the lower part of linkage group N13 (Figure 11). It was also observed that there were other ISLs with no significant effects for erucic acid content carrying donor segments from marker E44M50-122E to E44M50-115E (DH lines 984 and 1473) and on a terminal marker E32M47-188E (DH lines 495, 501 and 949). It indicates that the QTL was located between but not on or close to the marker E44M50-115E and E32M47-188E with a maximum interval of 20.1 cM. The QTL has an additive effect of 18.8% erucic acid content in the seeds. The positive sign of additive effect showed allele increasing the trait contributed by the donor parent ‘RS239’.

We could not map the second gene controlling erucic acid content in this analysis. It was already well studied that erucic acid content in *B. napus* is controlled by two genes mapped on linkage group N8 and N13 of *B. napus* chromosomes (Ecke et al. 1996; Thormann et al. 1996; Jourdren et al. 1996a; Fourmann et al. 1998; Peleman et al. 2005; Qiu et al. 2006). There were eight ISLs with significant effect for erucic acid content (Table 11) probably carrying the second gene for erucic acid content on N08 that remained unmapped since only 29.7 cM of linkage group N08 was covered by markers in our map. This was considerably smaller compared to the 70.9 cM covered in the cross of ‘MxS’ where we could map the gene for erucic acid content on N08 (see Figure 15).

Oil content: Nine QTL were identified for oil content showing the complexity of this trait (Table 9). All of the QTL showed negative additive effects indicating that the donor parent, 'RS239', contributed the alleles for reduction of the oil content. A QTL on linkage group N18a gave the highest effect in oil content and alone explained about 21% of total the additive effect detected in this analysis. The maximal intervals for the QTL positions ranged from 1.4-35.8 cM. A large maximum interval of more than 20 cM was identified on linkage groups N03, N10 and LG20.

Protein content: Six QTL were localized on linkage groups N07, N10, N12, N14 and N18a with positive additive effects ranging from 0.99-4.32% of seed protein content (Table 9). The positive additive effects showed donor alleles increasing the trait contributed by donor parent, 'RS239'. All additive effects together sum up to 11.8% of the seed protein content. The highest effect was detected on linkage group N18a with a contribution of 37% of the total additive effect. The QTL on linkage groups N10, N12, N14 and N18a were located in the same region of the linkage groups, where the QTL for oil content were mapped, indicating either a pleiotropic effect or closely linked QTL. Two other QTL on linkage groups N07 and N12 (Table 9) showed independent loci from the QTL for oil content with total additive effect of 2.5% for protein content.

Oleic acid content: Five QTL were identified showing a negative additive effect which resulted in a total of -22.3% of seed oleic acid content (Table 9). The negative signs of additive effects exhibiting donor alleles reducing oleic acid content in the seeds. Two major QTL were identified on linkage groups N13 and N15 which explained 66% of the total additive effect. These two QTL could be mapped in relatively small intervals with maximum interval of 6.6 and 1.8 cM, respectively. A pleiotropic effect on oil content or closely linked QTL were observed on linkage group LG20 and on N13 for oleic and erucic acid content.

Linolenic acid content: Two QTL were identified on linkage groups N07 and N15 with a total additive effect of -1.7% (Table 9), showing alleles reducing linolenic acid content contributed by the donor parent 'RS239'. The QTL on N15 showed a pleiotropic effect on oleic acid content or a close linkage to the major QTL for that trait.

Sinapate esters content: Four QTL were detected on linkage groups N06, N11, N13, and N15 with a total additive effect of -2.19 mg/kg showing donor alleles reducing sinapate esters content (Table 9). The QTL on N06, N13, and N15 exhibited pleiotropic effects on oleic acid content or close linkage QTL. The QTL on N13 was co localized with the erucic acid gene showing a pleiotropic effect or closely linked QTL on this trait.

Phytosterol content: Three QTL were mapped on linkage groups N11, N13, and N15 with a total additive effect of -983.69 mg/g of phytosterol content in the seeds (Table 9). The negative signs of additive effects showed allele decreasing phytosterol content contributed by the donor parent. Pleiotropic effects on sinapate esters and oleic acid content or closely linked QTL were detected for the QTL on linkage groups N06, N13, and N15. In addition the QTL on N13 also showed a pleiotropic effect on erucic acid content or close linkage QTL.

Table 9 QTL and their additive effects detected in the ISLs developed from the cross 'ExRS239'.

Trait	No.	LG	Marker/marker interval	Max. QTL Interval[1] (cM)	Position [2]	Additive effect[3]
Glucosinolate	1.	N01	E37M54-170E/ E37M54-171E – E44M50-078E	15.7	166.7-182.4	19.5
	2.	N10	E34M54-056E – E37M50-345E	11.2	29.0-40.2	9.6
	3.	N12	E33M54-331R – E32M49-480R	22.3	20.5-42.8	10.5
	4.	N19	E41M54-178R – E33M47-184E	5.6	87.4-93.0*	25.2
Erucic acid	1.	N13	E44M50-115E – E32M47-188E	20.1	79.3-99.4	18.75
Oil	1.	N01	E44M54-226E	8.1	137.6-145.7	-1.96
	2.	N03	E34M49-059E	35.8	137.7-173.5	-1.14
	3.	N09	E35M54-146E	14	57.8-71.8	-1.34
	4.	N10	E37M50-345E – E37M54-151E	30.2	29.0-59.2	-2.02
	5.	N11	E37M50-229E – E34M49-225E	1.9	66.1-68.0	-1.97
	6.	N12	E41M54-136E – E34M49-052E	8.9	48.2-57.1	-1.86
	7.	N14	E33M54-202R – E32M47-183R	10.6	131.7-142.3	-2.02
	8.	N18a	E41M50-211R – E33M49-099R	6.5	4.4-10.9	-4.13
	9.	LG20	E33M54-096R	24.7	24.7*	-2.06
Protein	1.	N07	E39M50-055R – E41M50-402R	2.5	84.1-86.6	1.48
	2.	N10	E37M50-345E – E37M54-151E	30.2	29.0-59.2	2.02
	3.	N12	E41M54-136E – E34M49-052E	8.9	48.2-57.1	1.24
	4.	N12	E32M54-153R	0.8	57.4-58.2	0.99
	5.	N14	E33M54-202R – E32M47-183R	10.6	131.7-142.3	1.66
	6.	N18a	E41M50-211R – E33M49-099R	6.5	4.4-10.9	4.32
Oleic acid	1.	N06	E32M47-102R – E32M49-059E	17.5	12.5-30.0	-3.30
	2.	N09	E35M54-146E	14	57.8-71.8	-1.91
	3.	N13	E44M50-115E – E32M47-188E	20.1	79.3-99.4	-6.49
	4.	N15	E34M49-056R	1.8	71.5-73.3	-8.13
	5.	LG20	E33M54-096R	24.7	24.7*	-2.52

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Table 9 Continued from page 42

Trait	No.	LG	Marker/marker interval	Max. QTL Interval[1] (cM)	Position [2]	Additive effect[3]
Linolenic acid	1.	N07	E44M54-068R	9.2	27.5-36.7	-0.54
	2.	N15	E34M49-056R	1.8	71.5-73.3	-0.53
Sinapate esters	1.	N06	E32M47-102R – E32M49-059E	17.5	12.5-30.0	-0.44
	2.	N11	E35M47-260E	14.1	19.9-34.0	-0.72
	3.	N13	E44M50-122E – E32M47-188E	20.1	79.3-99.4	-0.55
	4.	N15	E34M49-056R	1.8	71.5-73.3	-0.49
Phytosterol	1.	N11	E35M47-260E	14.1	19.9-34.0	-217.77
	2.	N13	E44M50-115E – E32M47-188E	20.1	79.3-99.4	-347.90
	3.	N15	E34M49-056R	1.8	71.5-73.3	-418.02

[1]: Size of maximal interval where the QTL could be located
[2]: Start and end point of maximal interval of QTL
[3]: For units see Table 1.
*: QTL position could also locate on end of the linkage group where the region is uncovered by the map

Table 10 ISLs with significant differences in seed glucosinolate content compared to the recurrent parent, of the cross 'ExRS239'

No.	DH lines	EIS[1] (µmol/g)	LG[2]	Group[3]	No. of donor segment[4]	Segment on linkage group[5]
1.	644	51.23	N19	a	1	**N19[62.4-93.0]**
2.	873	47.71	N19	a	2	**N19[62.4-93.0]**, LG21[0-14.2]
3.	954	44.22	N19	a	1	**N19[62.4-93.0]**
4.	955	45.95	N19	a	1	**N19[62.4-93.0]**
5.	1298	48.85	N19	a	1	**N19[62.4-93.0]**
6.	1301	49.10	N19	a	1	**N19[62.4-93.0]**
7.	1302	41.64	N19	a	1	**N19[62.4-93.0]**
8.	476	54.57	N19	b	1	**N19[76.6-93.0]**
9.	636	48.48	N19	b	2	N02[76.0], **N19[76.6-93.0]**
10.	645	49.13	N19	b	1	**N19[76.6-93.0]**
11.	1467	45.38	N19	b	1	**N19[76.6-93.0]**
12.	43	52.34	N19	c	2	N03[136.8], **N19[87.4-93.0]**
13.	45	60.46	N19	c	3	N03[136.8], N17[-3.3], **N19[87.4-93.0]**
14.	47	59.40	N19	c	4	N01[137.1], N03[136.8], N17[-3.3], **N19[87.4-93.0]**
15.	53	47.25	N19	c	3	N07[79.8], N11[-4.6], **N19[87.4-93.0]**
16.	103	53.02	N19	c	2	N07[79.8], **N19[87.4-93.0]**
17.	286	53.11	N19	c	2	N07[79.8], **N19[87.4-93.0]**
18.	287	54.52	N19	c	3	N07[79.8], N11[-4.6], N19**[87.4-93.0]**
19.	1502	51.37	N19	c	3	N04[46.5-84.2], N06[12.5-24.6], **N19[87.4-93.0]**
20.	49	50.68	N12, N19	d	3	N11[-4.6], **N12[17.8-20.5]**, **N19[87.4-93.0]**
21.	51	55.07	N12, N19	d	2	**N12[17.8-20.5]**, **N19[87.4-93.0]**
22.	98	58.22	N12, N19	d	4	N07[79.8], N11[-4.6], **N12[17.8-20.5]**, **N19[87.4-93]**
23.	848	51.47	N12, N19	d	6	N07[79.8], N15[72.2][74][74.4], **N12[17.8-20.5]**, **N19[87.4-93]**
24.	849	53.06	N12, N19	d	2	**N12[17.8-20.5]**, **N19[87.4-93]**
25.	1100	53.06	N12, N19	d	3	N11[-4.6], **N12[17.8-20.5]**, **N19[87.4-93]**
26.	1103	51.05	N12, N19	d	5	**N12[17.8-20.5]**[48.2][76.6-76.7][81.8], **N19[87.4-93]**

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No.	DH lines	EIS[1] (µmol/g)	LG[2]	Group[3]	Nr of donor[4]	Segment on linkage group[5]
27.	1116	52.49	N19	e	3	N07[79.8], N15[89.6-108.4], **N19[93.0**]
28.	1118	52.02	N19	e	3	N07[79.8], N15[89.6-108.4], **N19[93.0**]
29.	1453	59.89	N19	e	3	N01[166.7], N15[89.6-108.4], **N19[93.0]**
30.	1454	52.56	N19	e	3	N07[79.8], N15[89.6-108.4], **N19[93.0]**
31.	722	13.29	N12	f	5	N02[0.0], N11[-4.6], **N12[0-46.1]**[51.6-69.2][76.6-97]
32.	911	14.59	N12	f	5	N02[0.0], N11[-4.6], **N12[0-46.1]**[51.6-69.2][76.6-97]
33.	325	21.40	N12	g	3	**N12[0-20.5]**, N17[0.0][-3.3]
34.	715	30.11	N12	g	4	**N12[0-20.5]**[57.5], N17[0.0][-3.3]
35.	716	30.51	N12	g	5	N02[76], **N12[0-20.5]**[57.5], N17[0.0][-3.3]
36.	251	16.86	N01, N10	h	7	**N01**[27.3][83.7][133.3-137.1]**[138.4-166.7]**, **N10[40.2-61.5]**, N14[131.7-161][167.2-179.4],
37.	932	14.46	N01, N10	h	6	**N01**[83.7][133.3-137.1]**[138.4-166.7]**, N02[0.0-32.5], **N10[40.2-61.5]**, N17[83.1]
38.	933	24.49	N01, N10	h	6	**N01**[83.7] [133.3]**[138.4-166.7]**, N02[0.0-32.5], **N10[40.2-61.5]**, N17[83.1]
39.	1145	16.20	N01, N10	h	8	**N01**[27.3][83.7][133.3]**[138.4-166.7]**, N02[0.0-32.5], **N10[40.2-61.5]**, N14[131.7-133.6], N17[83.1]
40.	1154	24.95	N01, N10	h	6	**N01**[83.7][133.3-137.1]**[138.4-166.7]**, N02[0.0-32.5], **N10[40.2-61.5]**, N17[83.1]
41.	149	17.58	N01, N10	i	6	**N01**[27.3][83.7][133.3-137.1]**[138.4-166.7]**, N02[12.4-32.5], **N10[40.2-59.2]**
42.	1065	36.84	N01	j	7	**N01**[27.3][83.7]**[133.3-166.7]**, N06[35.4], N10[0.0], N18a[7.4-8.3], N18b[1-31.2]
43.	1066	41.18	N01	j	5	**N01**[27.3][83.7]**[133.3-166.7]**, N18b[1-31.2], LG21[0.0]
44.	445	12.16	?		4	N11[28.6-34][56.5-94.1], N14[126], N19[33.1]
45.	652	12.91	?		6	N02[0.0], N04[0.0], N05[86.0], N07[13.9-20.4], N09[32.3-37.6][37.9-51.7]
46.	957	26.90	?		1	N05[57.6]
47.	1435	22.94	?		1	N15[34.9-44.8]

[1]Effect of introgressed segment, see part 2.9

[2]Linkage group where the donor segment is relevant for the trait

[3]Group of lines with different length of relevant donor segment

[4]Number of donor segments carried by ISLs

[5]Linkage group respectively the donor segments; a parenthesis refers to a segment, expresses position of segment on LG as minimum length of donor segment; the donor segments relevant for the trait are written in bold, as an example DH line 716 carried 5 donor segment: 1 segment on N02, 2 segments on N12 and 2 segments on N17, and the donor segment relevant for the trait is **N12[0.0-20.5]** from position 0.0 to 20.5 cM on LG N12; The elimination of segments irrelevant for the trait, see part 2.9

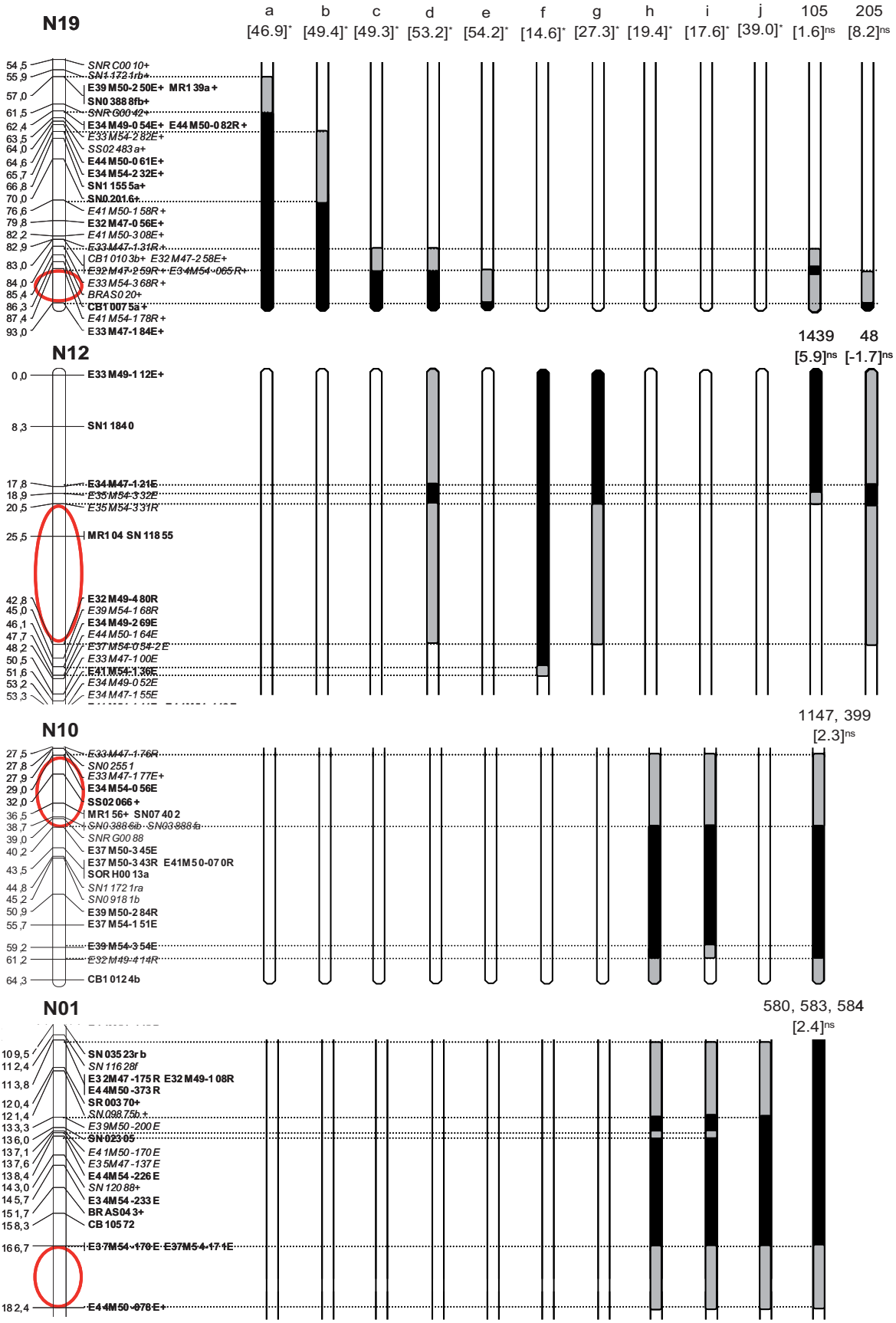

Figure 10 Analysis of QTL for seed Glucosinolate content using ISLs. Black bar: minimum length of donor segment from 'RS239' carried by the lines; grey bar: possible maximum extension of donor segments; red circle: maximal interval for the QTL localization; a-j: see Table 10; *lines with significant effect for seed glucosinolate content; nsDH-lines that have no significant effect; in parenthesis: average effect of seed glucosinolate content (µmol/g).

Table 11 ISLs with significant differences in erucic acid content compared to the recurrent parent, of the cross 'ExRS239'

No.	DH lines	EIS[1] (µmol/g)	LG[2]	Group[3]	No. of donor segment[4]	Segment on linkage group[5]
1.	49	37.17	?	-	3	N11[4.6], N12[17.8-20.5], N19[87.4-93.0]
2.	51	38.49	?	-	2	N12[17.8-20.5], N19[87.4-93.0]
3.	103	36.82	?	-	2	N07[79.8], N19[87.4-93.0]
4.	287	36.20	?	-	3	N07[79.8], N11[4.6], N19[87.4-93.0]
5.	848	37.50	?	-	7	N07[79.8], N12[17.8][20.5], N15 [72.2][74.0][74.4], N19[87.4-93.0]
6.	849	36.18	?	-	2	N12[17.8-20.5], N19[87.4-93.0]
7.	1100	36.41	?	-	3	N11[4.6], N12[17.8-20.5], N19[87.4-93.0]
8.	1502	37.72	?	-	3	N4[46.5-84.2], N06[12.5-24.6], N19[87.4-93.0]
9.	380	41.60	N13	a	6	N03[116], N04[64.8], N06[12.5], **N13[79.3-99.4]**, N14[23.9-75.5][81.5-94.5]
10.	405	38.07	N13	a	3	N01[27.3], N05[86.0], **N13[79.3-99.4]**
11.	491	40.28	N13	a	3	N11[68.0-94.1], **N13[79.3-99.4]**, N14[126]
12.	503	37.34	N13	a	4	N11[68.0-94.1], N14[126], **N13[79.3-99.4]**, N19[0.0]
13.	509	36.31	N13	a	1	**N13[79.3-99.4]**
14.	1192	42.80	N13	a	3	N11[68.0-94.1], **N13[79.3-99.4]**, N14[126]
15.	1308	35.19	N13	a	2	**N13[79.3-99.4]**, N19[0.0]
16.	1425	35.89	N13	a	4	N11[28.6-34.0][56.5-66.1], **N13[79.3-99.4]**, N19[0.0]
17.	1427	38.93	N13	a	3	N11[68.0-94.1], **N13[79.3-99.4]**, N14[126]
18.	1428	40.08	N13	a	5	N04[17.1-42.5], N11[68.0-94.1], **N13[79.3-99.4]**, N14[126], N19[0.0]
19.	515	37.10	N13	b	2	N01[27.3], **N13[79.3-83.7]**

[1]Effect of introgressed segment, see part 2.9
[2]Linkage group where the donor segment is relevant for the trait
[3]Group of lines with different length of relevant donor segment
[4]Number of donor segments carried by ISLs
[5]Linkage group respectively the donor segments (see note Table 10.)

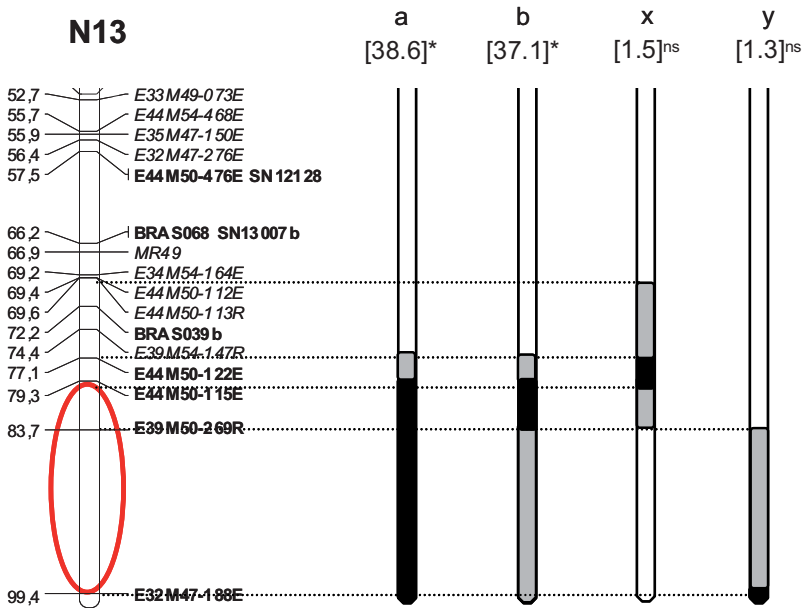

Figure 11 Localization of the erucic acid gene using ISL. Black bar: minimum length of donor segment from 'RS239' carried by the lines; grey bar: possible maximum extension of donor segments; red circle: maximal interval for the QTL localization; *lines with significant effect for erucic acid content (a, b: see Table 11); nslines that have no significant effect (x: DH lines 984 and 1473, y: DH lines 495, 501 and 949); in parenthesis: effect of erucic acid content (%).

3.6.2. Plant height and phenological trait

The results of QTL analysis for plant height and phenological traits are summarized in Table 12. A QTL for duration of flowering could not be detected in this analysis. It might be due to a very low heritability indicating low genetic variation or high genetic and environmental interaction and experimental error.

Begin of flowering: Three QTL were mapped on linkage groups N09, N14, and N18a (Table 12). The additive effects of the QTL ranged from 1.8 to 2.3 days showing that the donor parent contributes alleles for later flowering times.

End of flowering: Three QTL were detected on linkage groups N08, N09, and N18a with a total additive effect of 6 days (Table 12). The positive signs of additive effects showed alleles responsible for delaying end of flowering time contributed by the donor parent. A pleiotropic effect on begin and end of flowering was detected for the QTL on linkage group N18a.

Plant height at begin of flowering: Two QTL controlling plant height at begin of flowering were identified on linkage groups N13 and N19 (Table 12). Both QTL showed negative additive effects indicating that the donor parent contributes alleles for shorter plants at begin of flowering time. The QTL together sum up to an additive effect of -22.4 cm plant height at begin of flowering.

Plant height at maturation: Three QTL were mapped on linkage groups N13, N18a, and N19. All alleles contributed by the donor parent 'RS239' reduced plant height and resulted in a total negative additive effect of about -50.4 cm of plant height at maturation (Table 12). The major QTL detected on N18a explained about 45% of the total effect of all QTL. Pleiotropic effects on plant height at begin of flowering time and at maturation were detected on linkage groups N13 and N19.

Table 12 QTL for plant height and phenological traits and their additive effects detected in ISLs from the cross 'ExRS239'

Trait	No.	LG	Marker/marker interval	Max. QTL Interval[1] (cM)	Position [2]	Additive effect[3]
BOF	1.	N09	E33M54-082E	5.5	-5.5*-0.0	2.31
	2.	N14	E33M54-202R - E32M47-183R	16.3	126.0-142.3	1.80
	3.	N18a	E41M50-211R – E33M49-099R	6.5	4.4-10.9	2.09
EOF	1.	N08	E44M50-438R	11.0	5.5-16.5	1.75
	2.	N09	E33M54-082E	5.5	-5.5*-0.0	2.25
	3.	N18a	E41M50-211R – E33M49-099R	6.5	4.4-10.9	2.00
PH at BOF	1.	N13	E44M50-122E	4.9	74.4-79.3	-13.26
	2.	N19	E34M54-160E - E34M49-086E	13.0	9.8-22.8	-9.10
PH at maturation	1.	N13	E44M50-122E	4.9	74.4-79.3	-16.55
	2.	N19	E34M54-160E - E34M49-086E	13.0	9.8-22.8	-12.96
	3.	N18a	E41M50-211R – E33M49-099R	6.5	4.4-10.9	-24.08

[1]: Size of maximal interval where the QTL could be located
[2]: Start and end point of maximal interval of QTL
[3]: For units see Table 1.
*: QTL position could also locate on end of the linkage group where the region is uncovered by the map

3.7. Molecular analysis of the ISLs generated from the cross 'MxS'

One hundred thirty one ISLs were analysed using 20 AFLP marker combinations to characterize donor segments carried by the lines. Three ISLs had unclear amplifications products and could not be scored unambiguously. The number of donor segments carried by the ISLs is presented in Table 13. The ISLs carried 1 to 6 donor segments with the mean of 1.97 segments. Eight ISLs had undetected donor segments.

Table 13 Number of donor segments carried by the ISLs from the cross MxS

Number of substitution lines	Number of donor segments carried
8	0
42	1
42	2
29	3
5	4
4	5
1	6
Mean	1.97

The ISL represented a minimum of 515.5 cM and a maximum of 1095.3 cM corresponding to 38% and 80.5% of the AFLP genetic map developed in the cross of 'MxS' (Table 14). The donor segments were unevenly dispersed across the 19 linkage groups of the genetic linkage map developed in this cross. A lower minimum coverage of donor genome (below 30%) was observed on linkage groups N02 (6.1%), N04 (13.7%), N09 (15.5%), N10 (1.8%), and N18 (22.6%). Linkage groups N05 (0%), and N11 (0%) were estimated with minimum coverage 0% of donor genome (see Figure 3). A high percentage (100%) of donor genome coverage was observed on linkage groups N06 and N17. However they only covered 2.1 and 5.8 cM of the donor genome, respectively, since only small part of these linkage groups is covered by AFLP markers.

Table 14 The minimum, maximum and medium coverage of linkage group (LG) by donor segments in the ISLs from the cross 'MxS'

LG	Length of AFLP map[1] in cM	Minimum coverage[2] in cM (%)		Maximum coverage[2] in cM (%)		Medium coverage[2] in cM (%)	
N01	54.7	43.5	(79.5)	54.7	(100)	49.1	(89.8)
N02	112.7	6.9	(6.1)	60.3	(53.5)	33.6	(29.8)
N03	118.1	59.9	(50.7)	67.6	(57.2)	63.8	(54.0)
N04	53.2	13.7	(25.8)	53.2	(100)	33.5	(62.9)
N05	75.2	0.0*	(0)	33.5	(44.5)	19.2	(25.5)
N06	2.1	2.1	(100)	2.1	(100)	2.1	(100)
N07	48.7	48.7	(100)	48.7	(100)	48.7	(100)
N08	56	19.7	(35.2)	56.0	(100)	56.0	(100)
N09	105.9	16.4	(15.5)	84.9	(80.2)	56.5	(53.4)
N10	68	1.2	(1.8)	49.3	(72.5)	26.9	(39.6)
N11	83.2	0.0*	(0)	36.5	(43.9)	18.3	(21.9)
N12	65.3	38.9	(59.6)	65.3	(100)	59.6	(91.3)
N13	158	78.6	(49.7)	158.0	(100)	115.8	(73.3)
N14	101.6	74.6	(73.4)	101.6	(100)	89.5	(88.1)
N15	58.3	18.6	(31.9)	58.3	(100)	49.8	(85.4)
N16	61.5	41.8	(67.9)	61.5	(100)	61.5	(100)
N17	5.8	5.8	(100)	5.8	(100)	5.8	(100)
N18	60.2	13.6	(22.6)	25.5	(42.4)	20.2	(33.6)
N19	72.5	31.5	(43.4)	72.5	(100)	52.0	(71.7)
Sum	1361	515.5	(37.9)	1095.3	(80.5)	861.9	(63.3)

[1]Map without RFLP, SSR and RAPD markers, since the ISLs were only characterized with the AFLP markers

[2]Minimum, maximum and medium coverage of donor genome were calculated based on minimum, maximum and medium length of donor segments, respectively, see part 2.9 and Figure 4

*See part 2.9 and Figure 4

3.8. Phenotypic analysis of the ISLs generated from the cross 'MxS'

Two hundred seventy ISLs generated from the cross 'MxS' and the parental lines, 'Mansholt' and 'Samourai', were grown in the field for phenotypic data collection.

3.8 1. Seed quality trait

The test of the ISLs in the field showed that phenotypically most of the ISLs were similar to the recurrent parent and only a small number of lines significantly differed from the recurrent parent, 'Samourai'. It could be observed in the distribution of seed quality traits evaluated in the ISL population (Figure 12).

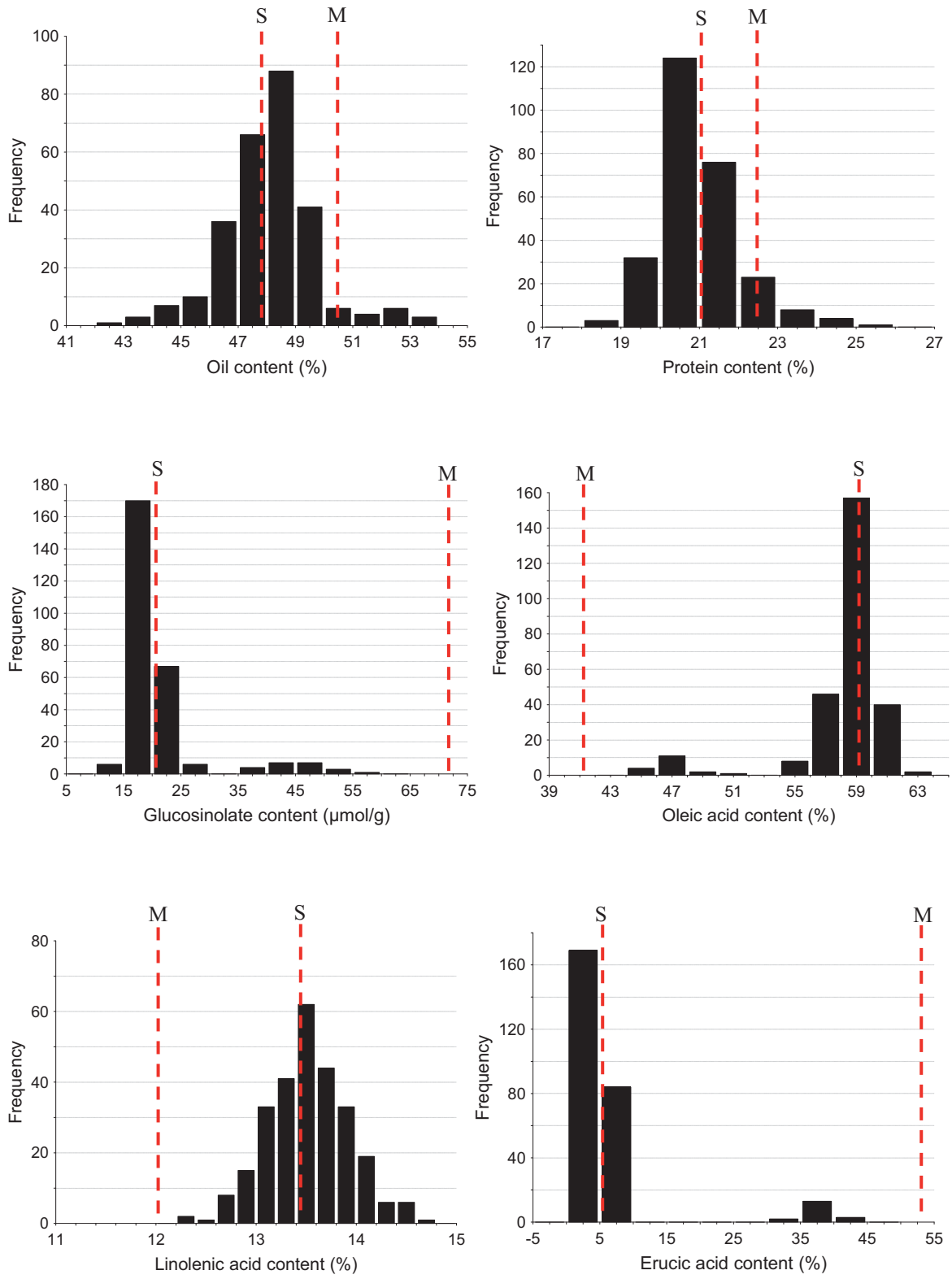

Figure 12 Distribution of the seed quality traits estimated by NIRS (n=270) in the ISL. Data represent mean values of the ISLs from 5 locations during 2 years. M: mean value of 'Mansholt'; S: Mean value of 'Samourai'. Continue to the next page.

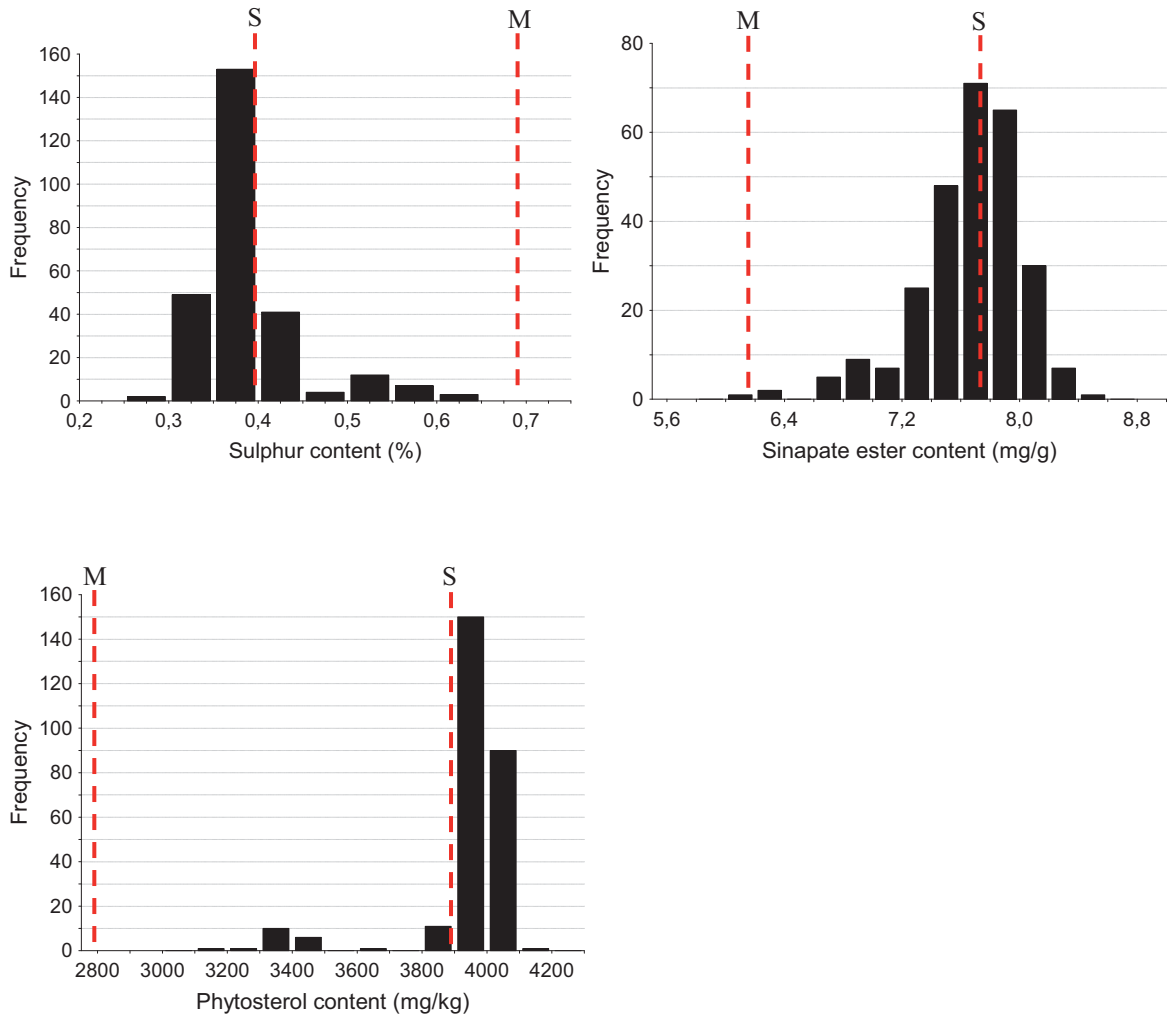

Figure 12 Continued from page 53

The mean value of ISLs is almost similar to the recurrent parent, 'Samourai', for all traits evaluated (Table 15). The donor parent, 'Mansholt', has high oil, protein, glucosinolate, erucic acid and sulphur content, and contains low oleic acid, linolenic acid, sinapate esters and phytosterol content in the seeds.

The analysis of variance and heritability of seed quality traits are summarized in Table 15. The effects of genotype, location within year and interaction between genotype and year were significant for all evaluated traits. The effect of year was not significant for seed quality traits except for linolenic acid content. The genotype variance was high for seed glucosinolate, oleic acid, erucic acid and phytosterol content resulting in high heritabilities of more than 90%.

Table 15 Analysis of variance and heritability of seed quality traits in the ISLs developed from the cross 'MxS'.

Traits[a]	Min[b]	Max[c]	Mean[d]	Mansholt[e]	Samourai[f]	σ^2_g	σ^2_y	$\sigma^2_{l(y)}$	σ^2_{gy}	h^2
Oil	35.8	58.4	48.1	50.3	47.7	1.75**	1.73	8.77**	0.70**	70.6
Protein	13.3	30.2	20.9	22.4	20.9	0.49**	-0.99	7.46**	0.55**	47.6
Glucosinolate	2.3	72.6	21.1	72.1	19.3	55.01**	-2.02	10.09**	4.54**	93.5
Oleic acid	37.7	75.5	58.3	40.7	59.4	9.11**	-0.20	6.64**	0.97**	90.6
Linolenic acid	8.4	18.7	13.5	11.9	13.3	0.08**	4.21**	1.24**	0.04**	52.9
Erucic acid	-10.6	51.0	6.8	54.1	4.5	68.53**	-2.19	11.25**	0.45**	98.6
Sulphur	0.2	0.8	0.4	0.7	0.4	0.003**	0.0001	0.003**	0.0005**	85.4
Sinapate esters	4.3	11.2	7.7	6.1	7.6	0.08**	0.42	0.74**	0.03**	60.7
Phytosterol	2962.2	4408.7	3942.6	2794.1	3879.7	24341.75**	-2516.99	2010.07**	2214.82**	92.6

Data observed at five locations during two years

**Significant at p=0.01

[a]For units see Table 1.

[b,c,d]Minimum, maximum and mean value of all ISLs, respectively

[e,f]Mean value of the donor parent 'Mansholt' and recurrent parent 'Samourai', respectively

σ^2_g, σ^2_y , $\sigma^2_{l(y)}$, σ^2_{gy}, h^2: Variance of genotype, variance of year, variance of location within year, variance of genotype *x* year interaction, and heritability, respectively

Correlations among seed quality traits in the ISLs are presented in Table 16. A significant positive correlation was observed between oil and erucic acid ($r=0.56^{**}$) content. Oil content was significantly negative correlated with protein ($r=-0.77^{**}$) and linolenic acid ($r=-0.56^{**}$) content. A positive correlation was shown between sulphur and glucosinolate ($r=0.92^{**}$) content. Erucic acid content was negatively correlated with phytosterol ($r=-0.96^{**}$), sinapate esters ($r=-0.59^{**}$) and oleic acid ($r=-0.92^{**}$) content. Phytosterol was positively correlated with oleic acid ($r=0.87^{**}$) and sinapate esters ($r=0.59^{**}$) content. A positive correlation was also observed between oleic acid and sinapate esters ($r=0.58^{**}$) content.

Table 16 Correlation among seed quality traits in the ISLs of the cross 'MxS'

	Oil	Protein	GSL	C18:1	C18:3	C22:1	S	SE
Protein	-0.77**							
GSL	-0.19**	0.21**						
C18:1	-0.37**	-0.12*	0.01					
C18:3	-0.56**	0.43**	0.14*	0.10				
C22:1	0.56**	-0.02	-0.05	-0.92**	-0.36**			
S	-0.48**	0.46**	0.92**	0.13*	0.29**	-0.21**		
SE	-0.19**	-0.14*	0.02	0.58**	0.43**	-0.59**	0.09	
Phyto	-0.42**	-0.15*	-0.03	0.87**	0.34**	-0.96**	0.07	0.59**

*, **: Significant at p=0.05 and p=0.01, respectively; Abbreviations: see table 1.

3.8.2. Plant height and phenological trait

Plant height at maturation and phenological traits, namely begin of flowering, end of flowering and duration of flowering were evaluated in the field trials. The distribution of these traits in the ISLs generated from the cross 'MxS' is shown in Figure 13.

Analysis of variance and heritability of plant height and the phenological traits are summarized in Table 17. The effects of genotype and location were highly significant for all traits. Environmental variances were higher than genotypic variances for begin of flowering, end of flowering and duration of flowering. Low heritabilities were detected for end of flowering, duration of flowering and plant height at maturation.

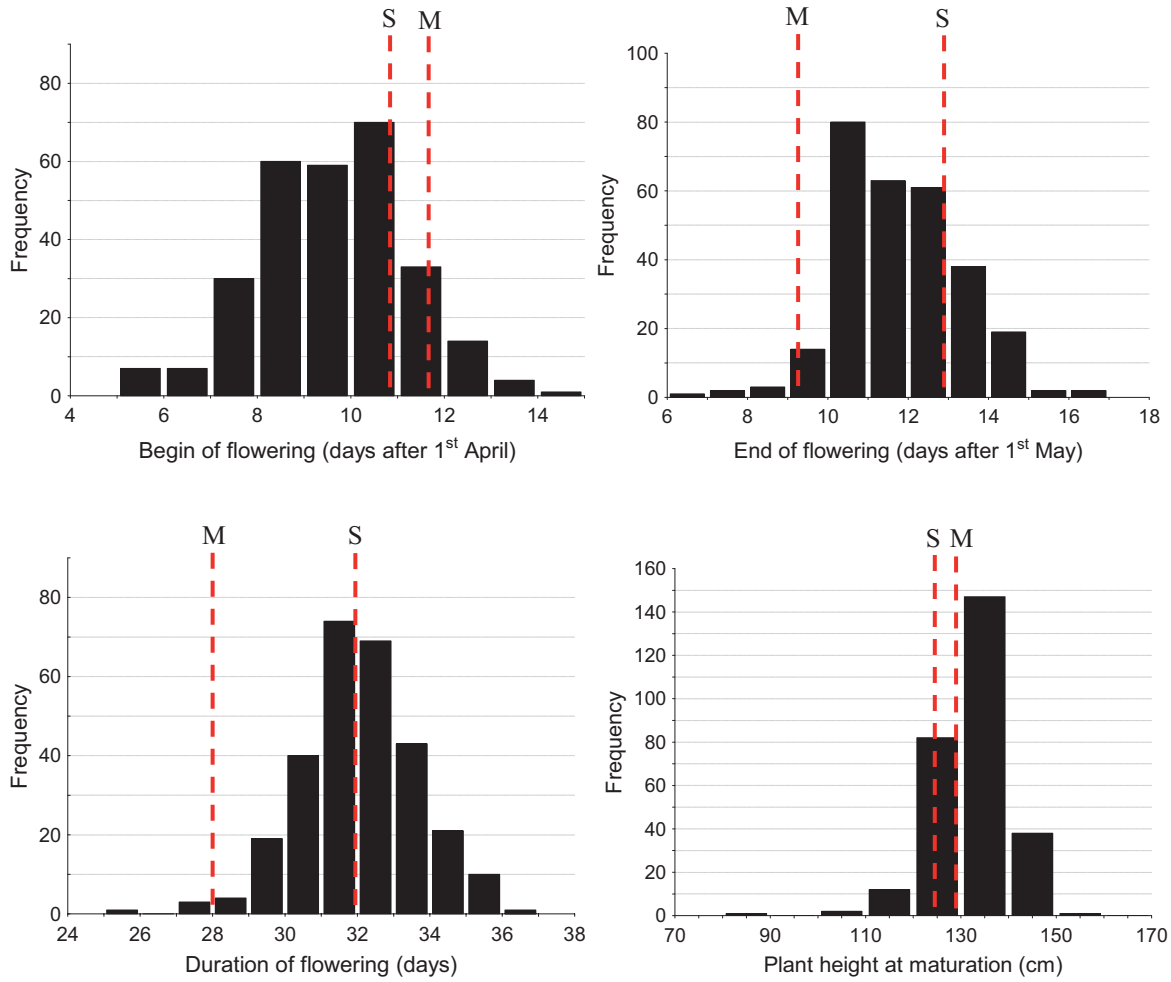

Figure 13 Distribution of plant height at maturation, begin of flowering, end of flowering and duration of flowering (n=284) in the ISLs. Data represent mean values of the ISLs from 2 locations (Goettingen and Grundschwalheim) in the growing season 2006/2007. M: mean value of 'Mansholt'; S: Mean value of 'Samourai'.

Table 17 Analysis of variance and heritability of plant height and phenological traits in the ISLs generated from the cross 'MxS'

Traits[a]	Min[b]	Max[c]	Mean[d]	Mansholt[e]	Samourai[f]	σ^2_g	σ^2_l	h^2
BOF	3.0	16.0	9.5	11.3	10.8	2.1**	4.9**	79.3
EOF	3.0	18.5	11.7	9.1	12.7	1.5**	4.8**	63.5
DOF	24.0	40.5	32.2	27.8	31.9	1.6**	19.7**	60.9
PH at maturation	91.0	173.0	132.6	126.2	124.7	32.2**	6.7**	55.1

Data observed at two locations: Goettingen and Grundchwalheim
**Significant at p=0.01
[a]For units see Table 1.
[b,c,d]Minimum, maximum and mean value of all ISLs, respectively
[e,f]Mean value of the donor parent 'Mansholt' and recurrent parent 'Samourai', respectively
σ^2_g, σ^2_l , h^2: Variance of genotype, variance of location and heritability, respectively

Correlations between the phenological traits and plant height at maturation are presented on Table 18. A significant negative correlation was observed between begin of flowering and duration of flowering (r=-0.58**). End of flowering was positively correlated with begin of flowering (r=0.47**) and duration of fowering (r=0.45**). No correlation was observed between plant height at maturation and phenological traits even though a significant negative correlation was detected between plant height at maturation and begin of flowering, but the value was very low (r=-0.17**)

Table 18 Correlation among plant height and phenological traits in the ISL of the cross 'MxS'

	BOF	EOF	DOF
EOF	0.47**		
DOF	-0.58**	0.45**	
PH at Maturation	-0.17**	-0.10	-0.09

Abbreviations: see table 1.

3.9. QTL Analysis in the ISLs generated from the cross 'MxS'

In the QTL analysis, mean value of all of intervarietal substitution lines was used as a check instead of recurrent parent, 'Samourai'. Considering that it would have a proximity value to the real mean value of the recurrent parent (see Table 15). Many of the recurrent parent were not survive through hard winter and showed bad development on the field.

3.9.1. Seed quality traits

The results of QTL analysis for seed quality traits evaluated in the ISLs are summarized in Table 19. QTL responsible for linolenic acid content was undetected in this analysis.

Glucosinolate content: The QTL for seed glucosinolate content were mapped on linkage groups N03, N09 and N12 with additive effects of 11.79, 26.99 and 11.72 μmol/g, respectively. The QTL on N09 showed the largest effect. However the effect could not be directly compared to the effect of the other QTL, since it was estimated in the selfed seed using HPLC. The ISL carrying the QTL was excluded from field experiment due to not enough seed available. All of the QTL showed alleles increasing seed glucosinolate content contributed by the donor parent.

Erucic acid content: Two genes responsible for erucic acid content could be mapped on linkage groups N08 and N13 in the maximal interval of 19.3 and 6.9 cM, with additive effects of 15.24 and 15.52%, respectively. The genes were mapped in a maximal interval of 19.3 cM and 6.9 cM on linkage group N08 and N13, respectively.

Oil content: Five QTL were detected on linkage groups N01, N08, N09, N12 and N13 (Table 19). Donor alleles decreasing oil content were identified on linkage groups N01 and N12 with negative additive effects of -1.49% and -1.69%, respectively. Three donor alleles increasing oil content were detected on linkage groups N08, N09 and N13, which together sum up to a total of 5.51% additive effect. The QTL on N08 and N13 represents the two of well investigated erucic acid genes.

Protein content: Two QTL were localized on linkage groups N01 and N09 (Table 19). These QTL together have a total additive effect of 3.2% seed protein content showing alleles increasing protein content contributed by the donor parent 'Mansholt'.

Oleic acid, sinapate esters and phytosterol content: Two QTL were detected on linkage groups N08 and N13 responsible for oleic acid, sinapate esters and phytosterol content (Table 19). The two QTL represent the two well studied erucic acid genes. All of the ISLs having significantly high erucic acid content were lines carrying donor alleles reducing oleic acid, sinapate esters and phytosterol content (Table 20). The two QTL showed negative additive effects, with a total of -10.98% on oleic acid, -0.92 mg/kg on sinapate esters and -691.64 mg/g on phytosterol content, showing alleles decreasing the traits contributed by the donor parent.

Table 19 QTL and their additive effects detected in the ISL from the cross 'MxS'

Trait	No.	LG	Marker/marker interval	Max. QTL Interval[1] (cM)	Position [2]	Additif effect[3]
Glucosinolate content	1.	N03	E32M61-218M-E33M50-165M	3.7	52.1-55.8	11.79
	2.	N09	E33M50-155M	9.6	-3.5-6.1	26.99[+]
	3.	N12	E38M59-243S/E32M50-339M	#	99.3	11.72
Erucic acid content	1.	N08	E33M59-564-M – E32M47-335-M	19.3	42.0-61.7	15.24
	2.	N13	E32M47-202S – E38M62-129M	6.9	114.1-121.2	15.52
Oil content	1.	N01	E38M59-289M	11.2	30.8-42.0	-1.49
	2.	N08	E33M59-564-M – E32M47-335-M	19.3	42.0-61.7	1.59
	3.	N09	E3363-204-S	19.8	68.5-88.3[*]	1.97
	4.	N12	E32M62-186S	8.9	45.4-54.3	-1.69
	5.	N13	E32M47-202S – E38M62-129M	6.9	114.1-121.2	1.95
Protein content	1.	N01	E38M59-289M	11.2	30.8-42.0	1.47
	2.	N09	E33M50-M001S/E35M60-312S	3.5	-4.7- (-1.2)	1.74
Oleic acid content	1.	N08	E33M59-564-M – E32M47-335-M	19.3	42.0-61.7	-5.42
	2.	N13	E32M47-202S – E38M62-129M	6.9	114.1-121.2	-5.56
Sinapate esters content	1.	N08	E33M59-564-M – E32M47-335-M	19.3	42.0-61.7	-0.51
	2.	N13	E32M47-202S – E38M62-129M	6.9	114.1-121.2	-0.41
Phytosterol content	1.	N08	E33M59-564-M – E32M47-335-M	19.3	42.0-61.7	-308.80
	2.	N13	E32M47-202S – E38M62-129M	6.9	114.1-121.2	-282.84

[1]: Size of maximal interval where the QTL could be located
[2]: Start and end point of maximal interval of QTL
[3]: For units see Table 1.
[*]: QTL position could also locate on end of the linkage group where the region is uncovered by the map
[#]: QTL interval could not be estimated
[+]: Effect was estimated in selfed seed produced in green house using HPLC

Table 20 ISL having significant differences in erucic acid, oleic acid, phytosterol and sinapate esters content compared to the recurrent parent according to Dunnet multiple comparisons at significance level of 5% in the cross 'MxS'

Nr	DH lines	Erucic acid (%)[1]	Oleic acid (%)[1]	Phytosterol (mg/kg)[1]	Sinapate esters (mg/g)[1]	Linkage group
1.	578	33.75	-10.82	-672.73	-0.53	N08
2.	789	29.32	-9.97	-544.05	-0.35[ns]	N08
3.	842	30.44	-13.61	-638.99	-0.71	N08
4.	873	31.76	-11.49	-681.42	-0.37[ns]	N08
5.	1150	27.02	-11.51	-559.28	-0.42[ns]	N08
6.	1572	30.55	-10.39	-630.53	-0.66	N08
7.	44	32.22	-12.80	-573.56	-0.55	N13
8.	156	31.22	-10.73	-563.60	-0.52[ns]	N13
9.	186	31.42	-9.77	-622.37	-0.36[ns]	N13
10.	187	26.98	-7.65	-524.58	-0.45	N13
11.	188	32.39	-10.67	-644.78	-0.29[ns]	N13
12.	189	31.38	-12.15	-549.16	-0.40[ns]	N13
13.	223	29.18	-12.06	-520.53	-0.42	N13
14.	814	31.96	-11.70	-535.28	-0.45[ns]	N13
15.	831	34.26	-12.26	-624.69	-0.41[ns]	N13
16.	863	28.83	-11.28	-546.27	-0.34[ns]	N13
17.	869	31.50	-11.20	-517.66	-0.52	N13

[ns]The effect was not significantly different compared to the check
[1]Effect of introgressed segment

3.9.2. Plant height and phenological traits

The mapping of QTL for plant height and phenological traits is summarized in Table 21. We could not identify QTL for end of flowering time in the ISLs. There was no ISL significantly differed to the check for end of flowering according to the Dunnet's multiple comparisons.

Begin of flowering: Three QTL were detected on linkage groups N05, N17 and N19. Two of the QTL on linkage groups N05 and N19 showed negative additive effects indicating that the donor alleles cause an earlier begin of flowering. The total additive effect of the two QTL was -3.64 days. A QTL with a positive additive effect of 1.54 days was observed on N17 showing donor allele delaying begin of flowering.

Duration of flowering: A QTL for duration of flowering was co localized on QTL for begin of flowering on linkage group N19. It indicates a pleiotropic effect or closely linked QTL for these traits. The positive additive effect of 2.08 days of the QTL showed allele for longer flowering time contributed by the donor parent.

Plant height at maturation: Three QTL were mapped on linkage groups N10, N12 and N16 with additive effects of 8.87, 13, and 8.81 cm respectively. The positive signs of additive effects showed alleles for higher plant height at maturation contributed by the donor parent. Together the QTL sum up to 30.68 cm of additive effect.

Table 21 QTL for plant height and phenological traits and their additive effects detected in the ISL from the cross 'MxS'

Trait	No.	LG	Marker/marker interval	Max. QTL Interval[1] (cM)	Position [2]	Additif effect[3]
Begin of flowering	1.	N05	E35M62-111S – E32M62-261S/E35M60-61S	8.7	46.3-50.4	-1.71
	2.	N17	E32M50-325S	5.8	22.5*-28.7*	1.54
	3.	N19	E32M49-409S	24.8	13.6-38.3	-1.93
Duration of flowering	1.	N19	E32M49-409S	24.8	13.6-38.3	2.08
PH at Maturation	1.	N10	E32M48-212S	1.2	64.5-65.7*	8.87
	2.	N12	E32M62-178M	12.7	34.0-46.7	13.00
	3.	N16	E40M60-199M – E33M59-276S	3.6	57.9-61.5*	8.81

[1]: Size of maximal interval where the QTL could be located
[2]: Start and end point of maximal interval of QTL
[3]: For units see Table 1.
*: QTL position could also locate on end or beyond of the linkage group where the region is uncovered by the map

3.10. Comparison of QTL detected in the ISLs and F_1DH population generated from the cross 'MxS'

The QTL mapped in the ISL population were compared with QTL that had been mapped in the F_1DH population from the cross of 'MxS (Uzunova et al. 1995; Ecke et al. 1996; Gül 2002; Amar et al. 2008).

Glucosinolate content: Three QTL for seed glucosinolate content were mapped on linkage group N03, N09 and N12 In the ISLs of the cross 'MxS' (Table 22). Uzunova et al. (1995) and Gül (2002) localized four QTL for seed glucosinolate content on linkage groups LG2, LG9, LG16 and LG18 corresponding to N16, N19, N09 and N12, respectively. Two QTL on N09 and N12 that had been mapped in the F_1DH population could be detected and mapped in the same region (Figure 14). The other two QTL on linkage group N16 and N19 mapped in the F_1DH population are undetected in the ISLs. The regions where the QTL on N16 and N19 had been mapped in the early mapping population were not covered by donor segments. A new QTL for glucosinolate content was identified on linkage group N03 in the ISLs that was not detected before in the segregating population.

It was observed that the additive effects of the QTL for seed glucosinolate content on N12 in the ISLs was 11.7, while in the F_1DH was estimated to be 4.3 µmol/g (Uzunova et al. 1995) or 6.0 µmol/g (Gül 2002). One of the major QTL with the largest effect of 7.8 µmol/g found on N09 in the F_1DH population was also detected as the QTL with the largest additive effect in the ISL with 26.99 µmol/g of seed glucosinolate content. Nevertheless, this result is not comparable since it was estimated in the selfed seed produced in the greenhouse using HPLC.

Erucic acid content: Two genes controlling the seed erucic acid content of *Brassica napus* were mapped using the F_1DH population on LG6 and LG12 (Ecke et al. 1996), corresponding to N8 and N13. In the ISLs, the two erucic acid genes were also mapped in the same region (Figure 15, Table 23). The erucic acid genes were mapped in the maximal intervals of 19.3 and 6.8 cM for the genes on N08 and N13, respectively. On the other hand, they were mapped in the larger interval of 22 and 14 cM, respectively, using interval mapping in the F_1DH population.

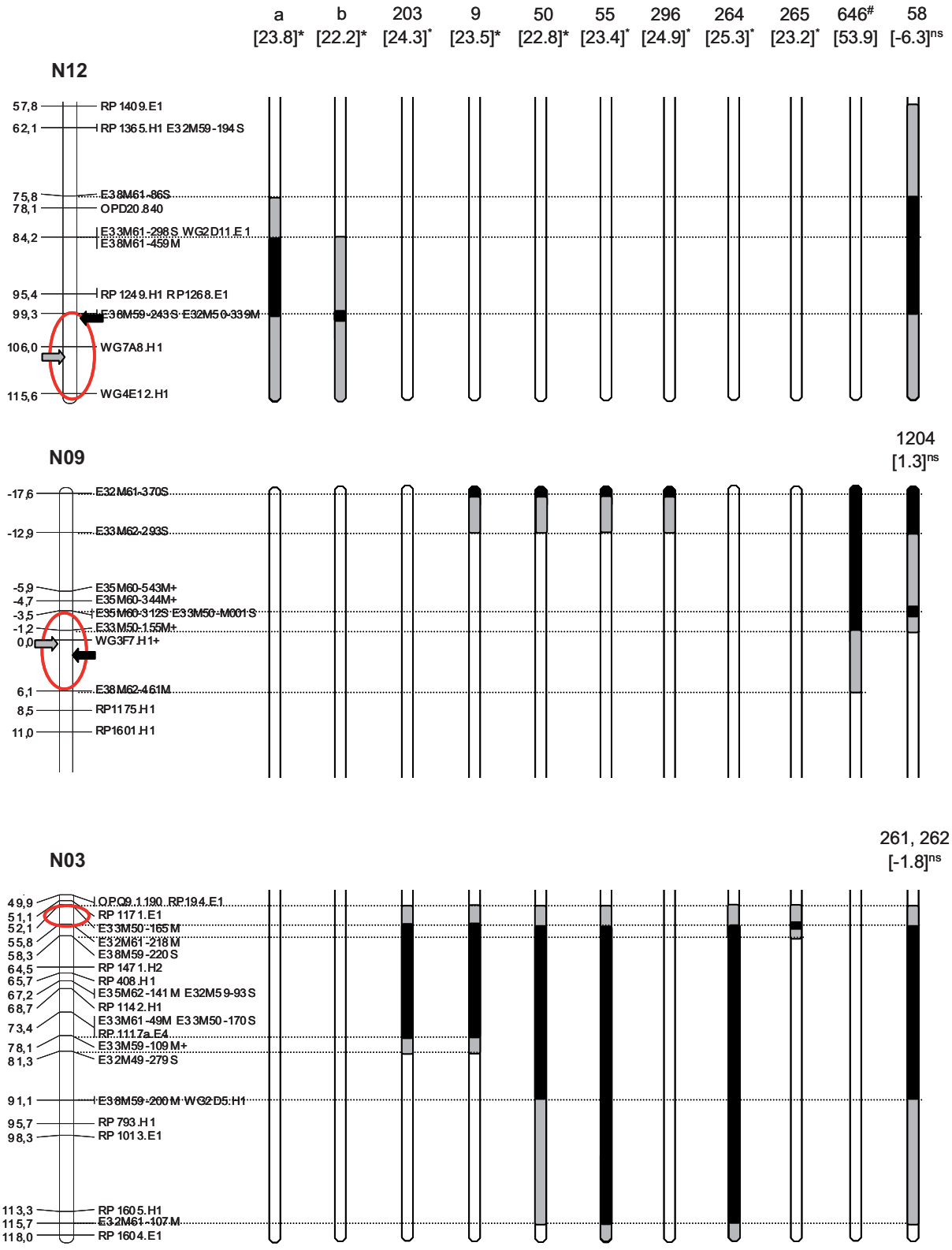

Figure 14 Analysis of QTL for seed glucosinolate content using the ISLs. Black bar: minimum length of donor segment ('Mansholt') carried by the lines; grey bar: possible maximum extension of donor segments; red circle: maximal interval for the QTL localization; black arrow: QTL position mapped using F_1DH population according to Uzunova et al. 1995, grey arrow: QTL position mapped using F_1DH population according to Gül 2002. *lines with significant effect for seed glucosinolate content (a, b: see Table 22); nslines that have no significant effect; #QTL effect was estimated in selfed seed produced in green house using HPLC; in parenthesis: QTL effect for seed glucosinolate content (µmol/g).

Table 22 ISL with significant differences in seed glucosinolate content compared to the check in the cross of 'MxS'

No.	DH lines	EIS[1] [µmol/g]	LG[2]	Group[3]	No. of donor segment[4]	Segment on linkage group[5]
1.	227	20.25	N12	a	3	N19[69.8], N11[14.9], **N12[99.3]**
2.	293	15.75	N12	a	4	N10[37.6], N13[-18.6- -9.0], N14[75.1-81.4], **N12[99.3]**
3.	387	30.55	N12	a	4	N19[13.6][38.3-69.8], N11[14.9], **N12[99.3]**
4.	117	26.85	N12	b	3	N01[42.0-67.2], N04[7.2], **N12[84.2-99.3]**
5.	257	18.32	N12	b	2	N09[40.5-42.6], **N12[84.2-99.3]**
6.	258	25.70	N12	b	2	N09[56.9], **N12[84.2-99.3]**
7.	260	21.87	N12	b	1	**N12[84.2-99.3]**
8.	294	24.56	N12	b	4	N09[72.6-84.2], N05[46.3-47.5], N04[7.2], **N12[84.2-99.3]**
9.	430	32.82	N12	b	5	N01[16.1], N14[75.1], N09[56.9], N04[7.2], **N12[84.2-99.3]**
10.	498	17.36	N12	b	3	N09[-18.6-84.2], N04[7.2], **N12[84.2-99.3]**
11.	499	31.82	N12	b	2	N09[40.5-84.2], **N12[84.2-99.3]**
12.	576	18.95	N12	b	1	**N12[84.2-99.3]**
13.	864	19.99	N12	b	3	N13[40.5-42.6], N09[56.9], **N12[84.2-99.3]**
14.	203	24.34	N03	-	3	N19[69.8], N11[14.9], **N03[55.8-78.1]**
15.	9	23.46	N03, N09	-	3	N13[139.4], **N03[55.8-78.1]**, **N09[-17.6]**
16.	50	22.56	N03, N09	-	2	**N03[55.8-91.1]**, **N09[-17.6]**
17.	55	23.44	N03, N09	-	3	N13[139.4], **N03[55.8-115.7]**, **N09[-17.6]**
18.	296	24.93	N09	-	2	N03[115.7], **N09[-17.6]**
19.	264	25.27	N03	-	1	**N03[55.8-115.7]**
20.	265	23.15	N03	-	1	**N03[55.8]**
21.	124	24.03	-	-	1	N13[-9.0]
22.	1034	6.62	-	-	0	-
23.	1395	9.27	-	-	1	N12[46.7-62.1]

[1]Effect of introgressed segment, see part 2.9
[2]Linkage group where the donor segment is relevant for the trait
[3]Group of lines with different length of relevant donor segment
[4]Number of donor segments carried by ISLs
[5]Linkage group respectively the donor segments (see note Table 10.)

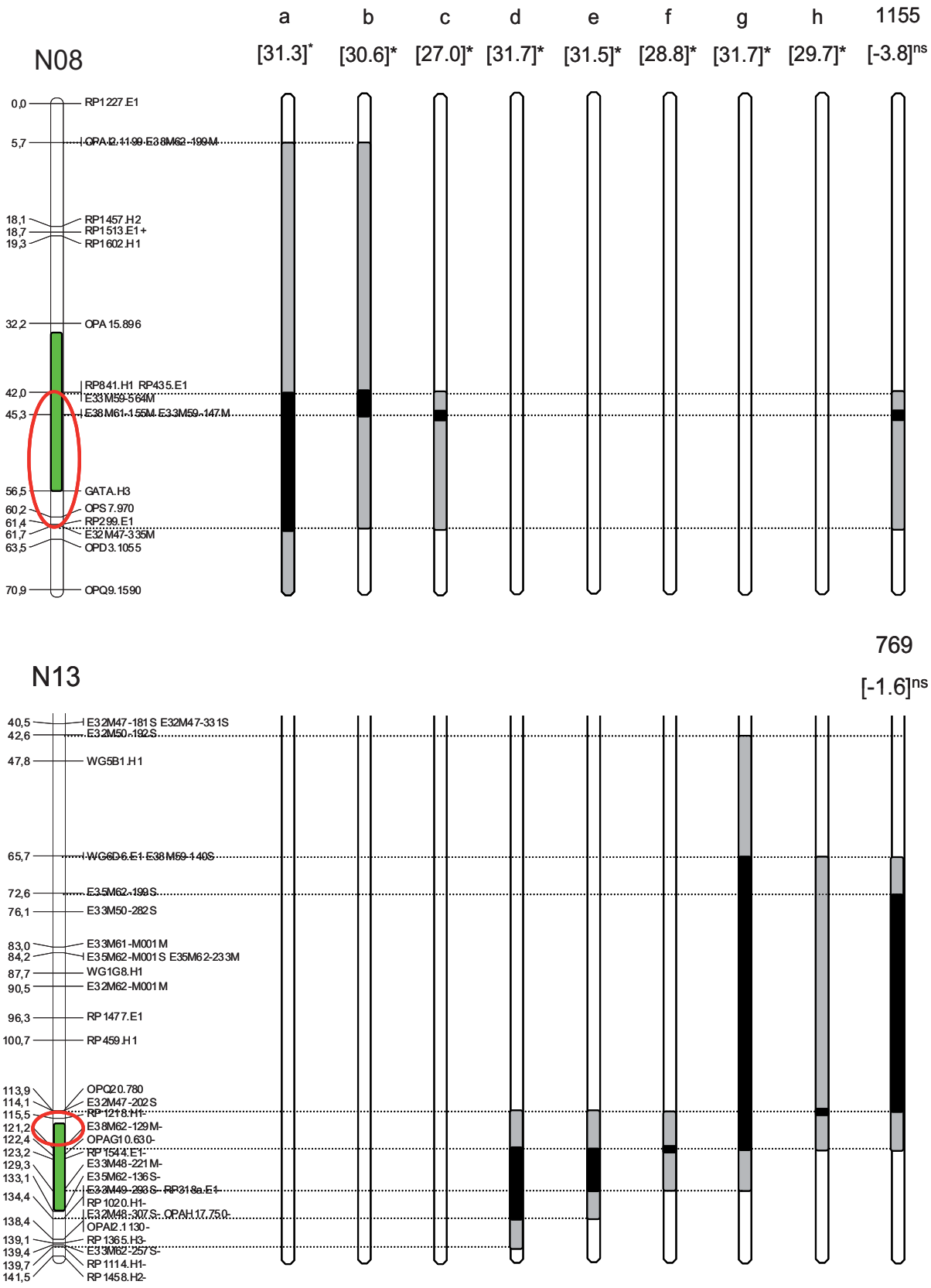

Figure 15 Localization of the erucic acid genes using the ISLs. Black bar: minimum length of donor segment ('Mansholt') carried by the lines; grey bar: possible maximum extension of donor segments; green bar in linkage group: QTL interval mapped using F_1DH population (Ecke et al., 1995); red circle: maximal interval for the QTL localization; *lines with significant effect for erucic acid content (a-h: see Tabel 23); nslines that have no significant effect; in parenthesis: effect of erucic acid content (%).

Table 23 ISL with significant differences in erucic acid content compared to the recurrent parent in the cross 'MxS'

No.	DH lines	EIS[1] (%)	LG[2]	Group[3]	No. of donor segment[4]	Segment on linkage group[5]
1.	578	33.75	N08	a	2	**N08[42.0-61.7]**, N13[133.41-134.4]
2.	789	29.32	N08	a	1	**N08[42.0-61.7]**
3.	842	30.44	N08	a	2	**N08[42.0-61.7]**, N12[46.7-54.3]
4.	873	31.76	N08	a	1	**N08[42.0-61.7]**
5.	1572	30.55	N08	b	1	**N08[42.0-45.3]**
6.	1150	27.02	N08	c	4	N18[2.4], **N08[45.3]**, N10[37.6], N14[0.0-81.4]
7.	156	31.22	N13	d	3	N10[65.7], **N13**[65.7-76.1]**[121.2-134.4]**
8.	223	29.18	N13	d	2	**N13**[76.1] **[121.2-134.4]**
9.	814	31.96	N13	d	3	**N13**[65.7-76.1]**[121.2-134.4]**, N12[45.4]
10.	831	34.26	N13	d	2	**N13**[65.7-83.0]**[121.2-134.4]**
11.	869	31.50	N13	e	2	**N13**[65.7-76.1]**[121.2-129.3]**
12.	863	28.83	N13	f	2	**N13**[65.7-76.1]**[121.2]**
13.	44	32.22	N13	g	3	N07[20.8-45.1], **N13[65.7-121.2]**, N09[-17.6--12.9]
14.	186	31.42	N13	g	3	N18[-23.1], N07[20.8-45.1], **N13[65.7-121.2]**
15.	189	31.38	N13	g	3	N07[20.8-45.1], **N13[65.7-121.2]**, N09[-17.6- -12.9]
16.	187	26.98	N13	h	4	N07[20.8-45.1], N01[67.2-70.8], **N13**[65.7]**[114.1]**
17.	188	32.39	N13	h	4	N07[20.8-45.1], N01[67.2-70.8], **N13**[72.6-76.1]**[114.1]**

[1]Effect of introgressed segment, see part 2.9
[2]Linkage group where the donor segment is relevant for the trait
[3]Group of lines with different length of relevant donor segment
[4]Number of donor segments carried by ISLs
[5]Linkage group respectively the donor segments (see note Table 10.)

Oil content: Ecke et al. (1996) mapped two QTL for oil content in the same interval of the two erucic acid genes. Gül (2002) mapped 5 QTL for oil content on linkage groups LG05, LG06, LG11, LG12, and LG15 corresponding to N01, N08, N06, N13, and N05, respectively. The two QTL on linkage groups N08 and N13 corresponding to the two erucic acid genes were also detected as the QTL responsible for oil content in the ISLs. The additive effects were 1.17% and 0.78% for QTL on N08 and N13, respectively. Higher additive effects were observed in the ISL population with 1.59% and 1.95% for QTL on N08 and N13, respectively. Other QTL on linkage groups N01, N05 and N06 mapped in the F_1DH population were not detected in the ISLs, since they were mapped in the regions that were uncovered by the donor segments. New QTL were detected on linkage groups N01, N09 and N12 using ISL population that were not detected in the F_1DH population (Table 19).

Protein content: Four QTL were mapped on linkage groups LG05, LG11, LG14, and LG15 (Gül 2002), corresponding to N01, N06, N03, and N05, respectively. All of these QTL were undetected in the ISLs. The QTL on linkage groups N05 and N06 could not be identified, because they were mapped in the regions that were uncovered by the donor segments. Two QTL on linkage groups N01 and N03 were detected as false positive QTL. The QTL could not be detected in the ISL population, although the region was covered by the donor segments (Figure 16). New QTL were mapped on linkage groups N01 and N09 (Table 19) using ISL population.

Phytosterol and Sinapate esters content: Two major QTL for phytosterol and sinapate esters content were mapped in the same interval with the two erucic acid genes in the F_1DH population (Amar et al. 2008). The same results were also found in this study, showing that QTL for phytosterol and sinapate esters content were co localized with the two erucic acid genes. The additive effects detected in the F_1DH for QTL on N08 and N13 were 205 and 172 mg/kg for phytosterol content, and 0.40 and 0.31 mg/g for sinapate esters content, respectively. In the ISLs the QTL were observed to be 309 and 283 mg/kg for phytosterol content, and 0.51 and 0.41 mg/g for sinapate esters content (Table 19), respectively, showing higher additive effects than that in the F_1DH population. The other minor QTL on linkage group N18 for total phytosterol content, and on N05 and N06 for total sinapate esters content that were mapped in the F_1DH population could not be detected in the ISL population. Since these regions were not covered by the donor segments.

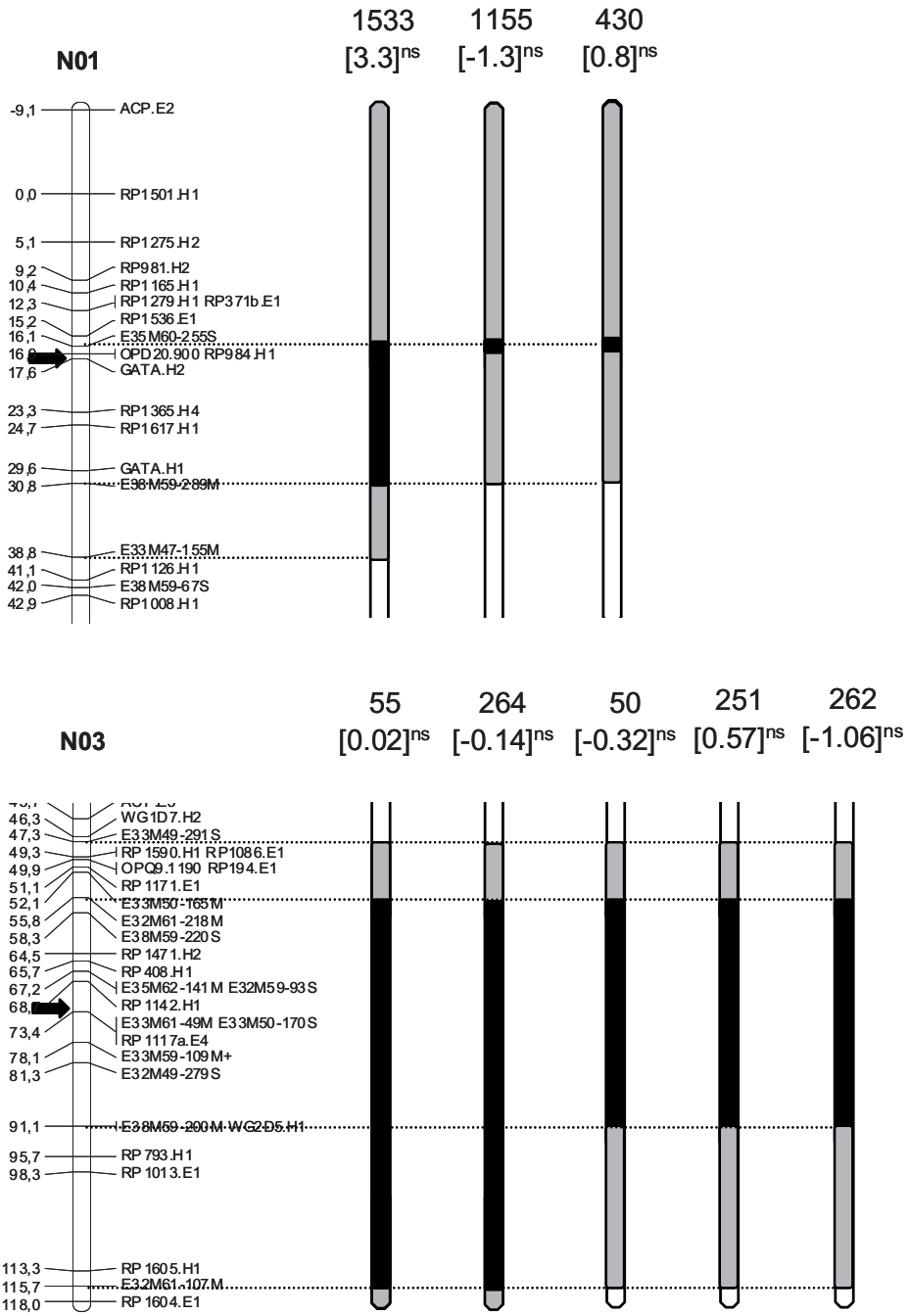

Figure 16 Detection of false positive QTL for protein content mapped using F_1DH population in the cross of 'MxS'. Black bar: minimum length of donor segment ('Mansholt') carried by the lines; grey bar: possible maximum extension of donor segments; black arrow: QTL position mapped using F_1DH population with additive effects of QTL on N01=[-0.55] and N03=[0.3] (Gül 2002); [ns]lines that have no significant effect ; in parenthesis: effect of protein content (%) carried by ISLs.

Plant height at maturation: Three QTL were mapped on linkage groups LG02, LG05, and LG19 (Gül 2002), corresponding to N16, N01, and N09. The QTL were undetected in the ISL population. The two QTL on linkage groups N01 and N09 were mapped in the regions that were uncovered by the donor segments, while QTL on N16 was detected as a false positive QTL (Figure 17).

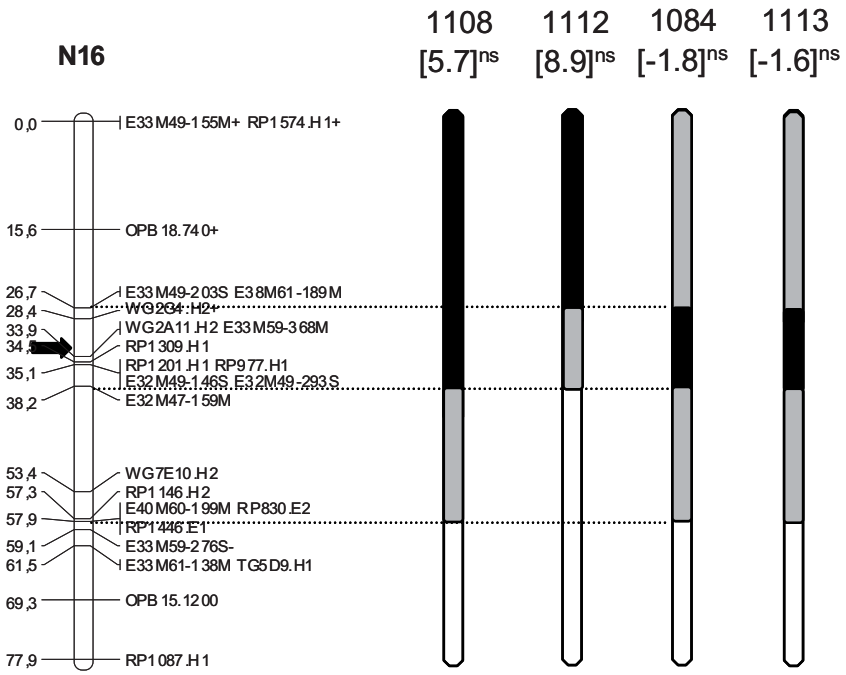

Figure 17 Detection of false positive QTL for plant height mapped using F1DH population in the cross of 'MxS'. Black bar: minimum length of donor segment ('Mansholt') carried by the lines; grey bar: possible maximum extension of donor segments; black arrow: QTL position mapped using F_1DH population with additive effects of QTL on N16=[2.4] (Gül 2002); ^{ns}lines that have no significant effect ; in parenthesis: effect of plant height (cm) carried by ISLs.

Phenological traits: Gül (2002) mapped four QTL for begin of flowering on linkage groups LG02, LG04, LG08 and LG09, corresponding to N16, N02, N10 and N19, respectively; four QTL for duration of flowering on N02 (on two position), N10 and N16; four QTL for end of flowering on LG03, LG07, LG09 and LG16, corresponding to N07, N17, N19 and N09. In this study, all of those QTL could not be detected, since they were mapped in the regions that were uncovered by the donor segments. For end of flowering, there was no line with significant difference compared to the recurrent parent according to Dunnets's multiple comparisons in this study.

4. Discussion

4.1. The genetic linkage map developed from the cross 'ExRS239'

The genetic linkage map constructed in the F1DH population from the cross 'ExRS239' covers 2003 cM of the rapeseed genome with 724 AFLP and SSR markers dispersed on 568 distinct loci resulting in a marker density one marker per 2.77 cM (Table 2). In a cross between the winter cultivar of resynthesized line 'R53' and 'Express', Radoev et al. (2008) developed a map with 363 markers, consisted of 243 SSR and 120 AFLP markers covering 1916 cM of the rapeseed genome at a marker density of one marker per 5.28 cM. The marker density between the maps was not directly comparable since the markers type and markers number applied was different.

Lombard and Delourme (2001) constructed three individual maps from a cross of winter type cultivar 'Darmor *bzh*' and a spring-type rapeseed 'Yudal' (DY), Two winter type lines 'Darmor' and 'Samourai' (DS), and two spring type lines 'Stellar' and 'Drakkar'. Their maps cover about 2023, 1574 and 1911 cM with 590, 344 and 340 mapped markers for each of the populations, respectively. The map developed from the cross between winter and spring type rapeseed (DY) showed the highest density map with an average marker distance of 3.6 cM per marker, compared to 5.0 and 6.2 cM per marker for the DS and SD maps. This might indicate that a wide cross species exhibiting high polymorphisms between the parental genotypes could result in a high density of markers mapped.

A high correlation of r=0.88 ($P=10^{-6}$) between the length and number of markers per linkage group was shown in the linkage map developed in this study. It indicates that the markers are evenly distributed across the genome. However, a non-uniform marker distribution was observed in some linkage groups, for instance, gaps of more than 20 cM were found on linkage groups N03, N14, LG20 and two of these gaps on N17 (Figure 5). On the other hand, some marker clusters were observed on linkage groups N02, N06, N09, N12, N15 and N19. The gap and the clustering of markers might be due to differences in the relation of physical distances and recombination frequencies in different parts of the genome (Tanksley et al. 1992; Lichten and Goldman 1995).

Lombard and Delourme (2001) estimated the range of the size of the rapeseed genome from 2.127 to 2.480 cM in a study on consensus linkage map construction. Based on this estimation, the map developed in this study with a length of 2003 cM covered about 81 to 94% of the rapeseed genome. A larger map of 2,619 cM was developed by Piquemal et al.

(2005). In relation to this map our genetic linkage map covers 76.5% of the largest rapeseed map developed so far. Nevertheless the size of the map is not easily comparable, since the type of mapping population and the mapping function applied were different.

In contrast to the genetic linkage map developed in an F_2 population by Piquemal et al. (2005), which could inflate the size of the map, the map developed in this study was based on the principle of a high fidelity (HF) map. In this HF map, all marker orders are supported by minimal log-likelihood difference of 3.0 with a maximal distance to the previous marker of 30 cM. Other markers were only placed individually relative to the markers of the HF map without changing the distances between the HF markers. The similar principle was applied by Qiu et al (2006) in the development of a comparative linkage map referred as a high stringency linkage map for the QTL analysis of seed oil and erucic acid content.

After an alignment to previously established maps (Lowe et al. 2004; Piquemal et al. 2005; Radoev et al. 2008; Ecke et al. 2010 and Sharpe and Lydiate, unpublished data;), linkage group N16 could not be identified, N18 remained split into two unlinked parts, and four additional small linkage groups could not be aligned to the reference maps (Figure 5). This indicated an incomplete coverage of the entire genome mapped in the cross used in this study.

In this study linkage group N16 of the C genome of rapeseed could not be identified. It might be that some of the unmapped markers actually refer to loci from N16. There were about 21% unmapped markers of which 96% were markers with strongly skewed segregation. The failure to detect N16 was already observed in the genetic linkage map developed by the breeding company KWS, who used the SSR markers in the cross of ’Express’x‘RS239’. These SSR markers have been integrated in our map and with an addition of AFLP markers which were two times SSR markers number, the linkage group N16 still can not be mapped. Butruille et al. (1999) who introgressed a winter-type cultivar into a spring-type cultivar of *B. napus* in the development of an inbred backcross lines population, observed that the population had unbalanced allele frequencies and no loci from N16 segregated in the population.

4.2. Distorted segregations

A high number of segregation distortions of the mapped markers (38%) were observed in this research. Additionally, there were about 21% unmapped markers in which 93% of it were markers with strongly skewed segregation. Segregation distortion has been documented in a number of species, including rapeseed (Thompson et al. 1991; Uzunova et al. 1995; Foisset and Delourme 1996; Foisset et al. 1997). There are two main hypotheses that could explain their existence: gametic selection or selection specific to in vitro microspore culture (Foisset et al. 1996). It was reported earlier that in microspore-derived DH populations, disturbed segregation was a common feature (Uzunova et al. 1995; Piquemal et al. 2005). A general explanation is due to specific parental alleles favourable for in vitro androgenesis or subsequent plant regeneration.

Ferreira et al. (1994) compared maps constructed from a doubled haploid and an F_2 population generated from the same F_1 plant derived from a cross between an annual canola cultivar (Stellar) and a biennial rapeseed (Major). In that study a disturbed segregation for 30% of the markers in the doubled haploid and for 24% in the F_2 population was observed. The high number of segregation distortions in the F_2 population showed that there were other factors besides the response to microspore culture influencing the selection of specific genotypes. In some populations developed from parental with very different responses to androgenesis, similar numbers of loci with non-Mendelian segregation in favour of the alleles of one or the other parent was observed (Foisset et al. 1996). Xu et al. (1997) observed that there were no significant differences of distorted markers between F_2, BC and DH populations in rice.

Radoev (2008) observed in a cross between the winter cultivar ‘Express’ and the resynthesized line ‘R53’, a high percentage of disturbed segregation markers of 31.4% of the mapped markers. In another study, Lombard and Delourme (2001) noticed a higher frequency of markers that did not segregate according to the expected Mendelian ratio in a cross between winter and spring type rapeseeds (24.6%) than in crosses of two winter (14%) or two spring type (8.8%) parents. In this study a cross between a spring type resynthesized rapeseed ‘RS239’ and an inbred line of winter type cultiver ‘Express’ exhibited about 38% of mapped markers showing significant deviation from the expected 1:1 segregation ratio. A high percentage of distorted segregation markers could be caused by the wide genetic distance between the parental used in the cross. McGrath and Quiros (1991) suggested that a relatively large number of disturbed segregation might be due to

genomic divergences between the parents of the crosses and the increasing of deviations in accordance with the level of divergence (Kianian and Quiros 1992). In rice, it has been also reported that wide crosses between sub-species can lead to a higher frequency of markers with distorted segregation (Lin et al. 1992, Lin and Ikehashi 1993).

Another factor that could explain the high number of markers with disturbed segregation observed in the cross of 'ExRS239' was the obstacle in the development of the mapping population. In the production of F_1DH lines as the mapping population, many of the microspore derived plants could not survive through the winter. The plants were grown in the field instead of in the green house for technical reason. One of the parental genotype used in this cross is a spring type rapeseed, resynthesize line'RS239', that has low winter hardiness and not well adapted to Germany growing condition.

4.3. Detection of duplicated regions

Rapeseed (*Brassica napus* L.) is an amphidiploid species with the A and C genomes derived from *Brassica rapa* (A genome) and *Brassica oleraceae* (C genome). Cytogenetic investigations indicated the relatedness between the basic *Brasssica* genomes (Attia and Röbbelen, 1986). The genomes are partially homologous and apparently derived from a common ancestral genome (Mizushima, 1950; Song et al. 1990, Lagercrantz and Lydiate 1996).

A high degree of duplications within the diploid genomes of *B. rapa* and *B. oleracea* have also been described in previous mapping studies (Song et al. 1991; Kianian and Quiros, 1992; Lan et al. 2000; Parkin et al. 2002). A high level of similarities between the homoeologous A and C genomes explaining the duplication of *Brassica napus* loci was reported in some investigations (Ferreira et al. 1994; Parkin et al. 1995; Uzunova et al. 1995; Scheffler et al. 1997; Mayerhofer et al. 2005; Udall et al. 2005). Analysis of synteny between the A, B and C genomes of the *Brassica* species identified the homoeologous linkage groups of the three genomes. It shows that the shape and ancestral blocks in all of the three diploid *Brassica* genomes remained unaltered during evolution and only minimal macro-level changes after polyploidization (Panjabi et al. 2008).

In this study, thirty-eight SSR primer pairs amplified two genetic loci in the linkage map showing the duplication of loci between the A and C genome, allowing the detection of regions where homoelogy is obvious. An unambiguous homoelogy involving 5 primer pairs between N1 and N11 covering 99 and 73 cM, respectively, with the same order of

markers (Figure 6) shows a large syntenious region between these two linkage groups. Piquemal et al. (2005) observed homoeology between linkage groups N1 and N11 covering regions of 100 and 80 cM, respectively, which is in line with results of Parkin et al. (2003) who showed that those two linkage groups are completely collinear.

The number of SSR markers is only 33.15% of the total markers mapped in the linkage map. Therefore the result found in this study is not optimal for homoeology analysis. Because of this, the detection of homoeologous regions between the A and C genome in this study has to be considered to be at the lower limit of the extent of duplications in the rapeseed genome.

4.4. Effect of colchicines re-treatment in increasing the frequency of doubled haploid plants production

Flow cytometry is a valuable tool for an early determination of ploidy level. The flower characteristic and the fertility of the plants observed in the greenhouse showed a correlation to flow cytometry results. All plants that were tetraploid according to flow cytometry had bigger flowers and buds compared to diploid plants. About 79% of the plants, which were diploid according to flow cytometry, were fertile (data not shown). No plant that was haploid according to flow cytometry showed seed set. Weber et al. (2005) also found a high correlation (R^2 = 0.97) between diploidization rates as determined by flow cytometry and plant fertility.

The haploid plants can be treated with colchicine by root immersion as soon as possible after the acclimatization, which will lead to a higher diploidization rate than at later developmental stages (Table 3). Foisset et al. (1997) applied colchicine treatment at the four-to-five-leaf stage of haploid plants before vernalization with three hours immersion of roots and achieved a higher diploidization rate compared to the results in our study with 57% of fertile plants.

The cutting immersion method can be used as an alternative to increase the number of homozygous lines. By immersing cuttings in 0.3% of colchicine for 3 hours the diploidization rate of haploid plants could be increased to 48% (Table 3). The high rate of diploidization obtained with this method might be because the absorption of the colchicine is more efficient through the wounded stem surface than by the root system. Another possible reason is because the size of the explants used is smaller and consequently the colchicine can more easily reach all of the cells.

An added advantage of this method is its easy applicability. The shoot with 2 or 3 leaves was simply cut compared to washing the root of the plant for root immersion. In addition, more than one cutting can be taken per plant to produce more seeds in one step. The efficiency rate of diploidization can be further increased by treating more cuttings with colchicine solution. Colchicine solution can be re-used in cutting immersion method in contrast to the root immersion, where the colchicine solution was more diluted and dirty because of the difficulty of cleaning the roots.

The number of doubled haploid plants regenerated also depends on the genotypes used. The number of plants regenerated and the fraction of fertile plants differed for the different donor plants (Figure 7). Large variation caused by genotypes was reported earlier (Zamani et al., 2000; Zhou et al., 2002). However, the 51 donor plants used in the present study are an ISL population, which have a very similar genetic background and differ only in small introgessed segments of the donor parent 'RS239'. Whether the presence of different 'RS239' segments in each microspore donor plant contributes to the different diploidization rates is still unclear. Another possible explanation is that the process of microspore culture was performed on different dates and at different age of donor plants and therefore the length of the donor plants under stress condition in controlled climate room was also different.

4.5. Characterization of the donor segments in the ISLs

Twenty AFLP marker combinations were applied in the development of genetic linkage map in the cross of the two winter rapeseed varieties 'Mansholt' and 'Samourai', resulting in 227 mapped markers covering 1361 cM of the rapeseed genome (Table 14). A higher degree of genetic polymorphisms was observed in the cross of the spring type resynthesized rapeseed line 'RS239' and the winter type rapeseed line 'Express' using 20 AFLP marker combinations resulting in 484 mapped markers covering 1800.9 cM of the rapeseed genome (Table 5). The high degree of polymorphisms in the second cross could be due to diverse characteristic of the reynthesized genotypes compared to the inbred cultivar.

The donor segments in the ISLs generated from the cross 'ExRS239' cover a minimum of 950.9 cM and maximum of 1587 cM corresponding to 52.8% and 88.1% of the genetic map of this cross. This coverage is larger than that in the ISL generated from the cross 'MxS', where the donor segments cover a minimum of 515.5 cM and a maximum of

1095.3 cM corresponding to 37.9% and 80.5% of the respective genetic map. The large factor of more than two was observed between the maximal and minimal coverage of donor genome in the ISLs of the cross 'MxS'. It might be explained by the low marker density of the map developed from this cross.

The large coverage of donor genome in the ISL of the cross 'ExRS239' might be due to the large number of the ISLs analysed, and a high number of markers applied in the characterization of the ISLs. Four hundred eighty four AFLP markers that were mapped in the new genetic linkage map (see part 3.1), were used in the characterization of the ISLs from the cross 'ExRS239' instead of the 220 AFLP markers mapped in the earlier genetic linkage map developed using the BC_1 population (see part 2.1.2.1). This resulted in a better characterization of the ISLs, since more markers were mapped in the new genetic linkage map. While only 227 AFLP markers mapped in the genetic linkage map developed using F_1DH population were used in the characterization of the ISLs developed from the cross 'MxS'.

In addition, the markers used in marker assisted selection (MAS) in the development of ISLs from the cross 'MxS' were fewer than of those in the ISLs from the cross 'ExRS239'. Only 161 AFLP markers were used as MAS of the cross 'MxS' to select BC_2 plants (see part 2.1.2.2). Many of these markers were observed as markers for recurrent parent alleles, which should be scored co-dominantly. Therefore, they could not be properly detected in performing MAS to select the backcross plants (Marschalek 2003). At the end, in the BC_4 plants selection only 120 AFLP markers could be used to characterize donor segments carried by the plants, due to the elimination of some doubtful, co-dominantly scored markers (Kebede, 2007). Contrary to that, most of the 220 AFLP markers used as MAS in the cross 'ExRS239' were markers specific for donor alleles. It resulted in a better characterization of the donor segments carried by the plants in the development of ISLs.

The number of donor segments carried by the ISL of the cross 'MxS' ranged from 1 to 6 (Table 13). About 92% of them carried less than 4 donor segments. It was observed that the number of donor segments carried by the ISL from the cross 'ExRS239' was larger, ranging from 1 to 9 (Table 4). About 23% of the ISLs carried more than 4 donor segments. The larger number of donor segments introgressed in the ISLs from the cross 'ExRS239' is probably explained by higher number of markers applied in the characterization of this set of ISL.

Another reason for the high number of donor segments in the ISLs maybe because of the presence of doubled crossovers between even closely linked markers, which were observed more frequently in the ISL of the cross 'ExRS239'. As an example, ISL 194 carrying 4 donor segments, is shown in Figure 18. This line carries two donor segments due to a doubled crossover around marker E35M47-201R on linkage group N17.

	Nr. of donor segment		3	3	2	4	4	1	1	8	2
		DH lines	1427	817	117	201	174	1074	671	1342	1028
Marker name	LG	Position									
E35M47-160E	N17	-3,3	E	E	R	R	E	E	E	E	E
E32M47-438R	N17	-2,4	E	E	E	R	E	E	E	E	E
E41M54-243E	N17	-2,2	E	E	E	R	E	E	E	E	E
E39M54-457E	N17	-2,2	E	E	E	E	R	E	E	E	E
E44M50-481E	N17	-2,2	E	E	E	E	R	E	E	E	E
E35M47-180R	N17	0,0	E	E	E	E	R	E	E	E	E
E35M47-201R	N17	0,0	E	E	R	R	E	E	E	E	E
E33M49-206E	N17	2,9	E	E	E	E	R	E	E	E	E
E35M47-171-2R	N17	17,8	E	E	E	E	R	E	E	E	E
E39M54-290R	N17	17,8	E	E	E	E	R	E	E	E	E
E39M54-083E	N17	25,4	E	E	E	E	E	E	E	E	E
E34M47-235E	N17	42,0	E	E	E	E	E	R	E	E	E
E34M47-154E	N17	46,9	E	E	E	E	E	R	E	E	E
E35M54-054R	N17	51,8	E	E	E	E	E	E	E	E	E
E41M50-173E	N17	83,1	E	E	E	E	E	E	R	E	E

Figure 18 The presence of doubled cross over observed in a very near genetic distance and within co-segregating markers on linkage group 17. E: scoring allele of 'Express'; R: scoring allele of 'RS239'

4.6. Detection of interesting QTL for rapeseed breeding in the ISLs from the cross 'ExRS239'

Exotic germplasms showed a high allelic diversity in their genomes. The utilisation of rapeseed artificially resynthesized from its parental species is a strategy to increase the genetic diversity of *Brassica napus* (Girke 2002; Lühs et al. 2002; Seyis et al. 2006). *B. napus* lines resynthesized from its diploid ancestors, *B. rapa* and *B. oleracea*, show a high degree of polymorphism compared to conventional rapeseed phenotypically and in marker analyses (Becker et al. 1995), indicating a high degree of allelic differences.

According to Engqvist and Becker (1994) resynthesized rapeseed is known to have low fertility, winter hardiness and oil content as well as high erucic acid and glucosinolate content. Besides these characters, resynthesized line 'RS239' also has low oleic acid, linolenic acid and phytosterol content. Nevertheless, this genotype also carries valuable alleles that could be very useful for breeding purposes. Some valuable traits detected are high protein content, that is needed for rapeseed meal as feedstuff, low sinapate esters, that

is required to improve rapeseed meal, and alleles decreasing plant height, that are very useful for lodging problem.

Six QTL were detected to have positive additive effect for seed protein content. The contribution of the donor alleles ranged from 1.98-8.64% of increased seed protein content in homozygous genotypes. Four of the QTL showed a negative pleiotropic effect with oil content, with the donor alleles reducing oil content. A highly significant negative correlation (-0.79^{**}) was observed between these traits. These traits share basic resources in the metabolic pathways (Röbbelen and Thies 1980) and are partially controlled by the same genes which causes a significant negative correlation. Several investigations observed the negative relationship between oil and protein content. Gül et al. (2002) detected three out of five QTL for oil content to be closely linked with QTL for protein content. Further, Zhao et al. (2006) found a clear evidence for a high genetic correlation between these two traits in which six QTL and nine epistatic locus pairs exhibited pleiotropic effects with opposite direction on both traits. In this study, it was found that two QTL on linkage group N07 and N12 controlling protein content independent from QTL for oil content. The two QTL showed positive additive effects of 2.5% for protein content (Table 9).

Four QTL with donor alleles decreasing sinapate esters content contributed by donor parent were mapped on linkage groups N06, N11, N13 and N15 in which the additive effects of the donor alleles sum up to -2.19 mg/kg in sinapate esters reduction (Table 9). This could be valuable for rapeseed breeding since the interest is grown on this trait for specific breeding programmes focused on the low sinapate esters content (Zum Felde et al. 2006). Sinapate esters are phenolic compounds make up 1-2% of the seed dry matter of rapeseed (Kozlowska et al. 1990; Shahidi and Naczk 1992; Bell 1993). They contribute to the bitter taste, astringency and dark colour of rapeseed products (Ismail et al. 1981). During seed oil processing, sinapate esters are oxidized and may form complexes with proteins, which can lower the digestibility of the rapeseed meal (Kozlowska et al. 1990; Shahidi and Naczk 1992; Naczk et al. 1998). Therefore the reduction of sinapate esters content is a substantial requirement for extending the use of rapeseed as a protein crop.

Susceptibility to lodging is a serious problem in hybrid breeding. Therefore the detection of QTL carrying alleles responsible to decrease plant height would be very useful to overcome this problem. Three QTL with donor alleles responsible for decreasing plant height at maturation were mapped on linkage groups N13, N18a, and N19, with a total

additive effect of -50.4 cm (Table 12). There are two explanations possible for the plant height reduction observed in this study. First, it is suspected that donor alleles controlling the plant height reduction causing dwarf plants were detected in the QTL found in this study. Second, the abnormal growth of plants due to winter susceptibility limits the availability of nutrient uptake and reduces plant height. For instance the QTL on linkage group N19 is carried by weak lines, which showed a bad performance due to winter damage. This may indicate that the observed plant height reduction could be caused by the susceptibility of the plants to cold stress during winter time rather than due to the presence of alleles reducing plant height. Nevertheless, these plants carried more than one donor segments. Therefore, it could also be possible that the plants carried donor alleles for winter susceptibility and alleles reducing plant height as well. The donor parent 'RS239' is a spring type rapeseed and not well adapted to German growing condition. To eliminate possible environmental effects on the ISLs showing reduced plant height, they could be further analysed by growing the lines under spring time condition, with artificial vernalization under controlled climate condition, and observe the plant height.

4.7. Undetected QTL in the ISLs of the cross 'ExRS239'

In the cross of 'ExRS239', the resynthesized line 'RS239', as the donor parent was observed to have low phytosterol content in its seed, which was affirmed by the detection of three QTL with donor alleles responsible for decreasing phytosterol content on linkage groups N11, N13 and N15 with additive effects of -217.7, -347.9 and -418.0 mg/g (Table 9), respectively. It was identified that one substitution line (DH line 73) has significantly increased phytosterol content (about 380 mg/g seed) (data not shown). It indicates that the donor parent carries an allele increasing phytosterol content in its genome. Unfortunately, the QTL could not be detected in the current analysis. It might be possible that the QTL is located in a region that is not covered by the map used to characterize the ISLs, for example linkage group N16 that could not be found at all or other linkage groups that are not fully covered.

In another case, Tanksley et al. (1996) was successful to identify a QTL carrying allele increasing fruit mass contributed by donor parent having low fruit mass. The QTL was mapped using near isogenic lines (NILs) containing segments of the *Lycopersicon pimpinellifolium*, a wild genotype, in the genetic background of an elite processing line of tomato. The allele increasing fruit mass (approximately 8% greater than recurrent parent,

L. esculentum) was contributed by the donor parent, L. *pimpinellifolium*, which only had approximately 2% of the recurrent parent fruit mass.

Another phenotypic effect with no detected QTL in the ISLs is the second gene controlling erucic acid content. It has been well studied that the erucic acid content in the seeds of *B. napus* is governed by two genes mapped on linkage groups N08 and N13 (Ecke et al. 1996; Thormann et al. 1996; Jourdren et al. 1996; Fourmann et al. 1998; Peleman et al. 2005; Qiu et al. 2006). In this study, one of the two erucic acid genes was mapped on linkage group N13 using twelve ISLs carrying donor segments on this linkage group (Table 11, Figure 11). The lines showed a mean increase of erucic acid content by 37.5%. There were eight additional ISLs having significantly higher erucic acid contents compared to the recurrent parent (Table 11). Erucic acid content in these lines is increased by an average of 37.0 %. It is assumed that these lines carry the erucic acid gene on linkage group N08 that could not be identified in our analysis. The linkage group N08 was mapped in a length of 29.7 cM in the map developed from the cross 'ExRS239' (Figure 5). The linkage group was considerably smaller than the 70.9 cM constructed in the cross 'MxS', in which the gene for erucic acid content can be mapped (Figure 15).

4.8. Comparison of QTL and their effect detected in the ISLs and the corresponding F_1DH population from the cross 'MxS'

A segregating doubled haploid population (F_1DH) from the cross of 'MxS' has been studied independently to detect QTL for important traits in rapeseed (Uzunova et al. 1995; Ecke et al. 1996; Gül 2002; Amar et al. 2008). In this study, comparison of QTL and their effects between the early (F_1DH) and advanced (ISL) mapping populations was done. It showed consistency, disappearance and new detection of QTL as well as the increase in QTL effects.

4.8.1. The confirmed QTL

In the earlier study with the F_1DH population, Uzunova et al. (1995) localized four QTL for seed glucosinolate content on linkage groups LG2, LG9, LG16 and LG18 corresponding to N16, N19, N09 and N12, respectively. Later, this conclusion was confirmed by Gül (2002). The two QTL on N09 and N12 could be detected and mapped in the same region (Figure 14). These two QTL were observed as consistent QTL for seed glucosinolate content. In other studies using different materials, Toroser et al. (1995)

mapped two major loci with the largest influence on total seed aliphatic glucosinolates in LG1 and LG20 corresponding to N09 and N12. Howell et al. (2003) detected 3 major QTLs mapped on linkage groups N9, N12 and N19, and Quijada et al. (2006) found three QTL on N2, N9 and N12.

The two erucic acid genes mapped using F_1DH population on LG6 and LG12 (Ecke et al. 1996), corresponding to N8 and N13, were confirmed in the ISL population (Figure 15). The genes were mapped in the same region on N08 and N13. These loci represent the two genes previously described genetically (Harvey and Downey 1964; Kondra and Stefansson 1965) and correspond to the two *FAE1* genes, *FAE1.1* (E1) and *FAE1.2* (E2) (Jourdren et al. 1996; Fourmann et al. 1998; Gupta et al. 2004) for elongation of oleic acid to erucic acid. The genetic variation in the ISLs showed two-class segregation of free and intermediate erucic acid content (Figure 12). The first class is a group containing zero-erucic acid with genotype e1e1e2e2. The second class is an intermediate erucic acid content group, characterized by E1E1e2e2 or e1e1E2E2 genotypes. None of the lines carried high-erucic acid content with genotype E1E1E2E2 by carrying both of the erucic acid genes on linkage groups N08 and N13.

Two QTL for oil content on linkage group N08 and N13 were mapped in the same interval of the two erucic acid genes by Ecke et al. (1996). Using the same population, Gül (2002) found five QTL for oil content, in which two of the QTL represented the two erucic acid genes on N08 and N13 confirming those found by Ecke et al. (1996). In this study, the two of five QTL mapped for oil content were also co localized with the two erucic acid genes (Table 19), supporting the results found by the previous authors. The QTL showed 'Mansholt' alleles increasing oil content and erucic acid content explaining the positive correlation between these two traits.

Amar et al. (2008) mapped two major QTL for phytosterol and sinapate esters content on linkage groups N08 and N13. The two QTL corresponded to the two erucic acid genes. Using the ISL population, the two QTL for phytosterol and sinapate esters content were also mapped in the same interval of the two erucic acid genes (Table 19), confirming the results found in the F_1DH population by Amar et al. (2008).

Concerning the effects of the QTL, it was observed that the additive effects of the QTL for seed glucosinolate, oil, protein, phytosterol and sinapate esters content detected in the ISL population were higher than in the F_1DH population. The magnitude of the effects in the ISLs is because of the lower genetic background variation and the limitation of donor

allele interactions to those between genes on the homozygous substituted donor segments (Eshed and Zamir 1995; Howell et al. 1996).

4.8.2. The undetected QTL

Many of the QTL that had been mapped in the F_1DH population could not be detected in the ISL population. The two QTL on linkage group N16 and N19 mapped by Uzunova et al. (1995) for seed glucosinolate content; three QTL for oil content on linkage groups N01, N05 and N06 mapped by Gül (2002); two QTL for protein content on linkage groups N05 and N06 (Gül 2002); QTL on linkage group N18 for total phytosterol content and on N05 and N06 for total sinapate esters content (Amar et al. 2008); two QTL for plant height on linkage groups N01 and N09, and all of phenological traits mapped by Gül (2002), were undetected in the ISL population.

The undetected QTL were probably mapped in the regions that were not covered by the donor segments. It shows the low ability of QTL detection due to the low coverage of the donor genome in the ISLs population of the cross 'MxS'. Since the ISLs cover only 38% of the mapped rapeseed genome in this cross using AFLP markers (Table 14).

In addition, the QTL mapping in the F_1DH population were mapped using RFLP and RAPD markers (Uzunova et al. 1995; Ecke et al. 1996; Gül 2002). The ISLs that were characterized by AFLP markers, represent the donor genome coverage of about 515 cM covering only 28% of the 1800 cM genetic linkage map developed in the cross of 'MxS' using RFLP, AFLP, SSR and RAPD markers (see part 2.1.2.2).

4.8.3. The detection of new QTL

In this study, some of new QTL for agronomically important traits were mapped using ISLs which were not detected earlier in the F_1DH population. A new QTL for glucosinolate content was identified on linkage group N03 in the ISLs. The indication for this QTL was large since there were six ISLs having significant effect for seed glucosinolate content which carried donor segment on this linkage group (Figure 14). Two of the ISLs (DH lines 264 and 265) only carried one donor segments on linkage group N03 (Table 22). Hasan et al. (2008) investigated the association of gene-linked SSR markers to seed glucosinolate content in rapeseed and found that the SSR primer Ol11-G11 amplified marker alleles with significant association to glucosinolate content which were mapped to two loci on N3 and N13.

Three new QTL were mapped on linkage groups N01, N09 and N12 for oil content in the ISLs. The QTL on N09 was observed as an independent QTL from erucic acid genes with a positive additive effect of 1.97% showed donor allele of 'Mansholt' increasing oil content (Table 19).

Two QTL for protein content were mapped on linkage groups N01 and N09. All of this QTL showed alleles increasing protein content contributed by donor parent. The QTL on N09 was observed as an independent QTL from oil content with an additive effect of 1.74% (Table 19).

The QTL for plant height on linkage groups N10, N12, and N16; QTL on linkage groups N05, N17 and N19 for begin of flowering; a QTL for duration of flowering on linkage group N19, were also observed as new QTL that were undetected earlier in the F_1DH population (Table 21).

The appearance of new QTL in the advance mapping population such as intervarietal substitution lines, which was not detected before in the early mapping population, was also observed in other studies (Tanksley et al. 1996; Finkers et al. 2007; Ramchiary et al. 2007).

4.8.4. The false positive QTL

Two QTL for protein content on linkage groups N01 and N03, and a QTL for plant height at maturation on N16 mapped in the F_1DH population by Gül (2002) were not identified in the ISL population. Interestingly, the regions where the QTL had been mapped were covered by donor segments in the ISLs (Figure 16 and 17). It was concluded that those QTL were detected as putatively false positive QTL. The ISLs carrying donor segments covering the QTL regions had no effects for those traits.

The use of an early generation mapping population such as a segregating doubled haploid population is a good starting point to detect QTL. Regarding to the accuracy of the estimation of effects and QTL positions, further evaluations should be conducted in an advanced generation population to get better conclusions on the QTL underlying the traits. In the early mapping population, the lines simultaneously segregate at the whole parental chromosome segments, therefore the use of an advanced population such as a set of ISL will simplify or eliminate the effects of the genetic background on the expression of QTL and facilitate the fine mapping of QTL.

4.9. Fine mapping

In this study, the QTL were mapped in the maximal intervals ranging from 1.8 to 35.8 cM in the ISLs of the cross 'ExRS239' and from 1.2 to 24.8 cM in the ISL of the cross 'MxS'. About 48% and 57% of the QTL were mapped in the maximal interval of less than 10 cM in the ISL from the cross of 'ExRS239' and 'MxS', respectively. In general about 70% of the QTL were mapped in the maximal intervals of less than 15 cM in both of the ISL populations. It should be considered that these are the maximal intervals for QTL locations which are estimated as the maximal region of the QTL are placed.

Contrary to that, usually in the segregating population confidence intervals have been estimated to be in the range of several ten cM depending on the size of the segregating population, the total variance of the character analysed and the QTL effect (Van Ooijen 1992; Darvasi et al. 1993; Hyne et al. 1995; Kearsey and Farquhar 1998). Delourme et al. (2006) mapped QTL for oil content in rapeseed using 445 DH lines derived from the cross 'Darmor-*bzh*' x 'Yudal', in the confidence interval ranging from 13-40 cM in the three trials. About 60% to 75% of the QTL were mapped in the confidence interval of more than 20 cM. In soybean, using 131 RILs (F_7 population) Hyten et al. (2006) mapped 67% of QTL for modifier fatty acid in the confidence interval of more than 15 cM.

The success of using ISLs in fine mapping compared to the interval mapping in the F_1DH population was in the localization of the erucic acid genes. The mapping intervals could be narrowed down to maximally 19.3 and 6.9 cM for the genes on N08 and N13, respectively, instead of the 22 and 14 cM using interval mapping in the F_1DH population (Table 19, Figure 15). The relatively large maximal interval of 19.3 cM for the gene on N08 should be considered as two successive intervals of less than 3.3 or 16.4 cM. The present of an ISL (line 1155) with no significant effect on erucic acid content carrying donor segment at marker E38M61-155M/E33M59-147M on 3.3 cM from the start point of maximal interval of the QTL confirmed that the gene was located either upward in a maximal interval of less than 3.3 cM or downward from those markers in the 16.4 cM interval.

By comparing overlapping donor segments in different substitution lines and their respective phenotypic values, QTL position can be narrowed down to a few centimorgans. The high resolution of the QTL mapping using ISL population can be achieved due to the present of smaller and overlapping donor segments. The successfully application of such an intervarietal substitution lines population to map QTL with a fine resolution by reducing

the size of the QTL interval and determine the phenotypic effects of segments with small differences (Paterson et al. 1990) were also reported in different studies. It verified the effects and refined the map positions of QTL in tomato (Eshed and Zamir 1995; Paterson et al. 1990; Monforte et al. 2001; Brouwer and Clair 2004), *Brassica* species (Rae et al. 1999; Burns et al. 2003; Peleman et al. 2005), rice (Wan et al. 2008) and soybean (Zhu et al. 2006).

Further applications of the use of ISLs in the mapping population is the benefits to allow reducing the size and number of introgressed donor segments in 'interesting' line and to map more markers to the linkage map where recombination is presumed to have occurred, by backcrossing the line with the recurrent parent for fine mapping of QTL.

4.10. Pleiotropy versus closely linked QTL

Some correlated traits showed QTL on the same donor segments. It was observed between erucic acid and phytosterol as well as sinapate esters content where the QTL for phytosterol and sinapate esters were co localized with the two erucic acid genes. Using the same cross, Amar et al. (2008) mapped two major QTL for phytosterol and sinapate esters content in the same interval of the two erucic acid genes and suggested that the two erucic acid genes exert a negative pleiotropic effect on phytosterol and sinapate esters content. The negative pleiotropic effect between these traits is explained based on biosynthetic pathway by the competition for cytoplasmic acetyl-CoA, an early essential precursor for erucic acid and phytosterol content (Fatland et al. 2005), and the competition for plastidic phosphoenolpyruvate, a common precursor for de novo fatty acid and sinapate ester biosynthesis (Fischer et al. 1997).

In the present study, strong negative correlations were observed between erucic acid and phytosterol content in the ISLs developed from the cross 'ExRS239' ($r=-0.93^{**}$, Table 7) and 'MxS' ($r=-0.96^{**}$, Tabel 16). We observed that the lines having significantly increased erucic acid content are the same lines with significantly reduced phytosterol content (Tabel 20 and Tabel 24). This suggested that pleiotropism rather than close linkage is the reason for the correlation of the traits.

It is not the case for the correlation between erucic acid and sinapate esters content. The significant correlations were observed between erucic acid and sinapate esters content in the ISLs developed from the cross 'ExRS239' ($r=-0.58^{**}$, Table 7) and ($r=-0.59^{**}$, Table 16) of the cross 'MxS'. We observed some lines with significantly increased erucic acid

content which did not have significantly reduced sinapate ester content (Table 20 and Table 24). Here the explanation for the correlation must be close linkage rather than pleiotropism. Nevertheless further investigations should be conducted to clearly prove the presence of closely linked QTL for these traits.

Table 24 ISL having significant differences in erucic acid, phytosterol and sinapate esters content compared to the recurrent parent according to Dunnet multiple comparisons at significance level of 5% in the cross 'ExRS239'

No.	ISIs	Erucic acid (%)[1]	Phytosterol (mg/kg)[1]	Sinapate esters (mg/g)[1]	LG
1.	161	37.17	-845.81	-1.39	?
2.	162	38.49	-834.60	-1.14	?
3.	165	36.82	-819.31	-0.82[ns]	?
4.	167	36.20	-729.50	-0.62[ns]	?
5.	168	37.50	-836.04	-0.98[ns]	?
6.	169	36.18	-827.90	-1.30	?
7.	170	36.41	-714.67	-1.27	?
8.	343	37.72	-795.03	-0.88[ns]	?
9.	208	37.34	-655.53	-1.35	N13
10.	209	35.19	-720.98	-1.00[ns]	N13
11.	211	35.88	-599.53	-1.35	N13
12.	212	38.93	-760.16	-1.21	N13
13.	214	36.30	-733.97	-1.38	N13
14.	215	37.10	-748.36	-0.43[ns]	N13
15.	216	40.08	-777.87	-1.51	N13
16.	217	38.07	-792.60	-0.66[ns]	N13
17.	219	40.28	-739.45	-1.37	N13
18.	220	42.80	-805.04	-1.63	N13
19.	303	41.60	-870.96	-0.99[ns]	N13

[ns]The effect was not significantly different compared to the check
[1]Effect of introgressed segment

4.11. Comparison of QTL in the ISL populations

Forty four QTL of agronomically important traits in rapeseed were mapped using the ISLs of the cross 'ExRS239'. In the ISLs from the cross 'MxS', only 24 QTL were mapped. The high number of QTL mapped in the ISLs of the cross 'ExRS239' is due to the larger genetic distance between the parents resulting in a larger number of functionally

different alleles segregating in the progeny of this cross. Another reason is the better coverage of donor genome in this set of ISL.

Some of the QTL mapped in the ISL of the cross ‘ExRS239’ were also detected in the same linkage group in the ISL of the cross ‘MxS’. For example, a major QTL for total seed glucosinolate content on linkage group N12, the erucic acid genes and QTL for oleic acid, phytosterol and sinapate esters content on linkage group N13, two QTL of oil content on N01 and N12 (Table 9 and Table 19). All of these QTL have the same signs of their additive effects. Nevertheless, it could not be concluded whether they are the same QTL or not. The alignment of the QTL could not be done due to insufficient number of markers that were mapped on both of the genetic linkage maps.

5. Summary

Intervarietal substitution lines (ISLs) having one or a few defined segments of a donor genome in the common genetic background of a recurrent parent can be used to search the genome for donor alleles affecting traits. A complementary set of substitution lines represents ideally the whole donor genome divided into a limited number of distinct segments, each carried by a different line. ISLs were suggested as an alternative to a segregating population for QTL mapping. An ISL population can be used to overcome the limitations of a segregating population in the accuracy of QTL localization. With overlapping donor segments in different substitution lines and their respective phenotypic values, QTL positions can be narrowed down to a few centimorgan (cM), allowing a high precision of QTL localization.

The main objectives of this study were:

- To develop set of intervarietal substitution lines (ISL) from the cross of 'Express' x resynthesized line 'RS239';
- To map QTL for some agronomically important traits in two sets of ISL populations developed from the crosses of 'Mansholt' x 'Samourai' and 'Express' x resynthesized line 'RS239';
- To compare QTL results mapped in the ISL population with QTL mapped in an earlier generation (F_1DH population) developed from the cross 'Mansholt' x 'Samourai';

For this, a set of ISLs was developed from a cross between the spring type resynthesized rapeseed line 'RS239' and the winter rapeseed variety 'Express' ('ExRS239'). A second set, developed from a cross between doubled haploid lines of the two winter rapeseed varieties 'Mansholt' and 'Samourai ('MxS'), was available from earlier work. The ISLs were developed through five backcross generations. Using marker assisted selection with AFLP markers, lines carrying a complementary set of donor segments were selected.

Three hundred fifty ISLs developed from the cross 'ExRS239' and 270 ISLs from the cross 'MxS', were grown in the field at five locations in the year 2009, respectively 2006 and 2007, in Germany and agronomically important traits were evaluated. Molecular marker analysis using AFLP markers was conducted to characterize the segments of the

donor parents carried by the ISLs. QTL were mapped by comparing the segments of the donor genome and their effects in the ISLs.

The donor segments in the ISLs generated from the cross of 'ExRS239' cover, depending on the method of estimation, a minimum of 950.9 cM or a maximum of 1587 cM, corresponding to 52.8% and 88.1% of the genetic map of this cross. This coverage is larger than that in the set of ISL generated from the cross 'MxS', where the donor segments cover a minimum of 515.5 cM or a maximum of 1095.3 cM, corresponding to 37.9% and 80.5% of the respective genetic map.

Forty four QTL of agronomically important traits in rapeseed were mapped using the ISLs of the cross 'ExRS239' and 24 QTL were mapped in the ISLs of the cross 'MxS'. The higher number of QTL mapped in the ISLs from the cross 'ExRS239' is due to the larger genetic distance between the parents resulting in a larger number of functionally different alleles segregating in the progeny of this cross. Another reason is the better coverage of donor genome in this set of ISL.

Some QTL valuable for breeding purposes and to improve rapeseed quality were identified in the ISL from the cross of 'ExRS239'. Six QTL were detected to have positive additive effects for seed protein content. Two of these QTL, the QTL on linkage groups N07 and N12, controlled protein content independently from QTL for oil content. The two QTL showed additive effects of 2.5% for protein content. Four QTL with donor alleles decreasing sinapate esters content were mapped on linkage groups N06, N11, N13, and N15. The additive effects of the donor alleles sum up to 2.19 mg/kg in sinapate esters reduction. Three QTL with donor alleles responsible for decreasing plant height at maturation were mapped on linkage groups N13, N18a, and N19 with a sum of additive effects of -50.4 cm.

The comparison of QTL of an early mapping generation, an F_1DH population, and the advanced generation, the ISLs, from the cross of 'MxS' confirmed some QTL mapped in the F1DH population in the ISLs: two QTL on linkage groups N09 and N12 for seed glucosinolate content; two genes controlling seed erucic acid content on linkage groups N8 and N13; and two QTL for phytosterol, sinapate esters and oil content on linkage groups N08 and N13 corresponding to the two erucic acid genes. Some of the QTL identified in the F_1DH population could not be detected in the ISL population because they had been mapped in regions that were not covered by the donor segments.

Several new QTL were identified in the ISLs that remained undetected earlier in the F_1DH population. On the other hand, two QTL for protein content on linkage groups N01 and N03, and a QTL for plant height on linkage group N16 mapped in the F_1DH population have to be considered as false positives. These QTL could not be detected in the ISL population, although ISLs carrying donor segments covering the regions where the QTL had been mapped were available.

It was observed that the additive effects of the QTL detected in the ISL population were higher than that in the F_1DH population. Using ISLs, the QTL interval could also be narrowed down compared to the interval mapping in the F_1DH population. The QTL were mapped in maximal intervals ranging from 1.8 to 30.2 cM in the ISL of the cross 'ExRS239' and from 1.2 to 24.8 cM in the ISL of the cross 'MxS'. About 70% of the QTL were mapped in maximal intervals of less than 15 cM in both of the ISL populations. This was considerably smaller than the confidence intervals usually estimated when using segregating populations.

The QTL for phytosterol and sinapate esters were co-localized with the two erucic acid genes. A pleiotropic effect between erucic acid and phytosterol content was identified. On the other hand, close linkage rather than pleiotropism was suggested as an explanation for the correlation between erucic acid and sinapate esters content.

Further fine mapping of QTL can be done by backcrossing ISLs to the recurrent parent to reduce size and number of introgressed donor segments. After fine mapping it will also be possible to better distinguish between pleiotropy and close linkage for QTL of correlated traits.

6. Zusammenfassung

Substitutionslinien mit einem oder wenigen definierten Segmenten eines Donorgenoms in dem genetischen Hintergrund eines rekurrenten Elters können verwendet werden, um das Genom nach Donorallelen zu durchsuchen, die phänotypische Merkmale beeinflussen. Ein komplementärer Satz von Substitutionlinien repräsentiert im Idealfall das gesamte Donorgenom unterteilt in eine begrenzte Anzahl von verschiedenen Segmenten, die jeweils durch eine Linie dargestellt werden. Substitutionslinien wurden als Alternative zu einer spaltenden Population für die QTL (Quantitative trait loci)-Kartierung vorgeschlagen. Mit einem Set von Substitutionslinien können die Grenzen der Genauigkeit bei der QTL-Lokalisierung in spaltenden Populationen überwunden werden da überlappenden Donorsegmente in verschiedenen Substitutionlinien und ihre phänotypischen Werte die Bestimmung der QTL Positionen bis auf wenige centiMorgan (cM) erlauben.

Das Ziel dieser Arbeit war es

- ein Set von Substitutionslinien einer Kreuzung von ’Express’ mit der resynthetisierten Rapslinie ’RS239’ herzustellen;
- QTL für agronomisch wichtige Merkmale in zwei Sets von Substitutionslinien der Kreuzungen ’Mansholt’ x ’Samourai’ und ’Express’ x ’RS239’ zu kartieren;
- die Ergebnisse der QTL-Kartierung in Substitutionslinien der Kreuzung ’Mansholt’ x ’Samourai’ mit der QTL-Kartierung in einer früheren Generation (F_1DH Population) dieser Kreuzung zu vergleichen.

Hierfür wurden ein Set von Substitutionslinien aus einer Kreuzung zwischen der resynthetisierten Sommerrapslinie ’RS239’ und der Winterrapssorte ’Express’ (’ExRS239’) hergestellt. Ein zweiter Set aus einer Kreuzung zwischen DH-Linien der Winterrapssorten ’Mansholt’ und ’Samourai’ (’MxS’) stand aus früheren Arbeiten zur Verfügung. Die Substitutionslinien waren über fünf Rückkreuzungsgenerationen entwickelt worden. Mit markergestützter Selektion durch AFLP-Marker wurden dabei Linien ausgewählt, die ein komplementäres Sortiment von Donorsegmenten enthalten.

Dreihundertfünfzig Substitutionslinien aus der Kreuzung ’ExRS239’ und 270 Substitutionslinien aus der Kreuzung ’MxS’ wurden in Feldversuchen an fünf Standorten im Jahr 2009, beziehungsweise 2006 und 2007, in Deutschland angebaut um agronomisch wichtige Merkmale zu erfassen. Die Donorsegmente in den Substitutionslinien wurden mit

AFLP-Markern charakterisiert. Die QTL wurden durch den Vergleich der Segmente des Donorgenoms und phänotypischen Effekten in den Substitutionslinien kartiert.

Die Donorsegmente im Set der Substitutionslinien aus der Kreuzung 'ExRS239' decken, abhängig von der Schätzungsmethode, minimal 950,9 cM oder maximal 1587 cM des Genoms ab, dies entspricht 52,8%, beziehungsweise 88,1%, der genetischen Karte von der Kreuzung 'ExRS239'. Diese Abdeckung ist größer als die im Set der Substitutionslinien der Kreuzung 'MxS'. Dort decken die Donorsegmente minimal 515,5 cM oder maximal 1095,3 cM ab, dies entspricht 37,9% und 80,5% der genetischen Karte dieser Kreuzung.

Vierzig QTL von agronomisch wichtigen Merkmalen bei Raps wurden in den Substitutionslinien der Kreuzung 'ExRS239' und 24 QTL in den Substitutionslinien der Kreuzung 'MxS' kartiert. Die höhere Anzahl von QTL in den Substitutionslinien der Kreuzung 'ExRS239' ist durch die größere genetische Distanz zwischen den Eltern der Kreuzung, die in einer größeren Anzahl von spaltenden, funktionell verschiedenen Allelen in der Nachkommenschaft dieser Kreuzung resultiert, zu erklären. Ein weiterer Grund ist die höhere Abdeckung des Donorgenom in diesem Set von Substitutionslinien.

Einige QTL zur züchterischen Verbesserung der Rapsqualität wurden in den Substitutionslinien der Kreuzung 'ExRS239' identifiziert. Sechs QTL wurden lokalisiert, die positive additive Effekte für den Eiweißgehalt in Samen zeigen. Zwei dieser QTL, auf Kopplungsgruppen N07 und N12, kontrollierten den Eiweißgehalt unabhängig von QTL für Ölgehalt. Diese beiden QTL zeigten zusammen eine additive Wirkung von 2,5% für den Eiweißgehalt. Vier QTL mit Donorallelen für einen geringeren Sinapinestergehalt wurden auf den Kopplungsgruppen N06, N11, N13 und N15 kartiert. Die additiven Effekte führten in der Summe der Donorallele zu einer Reduzierung des Sinapinestergehalts von 2,19 mg/kg. Drei QTL mit Donorallelen für eine reduzierte Wuchshöhe zur Reife wurden auf den Kopplungsgruppen N13, N19 und N18a kartiert, die Summe der additiven Effekte beträgt -50,4 cm.

Im Vergleich der QTL-Kartierung in der F_1DH Population (einer frühen Generation der entwickelten Substitutionslinien) mit den Ergebnissen der hier getesteten Substitutionslinien der Kreuzung 'MxS' wurden einige früher identifizierte QTL bestätigt: zwei QTL auf den Kopplungsgruppen N09 und N12 für Glucosinolatgehalt im Samen; zwei Gene die den Erucasäuregehalt in Samen kontrollieren auf den Kopplungsgruppen N08 und N13; zwei QTL für Phytosterol und Sinapinestergehalt sowie zwei QTL für

Ölgehalt auf den Kopplungsgruppen N08 und N13 in der Kartierungsregion der beiden Erucasäuregene. Einige der in der F_1DH Population identifizierten QTL konnten nicht in den Subtitutionlinien erkannt werden, da sie in Regionen kartiert wurden, die nicht durch Donorsegmente abgedeckt sind.

Mehrere neue QTL wurden in den Substitutionslinien identifiziert, die zuvor nicht in der F_1DH Population kartiert worden waren. Andere QTL aus der Kartierung in der F_1DH Population müssen als falsch-positive QTL bewertet werden: die QTL auf den Kopplungsgruppen N01 and N03 für Eiweißgehalt und auf Kopplungsgrupe N16 für Wuchshöhe. Diese QTL konnten nicht in den Substitutionslinien nachgewiesen werden, obwohl Substitutionslinien mit Donorsegmenten für die Regionen, in denen diese QTL kartiert wurden, zur Verfügung standen.

Es wurde beobachtet, dass die additiven Effekte korrespondierender QTL in den Substitutionslinien höher als die in der F_1DH Population sind. Mit Hilfe der Substitutionslinien konnten die QTL-Intervalle im Vergleich zu der Intervall-Kartierung in der F_1DH Population verringert werden. Die QTL wurden in Intervallen von maximal 1,8 bis 30,2 cM in den Substitutionslinien der Kreuzung 'ExRS239' und von 1,2 bis 24,8 cM in den Substitutionslinien der Kreuzung 'MxS' kartiert. Etwa 70% aller QTL in beiden Sets von Substitutionslinien wurden in maximal Intervallen von weniger als 15 cM lokalisiert. Diese waren deutlich kleiner als die üblicherweise in spaltenden Populationen geschätzten Konfidenzintervalle.

Die QTL für Phytosterol und Sinapinestergehalt wurden im gleichen Interval wie die beiden Erucasäuregene lokalisiert. Ein pleiotropher Effekt wurde zwischen Erucasäure und Phytosterolgehalt identifiziert. Andererseits deuten die Ergebnisse auf eine enge Kopplung statt Pleiotrophie als Erklärung für die Korrelation zwischen Erucasäure- und Sinapinestergehalt hin.

Die weitere Feinkartierung von QTL kann durch die Rückkreuzung von Substitutionslinien mit dem rekurrenten Elter erreicht werden, um die Größe und Anzahl der Donorsegmente zu reduzieren. Nach einer Feinkartierung wird es auch besser möglich sein zwischen Pleiotrophie und enger Kopplung von QTL für korrelierte Merkmale zu unterscheiden.

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Appendix

Appendix 1. *EcoRI* and *Mse*I primers with one and three selective nucleotides.

a) *EcoRI* and *Mse*I primers with one selective nucleotide

E01 5'-CTGCGTACCAATTCA-3'

M02 5'-GATGAGTCCTGAGTAAC-3'

b) *EcoRI* and *Mse*I primers with three selective nucleotides

E32 5'-CTGCGTACCAATTCAAC-3' (PET)

E33 5'-CTGCGTACCAATTCAAG-3' (NED)

E34 5'-CTGCGTACCAATTCAAT-3' (VIC)

E35 5'-CTGCGTACCAATTCACA-3' (FAM)

E35 5'-CTGCGTACCAATTCACA-3' (VIC)

E37 5'-CTGCGTACCAATTCACG-3' (PET)

E38 5'-CTGCGTACCAATTCACT-3' (FAM)

E39 5'-CTGCGTACCAATTCAGA-3' (NED)

E40 5'-CTGCGTACCAATTCAGC-3' (FAM)

E41 5'-CTGCGTACCAATTCAGG-3' (FAM)

E44 5'-CTGCGTACCAATTCATC -3' (VIC)

E45 5'-CTGCGTACCAATTCATG -3' (FAM)

M47 5'-GATGAGTCCTGAGTAACAA-3'

M48 5'-GATGAGTCCTGAGTAACAC-3'

M49 5'-GATGAGTCCTGAGTAACAG-3'

M50 5'-GATGAGTCCTGAGTAACAT-3'

M54 5'-GATGAGTCCTGAGTAACCT-3'

M59 5'-GATGAGTCCTGAGTAACTA-3'

M60 5'-GATGAGTCCTGAGTAACTC-3'

M61 5'-GATGAGTCCTGAGTAACTG-3'

M62 5'-GATGAGTCCTGAGTAACTT-3'

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