Increase of seed oil content in winter oilseed rape (*Brassica napus L.*) by using Chinese genetic resources

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To my beloved family

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List of Abbreviations

16:0	Palmitic acid
16:1	Palmitoleic
16:2	Hexadecadienoic
18:0	Stearic acid
18:1	Oleic acid
18:2	Linoleic acid
18:3	Linolenic acid
20:0	Arachidic acid
20:1	Eicosenoic acid
22:0	Behenic acid
22:1	Erucic acid
ACC	Acetyl-CoA carboxylase
ACP	Acyl carrier protein
AFLP	Amplified fragment length polymorphism
ANOVA	Analysis of variance
ASPE	Array-based allele-specific primer elongation
BC	Biotin carboxylase
BCCP	Biotin carboxyl carrier protein
BLAST	Basic Local Alignment Search Tool
BOF	Begin of flowering
bp	Base pair
BRAD	Brassica database
Cart	Cartographer
CI	Confidence interval
CIM	Composite interval mapping
CoA	Coenzyme-A
cond	Conditional phenotypic values
CT	Carboxyltransferase
DAG	Diacylglycerol
DAGTA	Diacylglycerol:diacylglycerol transacylase
DArT	Diversity array technology
DGAT	Diacylglycerol acyltransferase
DH	Doubled haploid
DM	Dry matter
DNA	Deoxyribonucleic acid
DY	Darmor-*bzh* x Yusal
ENR	Enoyl-ACP reductase
EOF	End of flowering
F1	First offspring generation
FA	Fatty acid
FAD	Fatty acid desaturase
FAE	Fatty acid elongase
FAS	Fatty acid synthase
FAT	Acyl-ACP/fatty acid thioesterase
FP	Flowering period
G	Gaoyou
G3P	Glycerol-3-phosphate
GC	Gas chromatography
gDNA	Genomic deoxyribonucleic acid
GPAT	Glycerol-3-phosphate acyltransferase

GSL	Glucosinolate
HAD	Hydroxyacyl-ACP dehydratase
HEAR	High erucic acid oilseed rape
HO	High oleic
HOLLi	High oleic low linolenic
idM	In defatted meal
KAR	3-ketoacyl-ACP reductase
KAS	3-ketoacyl-ACP synthase
kb	Kilo bases
KCS	β-ketoacyl-CoA synthase
LACS	Long-chain acyl-CoA synthetase
LEAR	Low erucic acid oilseed rape
LG	Linkage group
LLi	Low linolenic
LPAAT	2-lysophosphatidic acid acyltransferase
LPCAT	Lysophosphatidylcholine acyltransferase
LUFA	Long-chain unsaturated fatty acid
Mbp	Mega base pairs
MCMT	Malonyl-CoA:ACP malonyltransferase
MDA	multiple displacement amplification
MIM	Multiple interval mapping
NAD(P)H	Reduced form of nicotinamide adenine dinucleotide phosphate
NIRS	Near-infrared reflectance spectroscopy
PC	Phosphatidylcholine
PCR	Polymerase chain reaction
PDAT	Phospholipid:diacylglycerol acyltransferase
PH_EOF	Plant height at end of flowering
PP	Phosphatidate phosphatase
PR	Peoples republic
QTL	Quantitative trait locus/loci
RIL	recombinant inbred line
RNSL	Rapid x NSL96/25
S	Sollux
SAD	Stearoyl-ACP desaturase
SGDH14	DH line 14 derived from Sollux x Gaoyou
SGEDH	SGDH14 x Express617 doubled haploid
SNP	Single nucleotide polymorphism
SSR	Single sequence repeat/microsatellite
STS	Sequence tagged site
TAG	Triacylglycerol
TAIR	The Arabidopsis Information Resource
TILLING	Targeting induced local lesions in genomes
TKW	Thousand kernel weight
Win	Windows
WRI1	WRINKLED1 transcription factor

1 General Introduction

Brassica napus L. (*B. napus*; 2n = 38, AACC) commonly known as oilseed rape is one of the world's most important oil crops. Oilseed rape oil is used not only for human consumption but is also applied as primary source of industrial products. The seed oil of *B. napus* consists almost entirely of triacylglycerol (TAG) esters containing three fatty acids (FAs) varying in chain lengths from C16 to C22. The major fatty acid component of traditional oilseed rape oil is erucic acid (22:1), accounting for approximately 50 per cent of the total fatty acids. However, detrimental effects caused by the consumption of large amounts of erucic acid shown in animal experiments in the 1960s made erucic acid a possible risk for human health (Rocquelin *et al.* 1971, Abdellatif 1972). Hence breeders started to search for germplasm with reduced erucic acid content in the seed oil. This led to the development of oilseed rape containing essentially no erucic acid in seed oil during the 1970s (Stefansson *et al.* 1961, Kramer *et al.* 1983). In these new types loss of erucic acid led to an increase of oleic acid (18:1) in the seed oil (from around 15% to approximately 60%). Since the development of the first erucic acid free cultivars, further breeding attempts aimed at improving oil quality resulted in the development of so called HOLLi quality oilseed rape types with a high oleic acid (HO) and low linolenic acid (LLi) content. In parallel, an enormous reduction in seed glucosinolate (GSL) content was achieved, which enabled the use of defatted oilseed rape meal in animal feeding. This further increased the economic value of oilseed rape. Nowadays conventional *B. napus* cultivars, except for some HEAR (high erucic acid oilseed rape) varieties bred for industrial purposes, are of "00" (double low) quality. In Germany, this means <2% erucic acid in the oil and <25µmol/g glucosinolates in the seeds (Bundessortenamt 2015). The balanced fatty acid profile with beneficial healthy effects and an increasing demand for vegetable oil as renewable resource has turned increasing seed oil content into an important breeding aim in oilseed rape (Delourme *et al.* 2006). As a quantitative trait the genetic control of seed oil content is complex, and involved genes are expected to have only minor effects. However, intense scientific work during the last decades identified a number of quantitative trait loci (QTL) and putative candidate genes for seed oil content and breeding efforts already led to higher oil contents of current breeding material. But genetic diversity in *B. napus* is limited since it is a relatively young crop compared to other cultivated species like wheat (*Triticum aestivum* L.). Evaluations to discover new genetic resources to overcome this limitation revealed considerable genetic variation between Chinese and European oilseed rape germplasm (Shengwu *et al.* 2003). Based on this result and the fact that oilseed rape has been bred independently for high oil content in both China and Europe for decades, Zhao *et al.* (2005) investigated a DH population derived from a European x Chinese

cross of two high oil cultivars to develop genotypes with increased oil contents. Since erucic acid is positively correlated with oil content, two cultivars with high erucic acid content were crossed to each other, to avoid segregation of erucic acid alleles and confounding effects on oil content. The old European cultivar Sollux (S) and the Chinese cultivar Gaoyou (G) were chosen as parental lines, both of them also having high glucosinolate content in the seeds. In the study of Zhao *et al.* (2005) the DH population of 284 lines were tested in field experiments in one year at two locations each in Europe and in China. Results revealed DH line 14 (SGDH14) to be among those with the highest oil content and a combination of all favourable QTL alleles with additive main effects for oil content from both parental cultivars. These results made SGDH14 a promising candidate to further increase oil content in modern oilseed rape breeding material.
For the present study SGDH14 (++) was crossed to the inbred line 617 of the well-established winter oilseed rape cultivar Express (00) and a DH population of 212 DH lines segregating for erucic acid and glucosinolate content was generated from F1 plants. This SGEDH population was characterized with the following objectives:

- to analyse the genetic variation and inheritance for seed oil content and fatty acid composition as well as other relevant traits of the SGEDH population
- to construct a molecular marker map to identify quantitative trait loci (QTL) associated with seed oil content and fatty acid composition as well as other relevant seed quality traits of the SGEDH population
- to compare methods to correct for the effects of erucic acid on seed oil content including the conditional approach of Zhu (1995)
- to compare QTL for seed oil content of the SGEDH population with QTL for seed oil content of other related DH populations
- to compare evaluations from field trials conducted in Europe to field trials conducted in East China

2 Literature review

2.1 The oil crop *Brassica napus*

The allotetraploid *Brassica napus* L. (*B. napus*, oilseed rape; genome AACC, 2n = 38) is a member of the Brassicaceae (Cruciferae) family. It derived from a spontaneous interspecific hybridisation of the diploid species *Brassica rapa* L. (*B. rapa* syn. *campestris*; turnip rape; genome AA, 2n = 20) and *Brassica oleracea* L. (*B. oleracea*; cabbage; genome CC, 2n = 18) less than 10000 years ago (Parkin *et al.* 1995, Parkin *et al.* 2003). The family of Brassicaceae with more than 340 genera containing more than 3350 species (Price *et al.* 1994) is not only including the model plant *Arabidopsis thaliana* (L.) HEYNH. (*A. thaliana*), but also some of the worldwide most economically important crops for food production. Especially the species of the genus *Brassica* include a unique variety of agricultural and horticultural crops compared to any other plant genus (Wu and Raven 2001). Interspecific crosses and cytological analysis of chromosome conjugation in the progeny in the early 1930s, conducted by Morinaga, revealed the relationship between *B. napus* and its closest relatives (Prakash and Hinata 1980). U 1935 showed that *B. napus*, *B. juncea* and *B. carinata*, were amphidiploids derived from the monogenomic species *B. nigra*, *B. rapa* and *B. oleracea*. The latter themselves cytologically seemed to be secondary polyploids originating from a common ancestor with five to six chromosomes in the haploid stage (Prakash and Hinata 1980). Genetic mapping experiments confirmed the relationships of the *Brassica* species (Parkin *et al.* 1995, Axelsson *et al.* 2000) and revealed a conserved gene repertoire and a collinear order of chromosomal segments (not including translocations and insertions) within the genomes of *B. rapa*, *B. nigra* and *B. oleracea* indicating a common hexaploid ancestor (Lagercrantz and Lydiate 1996). Further comparative genetic analyses between *Brassicas* and their close relative *A. thaliana* proved the theory of the triplication of the genome of an original line of the diploid *Brassicas* (Lagercrantz *et al.* 1996, Scheffler *et al.* 1997, Cavell *et al.* 1998, Lagercrantz 1998, Parkin *et al.* 2002, Parkin *et al.* 2005). Through physical comparison it was possible to show that gene sequences of *Brassica* and *A. thaliana* were 80 – 88% identical (Bach 2007) considering *B. napus* as an ideal model crop in transferring information from the model species (Snowdon and Friedt 2004).

As a predominantly self-pollinating crop *B. napus* is producing 70 to 80% of the seeds by self-pollination (Olsson 1960). It does not need pollinating agents like wind and insects but it is very attractive to bees. The cross-pollination caused by bees does not have much effect on yield but research has reported that bees cause seed set to occur earlier, resulting in shorter, more

compact plants that ripen more uniformly (Williams 1978, Williams *et al.* 1987, Canola Council of Canada 2014). Besides, outcrossing rates of 5 - 55% have been identified (Timmons *et al.* 1995) and Becker *et al.* (1992) found that the rate of outcrossing can greatly be influenced by environmental conditions.

There are two crop forms of oilseed rape existing, a winter and a summer type. These are differentiated by a genetic mechanism controlling the requirement for vernalization (Snowdon *et al.* 2007). The winter type is predominately grown in North Europe and some countries of South America (the temperate zone) with a growing season from August to July, since this biennial type needs a vernalization period at temperatures near freezing to induce bolting and flowering (Kramer *et al.* 1983). The summer form does not need vernalization (growing season April to September) and is more sensitive to low temperatures. Thus, it is mostly grown in Asia but also in Canada and parts of Europe.

There has been evidence that a vegetable crucifer was widely cultivated as early as 10000 years ago but the production of oil from *Brassica* only started in northern Europe around the 13th century. Within the following 300 years oilseed rape became the major source of lamp oil in Europe and since the 18th century it started to capture significant cultivation areas (Kroll 1995, Kimber and McGregor 1995). From the end of the 19th century on oilseed rape oil was used as lubricant for industrial purposes, because of its quality and high quantity (up to 50%) of erucic acid. Beside a bitter taste, erucic acid was identified to lead to cardiac damage and related health problems in animal experiments, thus oilseed rape oil was rarely used as food oil. But in the 1970s this situation changed by the development of the first 0 and 00 oilseed rape varieties (Stefansson in Kramer and Sauer 1983, Röbbelen and Downey 1989, Downey 1990). First, the identification of a spontaneous mutant of the German summer cultivar Liho, containing less than 1% erucic acid, enabled the breeding of 0-quality oilseed rape (Stefansson and Hougen 1964). But the value of oilseed rape was still suppressed due to a high quantity of glucosinolates within its seeds, which limited the use of oilseed rape meal as feed for livestock. However, 1969 a low glucosinolate variety was found, the Polish summer oilseed rape cultivar Bronowski, which was used in an international backcross program to introduce this polygenic trait into high-yielding erucic acid-free material. As a result Tower, the first spring oilseed rape variety without erucic acid and low glucosinolate content (00-quality), was released in 1974 (Snowdon *et al.* 2007). After the successful introduction of the so called double low or canola cultivars, further breeding efforts aimed at the development of new varieties to increase the nutritional value of oilseed rape seed oil. As a result a magnitude of cultivars was developed with variation in the fatty acid composition of oilseed rape oil, focusing on the proportions of the polyunsaturated fatty acids

(PUFA), linoleic and linolenic acid. A major objective of oilseed rape breeding in recent years has been the production of hybrids, due to the observed advantages of heterosis in terms of yield and vigour (Gunstone 2009). Today oilseed rape is one of the leading oil crops in the world due to the diverse applications of oilseed rape seed oil, ranging from highly nutritional food oil to biodiesel, industrial lubricants, tensides for detergent and soap production as well as biodegradable plastics (Snowdon *et al.* 2007, Becker 2011). Efforts to increase seed yield of oilseed rape during the past two decades was more successful compared to increases in oil content, with seed oil contents between 40 to 50% for the majority of commercial cultivars. But still, an overall elevation in seed oil content by even a few per cent would enable a remarkable improvement in levels of oil production, since the worldwide oilseed rape production is constantly growing (Jiang *et al.* 2014).

2.2 The mature seed

The seeds of *B. napus* are round with an average diameter of 2.0 to 3.2mm and a weight between 3.5 and 5.5mg at maturity (Kimber and McGregor 1995, Dimov *et al.* 2012). The seed coat in this developmental stage is usually black, sometimes brown and in rare cases yellow and accounts for 10.5 to 20% of the seed weight of black seeded types. It consists of an outer epidermis, a palisade layer of thick-walled columnar-shaped cells, and a layer of crushed parenchyma (Beweley and Black 1984, Naczk *et al.* 1998). The larger part of the seed however is represented by the light yellow embryo, which makes up 84 to 88% of the seed. It is covered by a layer of crushed parenchyma and the endosperm, which consists of a single row of aleuron cells, together separating the embryo from the seed coat. The embryo itself consists of two cotyledons, an inner one, which is enclosed by a larger outer one. Both are attached to the short hypocotyl. Above the hypocotyl the epicotyl is located, from which the first true leaves and meristem will emerge. Below the hypocotyl the radicle is located, the root part of the embryo. The cotyledons are predominantly composed of cells with a wide lumen containing mainly storage particles for oil (oleosoms) and protein (aleuron bodies). The water content of oilseed rape seeds normally averages between 6 and 8%, which is due to their high oil content, varying between 39 and 45% depending on the cultivar and the environmental conditions, with contents of phosphor- and glycolipids accounting only for 0.5 to 1.5%. The protein content of the mature seed is ranging from 20 to 27% (Appelqvist *et al.* 1972, Theander *et al.* 1977, Anjou *et al.* 1977, Sosulski and Zadernowski 1981, Przybylski and Eskin 1991, Bell 1993). Seeds of yellow colour have a significantly thinner seed coat, decreasing the level of fibre and allowing a higher proportion of

embryo, which is increasing the oil and protein content of these seeds (Jonsson and Bengtsson 1970, Stringam *et al.* 1974, Suprianto 2014).

2.3 The oil

The most valuable component of the seed is its oil content. About 80% of the seed oil is located in the cells of the cotyledons, where it is stored in lipid droplets, the oleosoms. The hypocotyl and the root contain lower levels of oil, while in the seed hull and endosperm 7 – 12% of the total seed oil is found (Fowler and Downey 1970, Stringam *et al.* 1974). Temperature and moisture during seed development, nitrogen fertilisation and other factors are influencing the oil content. Usually cool and moist growing conditions favour high oil contents, while increasing rates of nitrogen fertilization reduce oil percentage per seed, but increase oil yield per acre. In general, seeds with highest oil content are harvested from winter types (Kramer and Sauer 1983, Kimber and McGregor 1995). More than 90% of the seed oil consists of triacylglycerols which are composed of three variable fatty acid chains bound to a glycerol-backbone. Fatty acids consequently being the major constituents of seed oil which makes fatty acid composition one of its most important properties.

In general, the fatty acid profile of oilseed rape consists of palmitic (16:0), palmitoleic (16:1), stearic (18:0), oleic (18:1), linoleic (18:2) and linolenic (18:3) acid. The traditional *B. napus* seed oil additionally contains the long-chain fatty acids arachidic (20:0), cis-11-eicosenoic (20:1), behenic (22:0) and a significant proportion of erucic (22:1) acid. The fatty acid composition is influenced by environmental conditions, resulting in a higher degree of unsaturated acyl groups with cooler climate and higher latitude, but it is mainly genetically determined (Kimber and McGregor 1995). Conventional breeding made use of this genetic variation and modified the fatty acid composition for various food, feed and industrial purposes. After first concerns about the nutritional intake of erucic acid in the mid-1950s (Kramer and Sauer 1983), with around 40 to 50% the main component of the *B. napus* seed oil, breeding efforts aimed on the development of low erucic acid varieties. A lot of research work and the restriction of the level of erucic acid content in oilseed rape oil for human consumption at the beginning of the 1970s finally lead to the introduction of low erucic acid oilseed rape (LEAR). Starting with a maximum of 5% the limit for erucic acid was lowered to a maximum of 2% in the early 1980s and industrial standards nowadays even aim to reach levels below 1% of erucic acid (Daun and Adolphe 1997, Gunstone 2009). Spotting oilseed rape oil as a highly valuable source of vegetable oil, further breeding attempts in the 1970s additionally reduced the level of another anti-nutritional component, the

glucosinolates, introducing the so called canola or double low quality oilseed rape (Daun 1984). This enormously increased the value of the by-product of oil extraction, the oilseed rape meal (Suprianto 2014). With a very similar fatty acid composition compared to LEAR oil, canola quality oil also had a high nutritional value. Its fatty acid composition is characterized by a high level of the monounsaturated oleic acid with up to 60% and a significant amount of linoleic (20%) and α-linolenic (10%) acid as well as a low proportion of saturated fatty acids of 6 – 7%. This breakthrough in modifying the fatty acid composition of *B. napus* seed oil initiated further alteration of the fatty acid composition to address specific industrial and nutritional needs. One of the following breeding challenges was to increase the amount of erucic acid in order to increase the value of oil used for industrial purposes, resulting in high erucic acid oilseed rape (HEAR) cultivars of summer type with over 50% of erucic acid in their oil (Latta 1990, Murphy and Sonntag 1991, Scarth *et al.* 1991). The challenge in breeding the high erucic acid oilseed rape (HEAR) is based on the inability of *B. napus* to place long-chain fatty acids in the *sn*-2-position of triacylglycerols, limiting the erucic acid content to a maximum of theoretically 66% (Taylor *et al.* 1992). Although several strategies were applied (Taylor *et al.* 1992), to date the limitation was only overcome in one line of *B. oleracea* (Taylor *et al.* 1994). Polyunsaturated fatty acids decrease the heat stability of oil, making it susceptible to oxidative changes during refining, storage and frying (Sasongko *et al.* 2003). With 8 to 10%, LEAR and canola oil contains a relatively high level of α-linolenic acid that may have some nutritional advantages, but is rapidly oxidized at higher temperatures and produces off flavours. Therefore, an additional breeding objective has been the reduction of α-linolenic acid to less than 3%, while maintaining or increasing the level of linoleic acid, an essential fatty acid in the human diet. An initial work of Rakow in 1973, who developed mutants with half the normal amount of linolenic acid, enabled the breeding of the *B. napus* cultivar Stellar with less than 3% linolenic acid and as much as 20% linoleic acid (Rakow and McGregor in 1973, Scarth *et al.* 1988).

2.4 Biosynthesis of storage lipids

Lipids are one of the most important groups of biological macromolecules in living cells. They have many important biological functions, including storing energy, signalling and acting as structural components of cell membranes (Fahy *et al.* 2009, Subramaniam *et al.* 2011). Thus they occur in many different forms, including fats, waxes, sterols, fat-soluble vitamins (such as vitamins A, D, E and K), monoglycerides, diglycerides, triglycerides, phospholipids and glycolipids. Beside surface lipids (1%) and membrane lipids (5%), most of the lipids in the seed, as the storage organ, are storage lipids (94%), which almost entirely consist of triacylglycerols

(TAGs) and are collected in oil bodies (Harwood 1996, Li-Beisson *et al.* 2013, Chen *et al.* 2015). Triacylglycerols are composed of three fatty acids connected to a glycerol backbone. The formation of TAG can be divided into three stages. Initially fatty acids are newly built in the plastid. Subsequently these are exported in the form of acyl-CoA thioesters to the cytoplasmic endomembrane system, where the modification of fatty acids and finally the assembly of storage lipids occurs (Roscoe 2005).

2.4.1 Fatty acid synthesis

Fatty acids are built *de novo* in every cell of a plant. The synthesis takes place in the plastids, therefore it is referred to as the prokaryotic part of lipid synthesis (Roughan and Slack 1982). Fatty acids are produced by the enzymes of the fatty acid synthase (FAS) complex. Using acetyl-coenzyme A (CoA) as precursor, a fatty acid carbon chain is elongated through sequential addition of two-carbon units. Each elongation process consists of four reactions: condensation, reduction, dehydration and reduction, with the acyl carrier protein (ACP) as cofactor of all reactions. Acetyl-CoA, as the major building unit of fatty acids, is mainly produced by the plastidial pyruvate dehydrogenase complex. The two carbon donor molecule necessary for fatty acid elongation is malonyl-ACP. In an ATP dependent two-step reaction acetyl-CoA and hydrogen carbonate are assembled to malonyl-CoA. This reaction is catalysed by a multisubunit heteromeric enzyme complex of prokaryotic type, the acetyl-CoA carboxylase (ACC) (Harwood 1996, Konishi *et al.* 1996). The first step of this reaction is catalysed by the biotin carboxylase (BC) domain of ACC, transferring CO_2 from bicarbonate to a biotin prosthetic group attached to a conserved lysine residue of the biotin carboxyl carrier protein (BCCP) domain of ACC. In a second step, the carboxyl group from carboxy-biotin is transferred to acetyl-CoA to form malonyl-CoA, catalysed by the carboxyltransferase (CT) domain of ACC. Two different subunits are forming the CT domain, α-CT and β-CT. β-CT being the only component in plant lipid metabolism encoded by the plastid genome (Ohlrogge and Browse 1995). Thus, implying a coordinated production of cytosolic and plastid subunits to build a functioning ACC. To finally enter the fatty acid synthesis cycle malonyl-CoA assembled by ACC is transferred to ACP by malonyl-CoA:ACP malonyltransferase (MCMT), forming malonyl-ACP. The initial elongation cycle is assembling acetyl-CoA and malonyl-ACP, starting with a condensation reaction yielding the four-carbon product 3-ketobutyryl-ACP. The condensing enzymes of fatty acid synthesis are 3-ketoacyl-ACP synthases (KAS), while the first condensation is catalysed by KAS III, the condensation reactions of following elongation cycles are catalysed by KAS I. Elongation from palmitoyl-ACP (C16) to stearoyl-ACP (C18) is catalysed by a third isoform, namely KASII

(Pidkowich *et al.* 2007). After condensation 3-ketobutyryl-ACP is reduced to 3-hydroxyacyl-ACP by 3-ketoacyl-ACP reductase (KAR). Hydroxyacyl-ACP dehydratase (HAD) then is dehydrating 3-hydroxyacyl-ACP leading to enoyl-ACP, which is finally reduced to C4 saturated fatty acid-ACP, the precursor for the next elongation cycle, by enoyl-ACP reductase (ENR) (Mou *et al.* 2000). Assembling of the C4 acyl-ACP with another malonyl-ACP initiates the next elongation cycle. The serial addition of two-carbon units to the growing fatty acid chain catalysed by the enzymes of the FAS complex is finally terminated by hydrolysation of 16:0-ACP and 18:0-ACP. Hydrolysation is catalysed by two different acyl-ACP thioesterases and results in free fatty acids. Fatty acid thioesterase B (FATB) produces palmitic acid by the hydrolysation of 16:0-ACP, while fatty acid thioesterase A (FATA) hydrolyses 18:0-ACP, which was produced by an additional cycle in the FAS machinery utilising KASII for condensation, releasing the 18:0 free fatty acid, stearic acid. Alternatively, 18:0-ACP can first be desaturated by Δ^9 stearoyl-ACP desaturase (SAD) producing 18:1-ACP, which is then hydrolysed by FATA to oleic acid. All free long-chain fatty acids produced are esterified with CoA by a long chain acyl-CoA synthetase (LACS) and exported from the plastid to the endoplasmic reticulum, where they are used to build storage lipids.

DNA microarray data indicated co-regulation of core enzymes of fatty acid synthesis at the transcriptional level (Mentzen *et al.* 2008) and further investigations identified the transcription factor WRINKLED1 (WRI1) directly activating the fatty acid biosynthesis pathway (Cernac and Benning 2004, Baud *et al.* 2007; Maeo *et al.* 2009). However, not only transcriptional control but also optimization of enzyme activity is regulating fatty acid biosynthesis (Buckhout and Thimm 2003).

2.4.2 Modifications of fatty acids

In *B. napus* two types of modification of fatty acids can be distinguished, the desaturation and the sequential elongation of oleic acid (18:1). Desaturation of 18:1 is conducted by two specialized microsomal membrane-associated desaturases, FAD2 (Δ^{12}) and FAD3 (Δ^{15}), which form 18:2 and 18:3, respectively. However, the elongation of 18:1 leads to the production of the long chain unsaturated fatty acids (LUFAs), eicosenoic (20:1) and erucic acid (22:1). The elongation from 18:1 to 22:1 takes place in the cytoplasm and is catalysed by the membrane-bound oleoyl-CoA elongation complex. Four successive reactions are included in the elongation process. In a first step β-ketoacyl-CoA synthase (KCS) is catalysing the condensation of malonyl-CoA with the long chain (18:1 or 20:1) acyl-CoA, resulting in the formation of β-ketoacyl-CoA. The second step comprises the reduction of β-ketoacyl-CoA by β-ketoacyl-CoA reductase using NAD(P)H as

reductant. The reduction leads to β-hydroxyacyl-CoA which in a third step is dehydrated to an enoyl-CoA by β-OH-acyl-CoA dehydratase. Enoyl-CoA finally undergoes a second reduction, which is mediated by trans-2,3-enoyl-CoA reductase also using NAD(P)H as reductant. The second reduction forms the long chain (20:1 or 22:1) acyl-CoA (Fehling and Mukherjee 1991, Harwood 1996). This elongation process is adding a two-carbon fragment to the carboxyl end of oleic acid forming eicosenoic acid in a first cycle and erucic acid after a second two-carbon addition (Downey and Craig 1964, Jönsson 1977, Sasongko *et al.* 2003). The erucic acid biosynthesis has been well characterized in *A. thaliana* identifying *fatty acid elongase 1* (FAE1), encoding the condensing enzyme KCS, as key-regulator (Lemieux *et al.* 1990, Kunst *et al.* 1992, Rahman *et al.* 2008).

2.4.3 Triacylglycerol synthesis

After fatty acids have been synthesised they are exported from the plastid to the ER were they enter the so called Kennedy pathway or glycerol phosphate pathway to form triacylglycerols (TAGs). Beside fatty acid chains the main component of these storage lipids is glycerol-3-phosphate (G3P). The first reaction of the glycerol phosphate pathway is the acylation of G3P at its *sn*-1 position, which is catalysed by glycerol-3-phosphate acyltransferase (GPAT). In a second acylation step 2-lysophosphatidic acid acyltransferase (LPAAT) is transferring a second fatty acid from the acyl-CoA pool to the *sn*-2 position. The resulting phosphatidic acid afterwards is dephosphorylated by phosphatidate phosphatase (PP) forming diacylglycerol (DAG). A particular characteristic of the *Brassica* LPAAT is its specificity, making the utilization of erucoyl-CoA as an acyl donor incapable (Bernerth and Frentzen 1990, Taylor *et al.* 1992). Thus, erucoyl moieties are typically excluded from the central sn-2 position of the triacylglycerol molecule in *B. napus* (Nath *et al.* 2008). The resulting DAGs are representing important intermediates not only for storage but also for membrane lipid synthesis. Therefore, the final acylation of the sn-3 position of the glycerol backbone is the unique and specific reaction in TAG biosynthesis. Depending on the acyl donor source three different mechanisms have been identified contributing to this step. Using a fatty acyl-CoA molecule acetylation of the sn-3 position of DAG is catalysed by diacylglycerol acyltransferase (DGAT). There have been two classes of DGAT identified, DGAT1 and DGAT2 respectively, which are differing in their sequence and membrane topology as well as their substrate discrimination. Up to now only DGAT1 has been shown to play a role in seed oil accumulation, while the role of DGAT2 remains to be confirmed. A second way of DAG acylation is catalysed by a phospholipid:diacylglycerol acyltransferase (PDAT) that is utilizing phosphatidylcholine (PC) as acyl source. PC is generated from lyso-PC by

lysophosphatidylcholine acyltransferase (LPCAT). Hence, TAG formation by PDAT depends on LPCAT activity. Dahlqvist *et al.* (2000) detected PDAT activity in plants and the gene encoding PDAT was identified in *A. thaliana* by Ståhl *et al.* (2004). Examination of mutants by Zhang *et al.* (2009) indicated that PDAT1 is capable of compensating absence of DGAT1, because double mutants of *PDAT1* and *DGAT1* were lethal, and RNAi suppression of either gene in a mutant background lacking the other gene resulted in severe defects in pollen and seed development, including greatly reduced oil bodies and oil content, but *dgat1* mutants only showed a minor reduction of oil content. Diacylglycerol:diacylglycerol transacylase (DAGTA) is catalysing the third reaction mechanism synthesising TAG by transferring a acyl group from one DAG to another (Roscoe 2005). Synthesised TAGs converge and are released from the ER enclosed by a phospholipid monolayer as the so called oil bodies or lipid droplets. The phospholipid monolayer is also containing different types of proteins, including oleosins, caleosins and steroleosins (Jolivet *et al.* 2004). Oleosins, which build the most abundant group, are regulating the size of oil bodies and thus enabling the mobilization of the TAG storage during seed germination by maximizing the surface-to-volume ratio of the oil bodies (Siloto *et al.* 2006, Shimada *et al.* 2008). While Caleosins also seem to play a role in TAG mobilisation during germination through mediating interactions with vacuoles (Poxleitner *et al.* 2006), steroleosins appear to play a role in signal transduction (Lin *et al.* 2002).

2.4.4 Candidate genes of oil biosynthesis

To reveal the mechanisms and characterize the genes involved in plant lipid biosynthesis a number of different genetic, molecular and biochemical studies were performed on the model plant and close relative of *B. napus*, *A. thaliana* (Ohlrogge *et al.* 2000). Information on the genes found to be involved in the lipid biosynthesis was thereupon collected in the *Arabidopsis* Lipid Gene Database (Beisson *et al.* 2003, http://aralip.plantbiology.msu.edu/pathways/pathways). The comparison of the *A. thaliana* wild type to collections of *A. thaliana* mutants identified the *tag1*-mutant (Zou *et al.* 1999) and the *wrinkled1*-mutant (Focks and Benning 1998) with reduced seed oil content. The *tag1*-mutant which additionally showed an altered fatty acid composition (Katavic *et al.* 1995) was traced back to the DGAT-gene. Seed-specific overexpression of the DGAT-gene increased the oil content (Jako *et al.* 2001). However, the decreased oil content in the *wrinkled1*-mutant of about 80% was caused by the mutation of the *wri1*-locus, presumably encoding a transcription factor (Cernac and Benning 2004b, Bach 2007). Microarray experiments of Ruuska *et al.* (2002) allowed the simultaneous investigation of >100 genes involved in lipid metabolism, enabling a broad overview of the transcriptional regulation

of the pathway. The researchers identified an expression cascade for specific groups of genes involved in oil biosynthesis during seed maturation. The comparison of wild type expression to the expression of the *wri1*-mutant identified 45 genes showing clear differences in their expression patterns. Most of these genes appeared to encode key regulators of fatty acid synthesis or carbon metabolism like BCCP2, KASI, enoyl-ACP reductase (ENR), two ACP isoforms, FAD2 (Ruuska *et al.* 2002). Thelen and Ohlrogge (2002) also realized the important role of the BCCP2-gene, since overexpression as well as antisense mediated reduction inactivated the plastid acetyl-CoA-carboxylase, which led to decreased oil content and changed fatty acid composition. Voelker and Kinney (2001) reviewing the "Variation in the Biosynthesis of Seed-storage Lipids" demonstrated that nearly every modification of the enzymes involved in oil biosynthesis, including fatty acid synthesis, modification and elongation as well as TAG formation, changes seed oil production.

2.5 Illumina's SNP beadchip technology

The Illumina SNP beadchip technology is an array-based whole-genome genotyping assay with single-tube sample preparation, which allows for accurate and robust genotyping in the context of full genomic complexity in a single array experiment (Gunderson et al. 2005). The bead-array method generally consists of four steps, (i) a whole-genome amplification, (ii) an array-based hybridization capture, (iii) an 'on array' enzymatic allele-specific primer extension and (iv) a signal detection step. The first generation of bead-arrays initially used a single-tube whole-genome amplification to generate a sufficient amount of the complex genomic DNA for hybridisation (Gunderson *et al.* 2005). The amplification of the genomic DNA was conducted by a method described by Dean *et al.* (2002) termed multiple displacement amplification (MDA). Compared to the commonly used random or degenerate oligonucleotide-primed PCR approaches to amplify genomic DNA and at the same time reduce its complexity, the MDA showed a lot of advantages. By the use of φ29 DNA polymerase and random exonuclease-resistant primers MDA became an isothermal, strand-displacing process yielding a highly uniform representation across the genome with product lengths of >10 kb and about 20 – 30 µg product from as few as 1 – 10 copies of human genomic DNA. MDA was even applicable to biological samples including crude whole blood and tissue culture cells. With high genome coverage of >95%, reproducibility of 98% and high concentration products the MDA was perfectly meeting the demands of the bead-array system. For hybridisation, amplified DNA was denatured and incubated together with bead-bound capture probes on the array. Hybridisation capture probes were composed of 75 base long oligonucleotides including 25 bases used for

decoding and 50 bases as target sequence for hybridisation. They were immobilized on activated beads of 3µm in diameter using a 5'-amino group. One bead loaded with multiple copies of one target sequence which was carrying a selective nucleotide at the 3'-terminal. After hybridisation an allele-specific primer elongation assay with high allelic discrimination was conducted to query the SNP. The use of two different bead-types (A and B) enabled the identification of all variants of the SNPs, since sequences were only extended and labelled if perfectly matching the target. To read the incorporated labels the allele specific primer elongation reaction is followed by a sensitive detection and signal amplification. Finally, intensity ratios between the two corresponding bead-types were captured by a custom imaging system and analysed to determine the genotype state of a given SNP locus (AA, AB or BB) using an appended software. By comparison of intensities between A and B allele probes the software assigns membership to archetypal clusters created by comparison of a large numbers of samples using a probabilistic model (Gunderson *et al.* 2004; Gunderson *et al.* 2005). Initially designed to genotype human DNA at thousands of SNPs simultaneously across the genome the Illumina SNP beadchip technology today is also available for a lot of animal and plant species due to enormous progresses in molecular biological techniques like "next-generation" DNA sequencing and computational methodology, as well as their dramatically reduction of costs (LaFramboise 2009, Edwards *et al.* 2013). The latest feature for SNP bead-chips is the individual and customised SNP selection, e.g. increases the power of association studies relative to random SNPs. In 2012 an international *Brassica*-SNP-consortium, established in collaboration with Illumina Inc. (San Diego, CA, USA) in 2011, produced a 60000 (60k) SNP genotyping array for *B. napus*. This introduced a very low-cost and efficient method for high-density, sequence-based, genome-wide polymorphism screening in *B. napus* populations (Liu *et al.* 2013). The consortium array contains 58464 SNPs designed to function well in *Brassica* A (28044 SNPs) or C (30420 SNPs) genome species (Sharpe 2012). The SNP content was derived from DNA sequence contributions by academic and commercial partners from Australia, China, Europe, North and South America, using preferentially single-locus SNPs identified from genomic and transcriptomic sequencing in genetically diverse *Brassica* germplasm (Snowdon and Iniguez Luy 2012, Liu *et al.* 2013). The recent publication of a first *B. napus* sequence (Chalhoub *et al.* 2014) finally enabled the use of these SNP marker information to navigate directly from genetic map positions to the genome sequence.

2.6 QTL mapping for oil content in *Brassica napus*

The genetic information of higher organisms is organised on chromosomes which carry genes in a fixed linear order. The specific position of a gene on the chromosome is called a locus. A gene can be represented by alternative forms, its alleles. To investigate the genetic control of a measurable phenotypic trait, a mapping population is used derived from a cross between parental lines which differ in the trait of interest. Within this mapping population recombination events between alleles of different loci are determined to estimate linkage values as a measure of genetic distance. The linkage relations among all chromosomes are used to create a genetic map of an organism. Combining genetic and phenotypic information, genetic regions influencing the phenotypic trait are identified. Depending on the mating type of the investigated species a suitable type of mapping population needs to be chosen (Meksem and Kahl 2006).

The seed-oil content varies quantitatively among germplasm of *B. napus*. This variation is attributed to the complex regulation of multiple genes that are involved in various aspects of seed-storage-oil metabolism (Ohlrogge and Browse 1995, Mekhedov *et al.* 2000, Barker *et al.* 2007). Thus, mapping the genetic loci that control the quantitative variation is a preliminary step to disclose the complex regulation of this trait (Jiang *et al.* 2014).

The initial study to identify QTL for seed oil content in *B. napus* was conducted by Ecke *et al.* (1995) using a RFLP (restriction fragment length polymorphism) map of a F1 microspore-derived DH population derived from a cross of Mansholt's Hamburger Raps and Samourai. Although the parental lines did not show significant difference in seed oil content, they detected three discrete loci for oil content within the transgressively segregating DH population. These QTL identified with MAPMAKER/QTL (Lincoln and Lander 1992) were located on linkage groups 6, 10 and 12 and together explained 51% of the total phenotypic variation for oil content. Their additive effects sum up to 4.8% of oil content for homozygous genotypes. Mansholt alleles increased seed oil content for QTL on linkage groups 6 and 12, while Samourai was contributing the positive allele increasing seed oil content on linkage group 10. Comparing the positions of QTL for oil content with estimated positions of the two erucic acid genes, Ecke *et al.* (1995) found a close association of QTL for both traits on linkage groups 6 and 12.

Butruille *et al.* (1999) were analysing canola quality inbred backcross lines of the German winter-type cultivar Ceres with the summer-type recurrent parent Marnoo (Australian) and Westar (Canadian) to map genomic regions of the donor parent that affect agronomic traits, identifying only one putative QTL affecting seed oil content on linkage group N1.

In 2003 Burns *et al.* investigated the inheritance of seed oil content and its fatty acid composition in a set of substitution lines formed from a cross between the winter oilseed rape varieties Tapidor and Victor. Like in Ecke *et al.* (1995) the parents of the substitution lines showed a similar seed oil content but differed in erucic acid content. With a model-fitting approach a total number of 13 QTL was identified distributed among ten linkage groups. While all QTL affected fatty acid composition, only seven of these QTL, which were located on the linkage groups N3, 6, 8, 13, 14, 18 and 19, influenced total seed oil content. And Burns *et al.* (2003) also suggested one of their seed oil-QTL located on linkage group N8 to be synonymous with a QTL for erucic acid, supporting the hypothesis of Ecke *et al.* (1995).

Studying an F1 derived doubled haploid population derived from a cross between the German cultivar Sollux and the Chinese cultivar Gaoyou, Zhao *et al.* (2005) detected eight QTL for seed oil content with additive main effects located on linkage groups N1, 7, 9, 11, 12, 14, 18, 19. The alleles increasing oil content for QTL on linkage group N7, 11 and 18 were derived from Gaoyou. QTL with additive main effects explained about 40% of the phenotypic variation and were summing up to 5.4% maximum difference in oil content between homozygous genotypes. Additionally, nine pairs of loci with additive x additive epistasis were identified which sum up to 5% maximum difference in oil content between homozygous genotypes, very similar to the results of QTL with additive main effects. All together the QTL with additive and epistatic main effects accounted for 80% of the phenotypic variation. Parental lines were chosen because of their high oil contents but they also contained high erucic acid contents. QTL were calculated by a mixed model approach of the mapping software QTLMapper (Wang *et al.* 1999) using microsatellite markers. Furthermore investigating the effect of additive x environment interaction, Zhao *et al.* (2005) identified five out of the eight seed oil-QTL with additive effects that showed significant interaction. And additional ten QTL with additive x environment interaction were observed showing no significant additive main effect. Since the alleles increasing oil content were dispersed between the parents, the authors suggested a marker assisted selection, considering epistatic effects and genotype x environment interactions, to recombine the positive alleles from both parental lines to further increase oil content in *B. napus*. Although the results of Zhao *et al.* (2005) indicated that the Chinese and European alleles identified were often more positive at their respective location, the authors also found four QTL where the allele from the German parent increased oil content in China and vice versa. In a subsequent publication including conditional mapping Zhao *et al.* (2006) identified six QTL and nine epistatic interaction pairs showing pleiotropic effects on oil and protein content, demonstrating the strong genetic relationship between these traits. In this study two additional QTL were identified which control oil content independently.

At the same time Delourme *et al.* (2006), who analysed two DH populations derived from the crosses Darmor-*bzh* x Yudal (DY) and Rapid x NSL96/25 (RNSL), investigated the 'Genetic control of oil content in oilseed rape'. Using composite interval mapping (CIM) with QTL Cartographer (Basten 2005), the authors found a total of fourteen and ten genomic regions involved in seed oil content in DY and RNSL populations, respectively. One of these QTL located on linkage group N3 was potentially common to both populations. While Darmor-*bzh* and Yudal had a quite similar oil content but carried different alleles at many QTL, causing a large distribution for oil content in the DY DH population, the oil content between Rapid and NSL96/25 differed more but alleles increasing oil content were mainly derived from the parent with higher oil content, and the derived DH population included only a few transgressive lines. These results showed that a combination of favourable alleles at different QTL positions can efficiently increase seed oil content. The authors also confirmed a significant environmental effect on average oil content beside the additive effects as main contributors, confirming the results of Zhao *et al.* (2005). But only little epistasis was observed. Furthermore, Delourme *et al.* (2006) recommended utilizing the available *Arabidopsis* genomic data to develop markers for *B. napus* and to identify candidate genes and the establishment of a consolidated map of QTL of different segregation populations to improve the investigation of traits of interest.

Qiu *et al.* (2006) developed a DH mapping population by crossing the genetically diverse oilseed rape lines Ningyou 7 and Tapidor, and constructed a linkage map based on a variety of different markers including sequence tagged site (STS), simple sequence repeat (SSR), restriction fragment length polymorphism (RFLP) and amplified fragment length polymorphism (AFLP) markers to enable the integration with existing *Brassica* linkage maps and with the *A. thaliana* genome. The parental lines again showed similar oil contents but different erucic acid contents. Using MapQTL (Van Ooijen 2004) the authors identified seven QTL for seed oil content, located on N1, 3, 4, 8, 12, 13 and 17, in total accounting for 54.6% of the phenotypic variation. The alleles increasing oil content on N1, 4 and 12 were contributed by Tapidor, while increasing alleles of N 3, 8 and 13 were contributed by Ningyou 7. Also analysing the genetic control of erucic acid, four QTL were detected which were found on linkage groups N1, 2, 8 and 13, all together explaining 87.9% of the variation within the population. The two major QTL for erucic acid on linkage groups N8 and N13 accounted for about 45 and 30% of the phenotypic variation, respectively. And QTL on linkage groups N1, 8 and 13 were co-located with QTL for seed oil content. Alignment of the QTL regions of these three loci to the genome sequence of *A. thaliana*, the *fatty acid elongase 1* (*fae1*) was identified as candidate gene regulating erucic acid biosynthesis and influencing oil content.

Three years later Yan *et al.* (2009) presented their results analysing seed oil content, seed hull content and seed coat colour in a recombinant inbred lines (RIL) population of *B. napus*, developed through successive selfing up to six generations from a cross between yellow-seeded female parent GH06 and black-seeded male parent P174 by single seed descent. Utilizing again different types of markers (AFLP, sequence related amplified polymorphism (SRAP), SSR and target region amplified polymorphism (TRAP)) and conducting composite interval mapping (CIM) by WinQTL Cartographer software version 2.5 (WinQTLCart 2.5; Wang *et al.* 2012a), eleven QTL controlling oil content were detected, accounting for 5.19 - 13.57% of the phenotypic variation. Alleles increasing seed oil content came from GH06 (N3, N4, N5, N7, N8 and N13) and P174 (N1 and LG14), respectively. Comparison of QTL of all three traits revealed the first common QTL region on N8. Like Zhao *et al.* (2005), the investigations of Yan *et al.* (2009) also showed a significant effect of the environment on seed oil variation.

Studying the genetic control of seed oil content, seed yield and days to flowering Chen *et al.* (2010) identified 27 QTL for oil content in six environments in Canada, distributed among 14 linkage groups (LG1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 13, 14, 16, and 18). The individual QTL for oil content explained 4.2–30.2% of the total phenotypic variance. WinQTLCart 2.5 was used to map QTL for all traits using its composite interval mapping method (CIM). The plant material of this experiments was consisting of a recombinant doubled haploid (DH) population derived from the cross of a high-oil content line and a low-oil content line. On LG7 a QTL for oil content, a QTL for seed yield, and a QTL for days to flowering were found coincidently located. The additive effects of these QTL were negative for both oil content and days to flowering, while a positive additive effect was detected for yield. And another QTL for oil content with a positive additive effect on LG2 was closely positioned with a QTL for days to flowering with negative additive effect.

In 2012, Zhao *et al.* presented a comparative mapping approach between *B. napus* and *A. thaliana* to identify potential candidate genes of the seed oil metabolism. On a refined version of their previous SSR map (Zhao *et al.* 2005) based on informative markers derived from *Brassica* sequences, which included orthologous genes of *A. thaliana* and were mostly related to genes of the acyl lipid metabolism, composite interval mapping of WinQTLCart 2.5 (Wang *et al.* 2012a) was conducted. This identified nine significant QTL for seed oil content on the linkage groups A1, A5, A7, A9, C2, C3, C6 and C8, together explaining 57.79% of the total phenotypic variation. Within the confidence intervals of six of these QTL a total number of 14 lipid related candidate gene loci were found, not including the prevailing transcription factors WRI1 (Cernac and Benning 2004b), LEC1 (Mu *et al.* 2008) and their homologous genes in *B. napus*, BnWRI1, BnLEC1 and LEC1-like gene BnL1L. Seven of the nine detected QTL were consistent to findings

of Zhao *et al.* (2005), and comparison with other populations (Burns *et al.* 2003, Delourme *et al.* 2006, Qiu *et al.* 2006, Chen *et al.* 2010) through alignment of common SSR markers also identified seven of the detected QTL in same or similar regions.

To make former seed oil-QTL results in *B. napus* more comparable, Jiang *et al.* (2014) constructed a high-density map of more than 700 markers using the DH population of Qiu *et al.* (2006) to align QTL among different populations. And since previous QTL analyses of seed oil content in *B. napus* were manly using DH populations or inbred lines which allow multiple repetitions of experiments, but revealed only additive effects and some epistatic effects of the seed oil-QTL, Jiang *et al.* (2014) additionally used a Tapidor and Ningyou 7 reconstructed-F2 population (TN; Shi *et al.* 2009) to enable determination of the dominance effects for QTL. Using composite interval mapping with WinQTLCart 2.5 (Wang *et al.* 2012a) and QTLMapper 2.0 software (Wang *et al.* 1999), 41 QTL including 20 QTL with dominance effects and 20 pairs of epistatic interaction loci were detected in the two populations, respectively. And the alignment of and comparison with selected genetic maps in total identified a number of 46 distinct QTL regions that control seed oil content on 16 of the 19 linkage groups of the *B. napus* genome represented by the genetic map of the TN population. 18 of these QTL regions were detected in different populations including a previous association study panel of breeding lines (Zou *et al.* 2009).

QTL analysis of seed oil content of the past two decades reflected the continuous development of molecular technologies and progress of research in understanding the mechanisms regulating seed oil content in *B. napus*. Now, the recent publication of the *B. napus* genome sequence by Chalhoub *et al.* (2014) represents a promising milestone to reveal detailed insides in the genetic control of oil content.

Recently, Teh (2015) mapped QTL for oil content and fatty acid content in a DH population derived from the two canola quality winter oilseed rape varieties Sansibar and Oase (SODH), investigating the two mega-environments Europe and China. Using AFLP, candidate-gene based, Diversity Array Technology (DArT), Silico-DArT, SSR, and SNP markers Teh identified five QTL for oil content in the European trial on linkage groups A01, A02, A07, C03 and C08 (Teh and Möllers 2015), and seven in the Chinese trial on linkage groups A04, A07, A09, C01, C03 (2) and C06 by applying the multiple interval mapping (MIM) of WinQTL Cartographer software. Two of these QTL were found consistent in both environments. Physical mapping based on the *B. rapa* genome allowed revealing the co-localisation of candidate genes of oil biosynthesis with QTL for different fatty acids.

2.7 Conditional mapping

The phenotypic variation of a lot of quantitative traits, like seed oil content, are affected not only by environmental conditions but also by genetic effects caused by gene action during specific plant developmental periods, making the evaluation of data from different points in time difficult. To solve this problem and allow the analysis of quantitative traits taking these effects into consideration, Zhu (1995) proposed a statistical procedure to analyse conditional genetic effects and conditional genetic variance components based on genetic models with time-dependent measures. This statistical method was not only suitable for additive-dominance models, but also applicable for other genetic models expressed in mixed linear models. For some genetic models with correlated genetic factors, such as seed or endosperm models, minimum norm quadratic unbiased estimation (MINQUE; Rao 1971) was recommended to estimate the variance and covariance components (Zhu and Weir 1994a, Zhu and Weir 1994b). In 1998 Yan *et al.* used the Zhu method in rice and combined it with a mapping program to identify conditional QTL for tiller number in different time intervals, while Yan *et al.* (1999) and Cao *et al.* (2001) used the software supplementation to map QTL with additive and/or additive by environment interaction effects for the developmental behaviour of plant height. Benmoussa (1998) further extended the conditional QTL mapping approach by analysing not time dependency anymore but the interrelationship of traits. Dissecting yield and yield components in rice molecularly, the contribution of each component to yield was revealed on QTL level. By combining the statistical procedures of Zhu (1995) and the new QTL mapping method based on mixed linear model approaches in QTLMapper version 1.0 of Wang *et al.* (1999), Cao *et al.* (2001) were able to analyse the dynamic behaviour of plant height at different stages of rice, and detect QTL not only with additive but also with epistatic effects as well as their QTL x environment interaction effects. Zhao *et al.* (2006) again used the mixed model approach of Zhu (1995) in combination with the QTL mapping software of Wang *et al.* (1999) to study the genetic interrelationship between QTL for oil content and the negatively correlated protein content as well as other plant developmental and yield related traits influencing oil content in *B. napus*. These investigations detected QTL that reflect the effects of oil content under exclusion of variation in a second potentially influencing factor. The conditional oil content was estimated for the situation of no variation for the secondary trait, a method very similar to the estimation of adjusted values in a covariance analysis, and analysed by QTL mapping in the same way as the original oil content. The comparison of unconditioned and conditioned oil-QTL revealed additional QTL with small effects which were undetectable in unconditioned mapping. Thus,

Zhao *et al.* (2006) suggested that for significantly correlated traits, the conditional QTL mapping method generally could be used to dissect the genetic interrelationship between traits at the level of individual QTL and reveal additional QTL undetected by unconditional mapping. This conclusion later on was confirmed by Li *et al.* (2007), who investigated the genetic relationship between popping expansion volume and two yield components in popcorn. Till today the conditional approach of Zhu (1995) is a prevalently utilized method to map conditional QTL in many crops like rice (Ye *et al.* 2009), wheat (Wang *et al.* 2012), soybean (Han *et al.* 2015) and oilseed rape (Wang *et al.* 2015)

3 Genetic variation and inheritance of oil content and relevant seed quality traits of the SGEDH population cultivated in Europe

3.1 Abstract

Breeding for increased oil content in oilseed rape (*Brassica napus* L.) has been pursued independently for many decades in Europe and in China. In previous work, the old German cultivar Sollux was crossed to the Chinese cultivar Gaoyou. Both cultivars have high oil content but have also high contents of erucic acid and glucosinolates ('++'-quality). A doubled haploid (DH) population was developed from this cross and tested in field experiments. From this population, line SGDH14 was identified as having the highest oil content under north-western German growing conditions and a combination of all QTL alleles increasing oil content from both parents. The objective of this work was to further investigate the inheritance of seed oil content and other seed quality traits in a new DH population derived from the cross of SGDH14 and the inbred line 617 of the German cultivar Express ('00'-quality), which is also known for its high oil content. The population (n=212) was tested in field experiments during three consecutive years in 14 environments in North Germany and South Sweden. Seed quality traits as well as phenological traits were determined. A framework map consisting of 19 linkage groups with 379 SNP, DArT and AFLP markers and covering 2651cM was developed. Mean trait values over the environments were used to map QTL. Analysis of variance revealed highly significant genotypic effects for all traits and heritabilities ranged from 0.76 to 1.00. Following the correction of oil content considering the effects of the erucic acid genes, three minor and one major QTL for oil content were detected on linkage groups A10, C03, C04 and C05, respectively. The direction of the additive effects showed that both parents contributed two alleles that increased oil content. Alignment of the genetic positions of the oil-QTL confidence intervals to their physical position on the *B. napus* Darmor-*bzh* reference genome allowed identification of lipid related candidate genes. However, only one candidate gene explaining the observed QTL effects on C04 was detected. The presented results contribute to on-going efforts to increasing oil content, and they delimit physically relevant genome regions in oilseed rape.

3.2 Introduction

Oilseed rape (*Brassica napus* L.; genome AACC, 2n = 38) is worldwide the second most important oil crop, with the European Union being its number one producer (USDA, http://apps.fas.usda.gov/psdonline/psdHome.aspx, date: 11.10.2015). Since the global oil consumption is forecast to continue to grow (FAO, http://www.fao.org/economic/est/est-commodities/oilcrops/en/, date: October 2015), one major breeding aim in oilseed rape is to continuously increase the seed oil content. As a quantitative trait, the variation in seed oil

content is attributed to the complex regulation of multiple genes (Jiang *et al.* 2014). To gain insight into the complex genetic control of oil content, a first molecular marker map was developed by Ecke *et al.* (1995) to identify QTL influencing seed oil content. Despite a limited number of RFLP markers, three QTL controlling seed oil content were detected. Two of these QTL were found closely associated with qualitative variation in erucic acid content. In subsequent studies mapping QTL combined parental lines with different seed oil content and quality type, and through developmental progress not only the marker systems but also computational methods evolved. Using microsatellite (SSR) markers Zhao *et al.* (2005) reported eight QTL in a DH population generated from a cross between a German and a Chinese cultivar, both of high oil, erucic acid and glucosinolate content. In following studies different types of markers were combined to improve genome coverage and to enable comparison with other genetic maps. Between seven and fourteen QTL for oil content were identified in a number of studies (Delourme *et al.* 2006, Qiu *et al.* 2006, Yan *et al.* 2009). Using a refined version of their previous map Zhao *et al.* (2012) presented a comparative mapping approach between *B. napus* and *A. thaliana* to identify candidate genes of seed oil metabolism. The availability of common SSR markers in the study of Zhao *et al.* (2012) additionally allowed the comparison of the nine detected QTL for seed oil content to QTL of different populations (Burns *et al.* 2003, Delourme *et al.* 2006, Qiu *et al.* 2006, Chen *et al.* 2010), which identified seven QTL mapped to the same or similar genetic regions. Although the study of Zhao *et al.* (2012) showed that the alignment of different populations of *B. napus* is possible, the lack of sufficient common markers usually prevented direct comparison between genetic maps. Those obstacles were overcome by the development of high throughput sequence-informative marker systems like DArT and SNP arrays. Another limitation for increasing seed oil content in *B. napus* is the restricted variation within the relatively young species compared to other crops (Hasan *et al.* 2005). Although breeding efforts to increase oil content in current European cultivars have been successful, the selection further narrowed the genetic basis. Alternative genetic resources could be useful to further increase seed oil content. In this regard Chinese oilseed rape material appears interesting, because it has been bred independently for high yield and oil content during the last decades. In previous work, the old German cultivar Sollux was crossed to the Chinese cultivar Gaoyou. Both cultivars have high oil content but have also high contents of erucic acid and glucosinolates (‘++’-quality). A doubled haploid (DH) population was developed from this cross and tested in field experiments. From this population, line SGDH14 was identified as having the highest oil content under north-western German growing conditions and a combination of all QTL alleles increasing oil content from both parental lines (Zhao *et al.* 2005). The objective of this work was to further investigate the inheritance of seed oil content and other seed quality traits in a new DH population derived from the cross of SGDH14 and the inbred line 617 of the

German cultivar Express ('00'-quality), which is also known for its high oil content. Sequence informative SNP and DArT markers were used for genotyping the population to allow for identification of candidate genes for oil content using the *B. napus* genome sequence (Chalhoub *et al.* 2014).

3.3 Materials and methods

3.3.1 Plant material

The plant material consisted of 212 DH lines developed from F1 plants of the cross SGDH14 x Express617 by microspore culture (Georg-August-Universität Göttingen, Department of Crop Sciences, Division of Plant Breeding, Göttingen, Germany), further referred to as SGEDH population. SGDH14 is a DH line derived from the cross Sollux x Gaoyou (SG) analysed by Zhao *et al.* (2005). Sollux is an old German cultivar, whereas Gaoyou is an old Chinese cultivar. Both lines are characterised by high erucic acid and high glucosinolate content ('++'-quality) as well as high seed oil content. SGDH14 was chosen as parental line since it showed highest oil content at the German locations tested, and characterization by molecular markers revealed SGDH14 to combine all favourable QTL alleles for oil content from Sollux and Gaoyou (Zhao *et al.* 2005). Marker genotype distribution of SGDH14 was generally balanced, showing 52.4% Sollux and 47.6% Gaoyou alleles in a set of 473 markers (Prof. Jianyi Zhao, Zhejiang Academy of Agricultural Sciences, Hangzhou, PR China, personal communication). Like its parental lines, SGDH14 has high erucic acid and high glucosinolate content. Express617 is an inbred line of the German winter oilseed rape cultivar Express. It was chosen as parental line due to its low erucic acid and low glucosinolate content (canola-/'00'-quality) and high oil content.

3.3.2 Field experiments

The plant material was tested in the three consecutive growing seasons 2009/10, 2010/11 and 2011/12 in 14 environments located in northwest Germany and south Sweden (Table 3.1).

Table 3.1: Environments of field experiments of SGEDH population

Year	2010	2011	2012
Location	Göttingen, Germany	Göttingen, Germany	Göttingen, Germany
	Biehmsen, Germany	Asendorf, Germany	Svalöv, Sweden
	Thüle, Germany	Einbeck, Germany	Hovedissen, Germany
	Nienstädt, Germany	Bad Salzuflen, Germany	Nienstädt, Germany
	Rosenthal, Germany		Rosenthal, Germany

Field experiments were conducted as group-wise randomized block design in small plots without replications. The three different groups within the experiment represented genotypes with low (L; <10%), medium (M; 10-35%) and high (H; >35%) erucic acid content predetermined by a half seed analysis for fatty acid composition using gas chromatography. For each genotype seed samples were harvested at maturity from the main raceme of ten open pollinated plants. Seeds were bulked and seed quality traits were analysed by near-infrared reflectance spectroscopy (NIRS) and gas chromatography (GC). During growing seasons begin of flowering (BOF) and end of flowering (EOF) were scored, plant height at end of flowering (PH_EOF) was measured and flowering period (FP) was calculated. BOF and EOF were measured as days from 1st of January to BOF and EOF, respectively. BOF was scored when 10% of the plants within the plot had at least one open flower. EOF was scored when at least 10% of the plants within the plot finished flowering. FP was calculated from the difference between EOF and BOF. Plant height was measured from the soil surface to the shoot tip at EOF of each plot.

3.3.3 Phenotypic analysis

3.3.3.1 Near-Infrared Reflectance Spectroscopy

To determine seed quality traits about 3g of bulked seeds were scanned between 400 to 2498nm by a NIRS monochromator (FOSS NIR Systems model 6500, NIRSystems, Inc., Silversprings, MD, USA). Absorbance values log at 2nm intervals were recorded to create a NIR spectrum for each sample. With WinISI II software (version 1.50) seed quality traits were determined using the calibration raps2012.eqa provided by VDLUFA Qualitätssicherung NIRS GmbH (Teichstr. 35, 34130 Kassel, Germany, http://h1976726.stratoserver.net/cms/). Oil (Oil) and protein (Protein) content were determined in per cent at 91% seed dry matter. Glucosinolate content (GSL) was measured in µmol/g at 91% seed dry matter. Fatty acid content (18:1, 18:3 and 22:1) was determined in per cent of the total fatty acid content.

3.3.3.2 Gas Chromatography

Fatty acid profile analysis was performed by gas chromatography according to the method of Thies (1971) as described by Rücker and Röbbelen (1996) with modifications as follows: Two 4mm stainless steel metal balls and 200mg seeds per genotype were filled into a 3ml polypropylene tube. 1000µl of Na-methylate in methanol (0.5mol/l) were added to each tube and tubes were tightly closed with a screw cap. To extract and transmethylate fatty acids seeds were milled by 2min shaking using a custom-built vertical homogenizer (Institute of Applied Plant Nutrition, Georg-August-Universität Göttingen, Göttingen, Germany). Grounded seeds were then incubated for 15min at room temperature while shaking every 5min. 300µl 5% $NaHSO_4$ as well as 500µl isooctane were added and tubes were vortexed for salt precipitation and extraction of fatty acid methyl ester in isooctane followed by 1min centrifugation at 1000rpm (150 x g). 200µl of the upper phase were pipetted into septum vials and closed with crimp caps. 2µl of the extract were injected into a gas chromatograph (Trace GC ultra, Thermo Electron corporation) with a 25m x 0.25mm I.D. FFAP column (Macherey & Nagel, 0.25µm film thickness, 210°C, split injection 1:70, carrier gas: 150kPa H_2, injection/detector: temperature 230°C). Palmitic (16:0), palmitoleic (16:1), hexadecadienoic (16:2), stearic (18:0), oleic (18:1), linoleic (18:2), linolenic (18:3), arachidic (20:0), eicosenoic (20:1), behenic (22:0) and erucic (22:1) acid were determined by the GC chromatogram of each sample. Fatty acids were expressed as per cent of the sum of all fatty acids.

3.3.3.3 Adjustment of NIRS predicted erucic acid contents

Comparing NIRS predictions and GC measures of erucic acid content within the SGEDH population a conspicuous underestimation of erucic acids contents of NIRS data was identified (cf. Figure 3.1a). Comparing the differences between GC and NIRS values, different deviations were observed within the group of genotypes with no or low erucic acid, determined as erucic acid free, and the one of genotypes with medium and high erucic acid. Because of the different deviations both groups were adjusted individually. Erucic acid free genotypes, showing generally negative NIRS predictions, were adjusted by subtracting a group specific correction factor of -6.35%. This factor was calculated as the mean difference between GC and NIRS erucic acid content of means over 14 locations of 70 genotypes showing no or low erucic acid content determined by GC. NIRS erucic acid content of genotypes with medium and high GC erucic acid content were adjusted using the regression equation of this group, $y = 1.50x - 5.05$ (Figure 3.1b).

The comparison of adjusted NIRS predicted erucic acid contents and GC erucic acid contents (Figure 3.1c) showed a high coefficient of determination with $R^2 = 0.99$.

3.3.3.4 Thousand kernel weight

Thousand kernel weight (TKW) was obtained from weight conversion of 500 seed. Seeds were counted using a Contador seed counter (Pfeuffer GmbH, D-97318 Kitzingen, http://www.pfeuffer.com).

3.3.3.5 Protein content and glucosinolate content in the defatted meal

Protein content in the defatted meal (Prot.idM) was calculated by using NIRS predicted seed oil and seed protein content (both at 91% dry matter) as:

% protein in the defatted meal (Prot.idM) = [% protein / (100 - % oil)] * 100

Glucosinolate content in the defatted meal (GSLidM) was calculated by using NIRS predicted seed oil and seed glucosinolate content (both at 91% dry matter) as:

µmol/g glucosinolates in the defatted meal (GSLidM) =
[µmol/g glucosinolates / (100 - % oil)] * 100

3.3.3.6 Correction of oil content considering erucic acid content

SGEDH population segregated for erucic acid content, furthermore erucic acid and oil content showed a strong positive correlation. Thus, to be able to compare the oil content of genotypes with varying erucic acid contents, oil contents were corrected by eliminating the effect of erucic acid on oil content in three different ways.

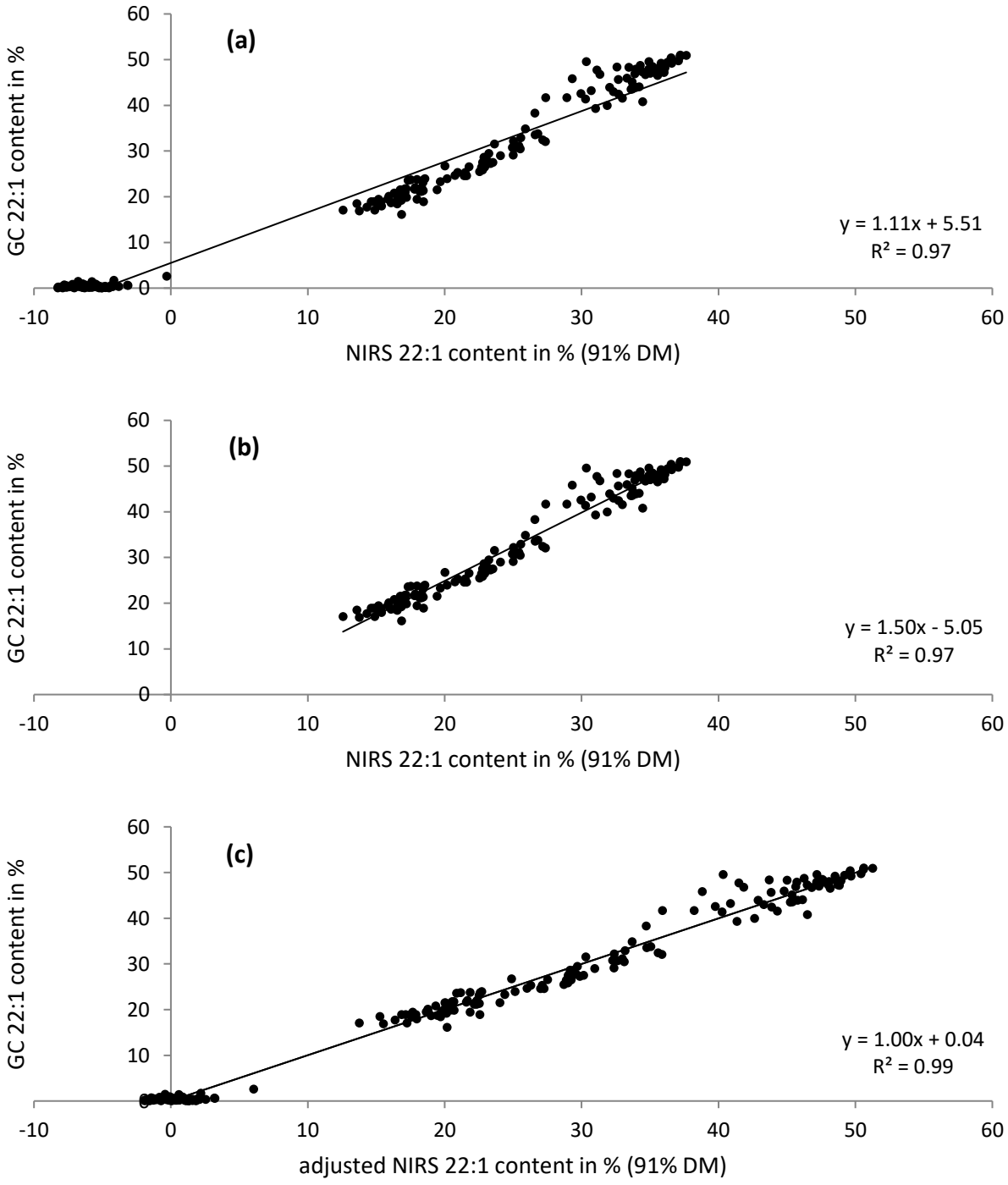

Figure 3.1: xy plots of (a) erucic acid content measured by gas chromatography and NIRS predicted erucic acid content for whole SGEDH population, (b) for genotypes with medium and high erucic acid contents, and (c) erucic acid content measured by gas chromatography and adjusted NIRS values of the SGEDH population. Data are presented as means over 14 environments

Correction by regression

For the correction of oil content by regression information, the linear regression between GC erucic acid content and NIRS oil content from trait means over 14 locations was calculated. The slope of this regression was used to calculate the regression corrected oil content the following way:

NIRS oil content – (slope of linear regression between GC erucic acid content and NIRS oil content * GC erucic acid content)

Correction by molecular weight

De novo fatty acid biosynthesis until oleoyl-CoA takes place in the chloroplasts. However, oleoyl-CoA elongation to erucoyl-CoA via eicosenoyl-CoA and triacylglycerol (TAG) assembly takes place in the cytoplasm. If one molecule oleoyl-CoA is elongated to eicosenoyl-CoA and subsequently to erucoyl-CoA, this leads to an increase of molecular weight of 10 and 19%, respectively. Therefore, if the number of fatty acid molecules produced by the *de novo* fatty acid biosynthesis remains the same an increase in erucic acid content in the seed oil should results in a proportional increase in seed oil content. Following this simplified theoretical assumption, the molecular weight of eicosenoic acid and erucic acid can be reduced on the basis of oleic acid by 9 and 16.6% to eliminate their effects on oil content. Hence the molecular correction of oil content was calculated from trait means over 14 locations as:

NIRS oil content – [0.09 * (GC eicosenoic acid content * NIRS oil content / 100) + 0.166 * (GC erucic acid content * NIRS oil content / 100)]

Correction by conditioning

The mixed model approach for the conditional analysis of quantitative traits described by Zhu (1995) was applied to calculate oil content independent of erucic acid contents. The conditioning allows analysis of correlated traits independently of variation in the secondary trait. This method is very similar to the estimation of adjusted values in a covariance analysis. Required software tools were provided by Prof. Jianyi Zhao (Zhejiang Academy of Agricultural Sciences, Hangzhou, PR China). The software was used to calculate the conditional phenotypic

values (cond) from trait means over 14 locations of NIRS predicted oil content at 91% seed dry matter conditioned by erucic acid content determined by gas chromatography.

3.3.3.7 Correction of protein content in defatted meal considering erucic acid content

The SGEDH population showed a strong positive correlation between erucic acid and protein content in defatted meal. To investigate protein content in defatted meal independent of the effect caused by erucic acid, regression and conditional correction of protein content in defatted meal was conducted (cf. section 3.3.3.6).

Correction by regression

For the correction of protein content in defatted meal by regression information, the linear regression between GC erucic acid content and protein content in defatted meal was calculated. The slope of this regression was used to calculate the regression corrected protein content in defatted meal the following way:

Protein content in defatted meal – (slope of linear regression between GC erucic acid content and protein content in defatted meal * GC erucic acid content)

Correction by conditioning

Conditional correction of protein content in defatted meal was conducted using the method of Zhu (1995) as explained in section 3.3.3.6.

3.3.4 Statistical analysis

PLABSTAT software version 3A (Utz 2011) was used to perform analysis of variance (ANOVA) applying the following general model:

$$Y_{ij} = \mu + g_i + e_j + ge_{ij}$$

where Y_{ij} is the trait value of genotype *i* in environment *j*, μ is the general mean, g_i is the effect of *i*th genotype, e_j is the effect of *j*th environment, and ge_{ij} is the interaction between *i*th genotype

and *j*th environment. Genotypes were considered fixed in the analysis, whereas environments were treated as random variables. The data were tested for outliers by a modification of the Anscombe and Tukey method (1963) based on the detection of extreme residuals. After examining the list of detected outliers, the measured values of the outliers with highest standardized residual were checked for errors and the ANOVA was repeated considering missing values for extreme outliers. The adjusted results were used in the subsequent analyses. Heritability (h^2) was calculated as:

$$h^2 = \frac{\sigma_g^2}{(\sigma_g^2 + \frac{\sigma_{ge}^2}{E})}$$

where σ^2_g and σ^2_{ge} are variance components for *g* and *e*. *E* refers to number of environments.

Spearman's rank correlation coefficients between traits mean values of the genotypes across the environments were calculated using PLABSTAT's BASIC command. The partial correlation coefficient ($r_{s\_partial}$) between a trait X and Y both correlated to a trait Z was calculated using the equation of Fuente *et al.* (2004):

$$r_{(X,Y)/Z} = \frac{r_{XY} - r_{XZ} * r_{YZ}}{\sqrt{(1 - r_{XZ}^2)(1 - r_{YZ}^2)}}$$

The BASIC command was used to calculate the skewness and kurtosis of the distribution of each trait analysed.

3.3.5 Molecular markers

3.3.5.1 DNA extraction and measurement

For DNA extraction 90 – 110mg of leaf material was collected from 3 – 4 weeks old plantlets grown in the greenhouse. After weighing, leaf tissue was directly frozen in liquid nitrogen and stored at -20°C until further processing. Genomic DNA for AFLP analyses was extracted using illustra™ Nucleon Phytopure Genomic DNA Extraction Kit (GE Healthcare) following the manufacturers protocol for small samples (RPN8510). For DArT and SNP analyses was extracted using innuPREP Plant DNA Kit (Analytik Jena, Germany) following the manufactures user manual. DNA concentration was measured with a Bio-Rad Versa Fluor™ Fluorometer

(http://www.bio-rad.com/en-us/product/versafluor-fluorometer) using Bio-Rad Fluorescent DNA Quantification Kit (Bio-Rad Laboratories CA, USA).

3.3.5.2 AFLP markers

Amplified fragment length polymorphism (AFLP) analyses were performed following the method of Vos *et al.* (1995), described in Radoev (2007) with minor changes as follows: In preamplification 0.45mM dNTPs were used for the respective polymerase chain reaction (PCR) and the elongation time in the thermo profile was extended to 2min at 72°C. For the main amplification 0.36mM dNTPs were applied and a multiplex reaction was performed using *Eco*RI-Primer + 3 nucleotides with four different fluorescence labels in the following concentrations: 2pmol FAM labelled primer, 2pmol VIC labelled primer, 4pmol NED labelled primer and 6pmol PET labelled primer. The thermo profile of the main amplification reaction was modified by not decreasing the annealing temperature for 0.7°C/cycle in the first 12 reaction cycles and not increasing the elongation for 1 sec/cycle in the second 25 reaction cycles. The following 12 primer combinations were used:

E32M47	E32M49	E35M62
E33M47	E33M49	E37M62
E34M47	E34M49	E39M62
E35M47	E45M49	E44M62

To analyse AFLP fragments 2µl of PCR main amplification product were diluted 1:5 with 8µl of a Hi-Di formamide + GeneScan LIZ500 (Applied Biosystems) mix (prepared from 940µl Hi-Di formamide + 60µl LIZ500), denatured for 2min at 95°C in a thermocycler and separated on a ABI PRISM 3100 genetic analyser (Applied Biosystems) using 36cm-capillary arrays. The results were analysed with GeneScan software version 3.7 (Applied Biosystems) and scored using the program GenMapper version 3.7 (Applied Biosystems).

3.3.5.3 DArT markers

The SGEDH population as well as the parental lines were genotyped with the *Brassica napus* v1.0 Diversity Arrays Technology (DArT) microarray including a set of 3072 markers. DArT marker analysis was performed by Diversity Array Technology Pty Ltd, Yarralumla, Australia. Marker

names are carrying the prefix "brPb" and marker sequences are available at http://www.diversityarrays.com/dart-map-sequences.

3.3.5.4 SNP markers

The Illumina Infinium Brassica 60k SNP array (Illumina, Inc., http://www.illumina.com) with a total number of 58464 markers was used to genotype the SGEDH population and its parental lines at TraitGenetics GmbH, Gatersleben, Germany (http://www.traitgenetics.com). The assay data was analysed with Illumina's GenomeStudio® Software v2011 (Illumina, Inc.) applying proprietary cluster files. Heterotic genotype calls were manually set to missing values. SNP marker sequences were provided by Isobel Parkin (AAFC, Saskatoon, Canada).

3.3.6 Linkage mapping

A SNP linkage map was constructed with JoinMap® 4.1, Kyazma® (Stam 1993, Van Ooijen 2011), by TraitGenetics GmbH, Gatersleben, Germany (http://www.traitgenetics.com). Using a set of about 6000 selected markers from the polymorphic markers (15474) identified for the SGEDH population, an anchor marker map was built. In a second step remaining polymorphic markers were added to this anchor marker map to create a map including all SNP markers. Anchor marker selection and map construction were reviewed by comparing results with a *B. napus* consensus map property of TraitGenetics GmbH. The reviewed SNP map (15474 markers) was extended by AFLP (159) and DArT (546) markers polymorphic for SGDH14 and Express617. AFLP and DArT markers were subsequently placed at their most likely position using JoinMap® 4.1. Placed AFLP and DArT marker haplotypes were compared to adjacent SNP marker haplotypes and conspicuous marker genotypes were set to missing data points manually. In the refined full map 94 SNP, 232 DArT and 43 AFLP markers could not be mapped or were excluded from the map because of too many missing data points. Final marker distances were calculated with Map Manager QTX Version 0.30 (provided by Dr. Jörg Plieske, TraitGenetics GmbH, Gatersleben, Germany) applying Kosambi map function, resulting in the full map of the SGEDH population with 15810 markers. The segregation of each marker was tested by χ^2 test (p=0.05) to verify the consistency of the expected DH population marker segregation ratio (1:1). Markers significantly deviating from 1:1 segregation ratio were defined as skewed segregating markers. For QTL mapping a framework map was developed from the full map. To construct the framework map one representative marker with lowest possible number of missing data was chosen for each locus, eliminating all other co-segregating marker if present. From this set of

markers, in a second step, marker with mean distance of 5 – 10cM between each other were selected to obtain a framework map with even marker distribution. The framework map consisted of a selected subset of 379 full map markers.

3.3.7 QTL mapping

QTL computation was conducted by QTL Network software version 2.1 (Yang *et al.* 2008) using a systematic mapping strategy to search for QTL with and/or without epistatic effects in a mixed linear model introduced by Yang *et al.* (2007). At first a one-dimensional genome scan is performed by a composite interval mapping (CIM) approach proposed by Zeng (1994) to identify putative QTL with the help of selected candidate marker intervals as cofactors (Piepho and Gauch 2001). In a second step epistatic effects for QTL with additive main effects are detected by a two-dimensional genome scan. *F*-test based on Henderson method III is performed to adjust for the critical threshold value to control the experiment-wise false positive rate. A permutation testing (Doerge and Churchill 1996) with 1000 permutations is employed to determine an empirical threshold value of the *F*-statistic (Appendix 3). QTL names were defined as sting of "E_" for the environment Europe, "trait_name" or an abbreviation explaining the trait and a continuous "QTL-number". QTL explaining ≥25% of the phenotypic variance were considered as major QTL.

3.3.8 Physical mapping

To determine physical positions of sequence-informative SNP and DArT markers and candidate genes *Brassica Database* (BRAD; http://brassicadb.org/brad/index.php) BLAST search was used. All sequences were aligned against the Darmor-*bzh B. napus* reference genome v4.1 (Chalhoub *et al.* 2014). Most likely positions were selected from the BLAST hits considering best matching and highest possible E-value as well as genetic map data information correspondence. *A. thaliana* candidate gene sequences were chosen from *The Arabidopsis Information Resource* (TAIR; https://www.arabidopsis.org/index.jsp) using the gene search. *B. napus* homologs were identified with the annotations search function of BRAD.

3.4 Results

3.4.1 Phenotypic analysis

Highly significant effects for the genotype and the environment were detected for all traits in the SGEDH population. Since the population was segregating for canola quality large variance components were found for glucosinolate and fatty acid contents (Table 3.2.). No significant effect could be detected for the genotype x environment interactions because field experiments were performed without replications. Heritability estimates were high for all traits, ranging from 0.76 for end of flowering to 1.00 for the fatty acids 18:1, 20:1 and 22:1. In the DH population the oil and protein content ranged from 43 to 51% and from 17 to 20%, respectively (Table 3.3). Transgressive segregation was observed for most of the traits in the DH population. The lowest genotype had a 2.6% lower oil content compared to Express617, the lower parent. The best genotype had a 2.2% higher oil content compared to SGDH14. A large difference of 11 days for begin of flowering was observed in the population compared to a one day only difference between the parental lines. At end of flowering plant height varied from 115 to 156 cm in the population. Frequency distribution of oil content showed a significant kurtosis of -0.67* (platykurtic), whereas skewness was positive, but not significant (0.23^{ns}; Figure 3.2a). Following correction by regression of the effect of the erucic acid content on oil content, the frequency distribution improved to a skewness of zero (0.00^{ns}) and to a kurtosis of -0.44^{ns} (Figure 3.2b). The mean value (46.3%) and the standard deviation (1.92%) of uncorrected oil contents were both reduced by correction. Corrected oil contents showed a mean value of 44.4%, and a standard deviation of 1.09%.

Table 3.2: Components of variance and heritabilities for contents of seed oil (%), protein (%), protein in defatted meal (Prot.idM in %), glucosinolates (GSL in µmol/g), glucosinolates in defatted meal (GSLidM in µmol/g), fatty acids (%), and for thousand kernel weight (TKW in g), begin of flowering (BOF), end of flowering (EOF), flowering period (FP in days) and plant height at end of flowering (PH_EOF in cm) in the SGEDH population

Trait	Variance components			Heritability
	σ^2_g	σ^2_e	σ^2_{ge}	h^2
DF	211	13	2603	
Oil	3.53**	6.54**	2.09	0.96
Protein	0.36**	3.75**	1.12	0.82
Prot.idM	2.56**	4.63**	1.80	0.95
Oil+Protein	3.83**	0.82**	0.67	0.99
GSL	160.60**	70.77**	60.89	0.97
GSLidM	562.48**	176.78**	203.08	0.97
16:0	0.29**	0.10**	0.10	0.98
18:0	0.06**	0.01**	0.02	0.97
18:1	377.94**	4.51**	10.64	1.00
18:2	6.82**	0.28**	1.02	0.99
18:3	0.68**	0.45**	0.42	0.96
20:1	47.98**	0.31**	2.33	1.00
22:1	327.87**	2.90**	12.57	1.00
TKW	0.09**	0.23**	0.21	0.85
BOF[a]	8.26**	24.40**	4.89	0.95
EOF[a]	0.73**	48.34**	1.85	0.76
FP	4.40**	35.47**	4.83	0.88
PH_EOF	62.52**	352.43**	81.68	0.91

σ^2_g = genetic variance; σ^2_e = environmental variance; σ^2_{ge} = variance of genotype x environment interaction; DF = degrees of freedom; ** denotes significance at $P < 1\%$
a days counted from 1st of January

Correlation analysis revealed significant close positive correlations between oil content and the long chain fatty acids 20:1 and 22:1, whereas negative correlations were found between palmitic acid (16:0) and all C18 fatty acids (Table 3.4 and Figure 3.3a). Close correlations were determined among the C18 fatty acids. No correlation was found between oil and protein content. While oil content was instead positively correlated with protein content in defatted meal, which itself was also positively correlated with erucic acid content. Calculation of partial correlations for these three traits indicated no direct effect of oil content on protein content in defatted meal (if 22:1 is kept constant, then $r_{s\_partial}$ =-0.013). This was affirmed by the detection of no more correlation (r_S = -0.03) between oil content and regression corrected protein content in defatted meal. Correlation between begin of flowering and end of flowering was close and both traits were negatively correlated to flowering period, but positively correlated to plant

height. Plotting oil content against erucic acid content showed segregation of the DH population for erucic acid into three groups (Figure 3.3a).

Table 3.3: Minimum, maximum and mean values for contents of seed oil (%), protein (%), protein in defatted meal (Prot.idM in %), glucosinolates (GSL in µmol/g), glucosinolates in defatted meal (GSLidM in µmol/g), fatty acids (%), and for thousand kernel weight (TKW in g), begin of flowering (BOF), end of flowering (EOF), flowering period (FP in days) and plant height at end of flowering (PH_EOF in cm) in the SGEDH population

Traits	Min	Max	Mean	*F*-value	LSD 5%	Parents	
						SGDH14	Express617
Oil	42.5	50.9	46.3	24.6**	1.1	48.7	45.1
Protein	16.6	20.0	18.0	5.5**	0.8	18.1	17.8
Prot.idM	30.0	37.0	33.4	20.9**	1.0	35.1	32.4
Oil+Protein	60.3	68.4	64.3	81.6**	0.6	66.8	62.9
GSL	18.2	74.6	51.5	37.9**	5.8	65.8	26.0
GSLidM	32.7	141.8	95.6	39.78**	10.56	128.0	46.9
16:0	3.3	5.4	4.4	40.5**	0.2	3.6	4.9
18:0	0.7	1.7	1.2	39.6**	0.1	0.8	1.4
18:1	12.7	65.5	34.5	498.3**	2.4	14.7	61.2
18:2	11.7	23.3	16.5	94.2**	0.8	14.0	18.3
18:3	7.5	11.6	9.3	23.8**	0.5	9.4	9.8
20:1	1.2	22.3	10.2	289.8**	1.1	11.7	1.8
22:1	0.0	51.0	22.1	366.3**	2.6	43.3	1.0
TKW	4.1	6.0	5.1	6.9**	0.3	5.3	5.4
BOF[a]	107.6	120.9	114.9	19.6**	1.9	116.1	114.7
EOF[a]	141.9	146.9	144.5	4.2**	1.3	144.5	143.6
FP	25.0	35.9	29.9	8.3**	2.2	29.0	29.3
PH_EOF	115.0	156.2	132.1	11.0**	7.0	138.9	130.0

LSD 5% = least significant difference at $P < 5\%$; ** denotes significance at $P < 1\%$
a days counted from 1st of January

One group of 70 genotypes with 0 to 2.6% erucic acid showed oil contents of 42.5 to 46.5%, a second group including 84 genotypes with an erucic acid content of 16.1 to 34.8% had oil contents between 44.2 and 49.1% and in a third group of 58 genotypes with 38.3 to 51.0% erucic acid, oil content ranged from 45.6 to 50.9%. Within the group of genotypes with no or low erucic acid content the DH line 172 (SGEDH172) was identified with highest oil content (46.5%). Regression and molecular correction of oil contents (Figure 3.3b+c) both nearly eliminated the effect of erucic acid on oil content, reducing the correlation coefficient to -0.00017 and -0.035, respectively.

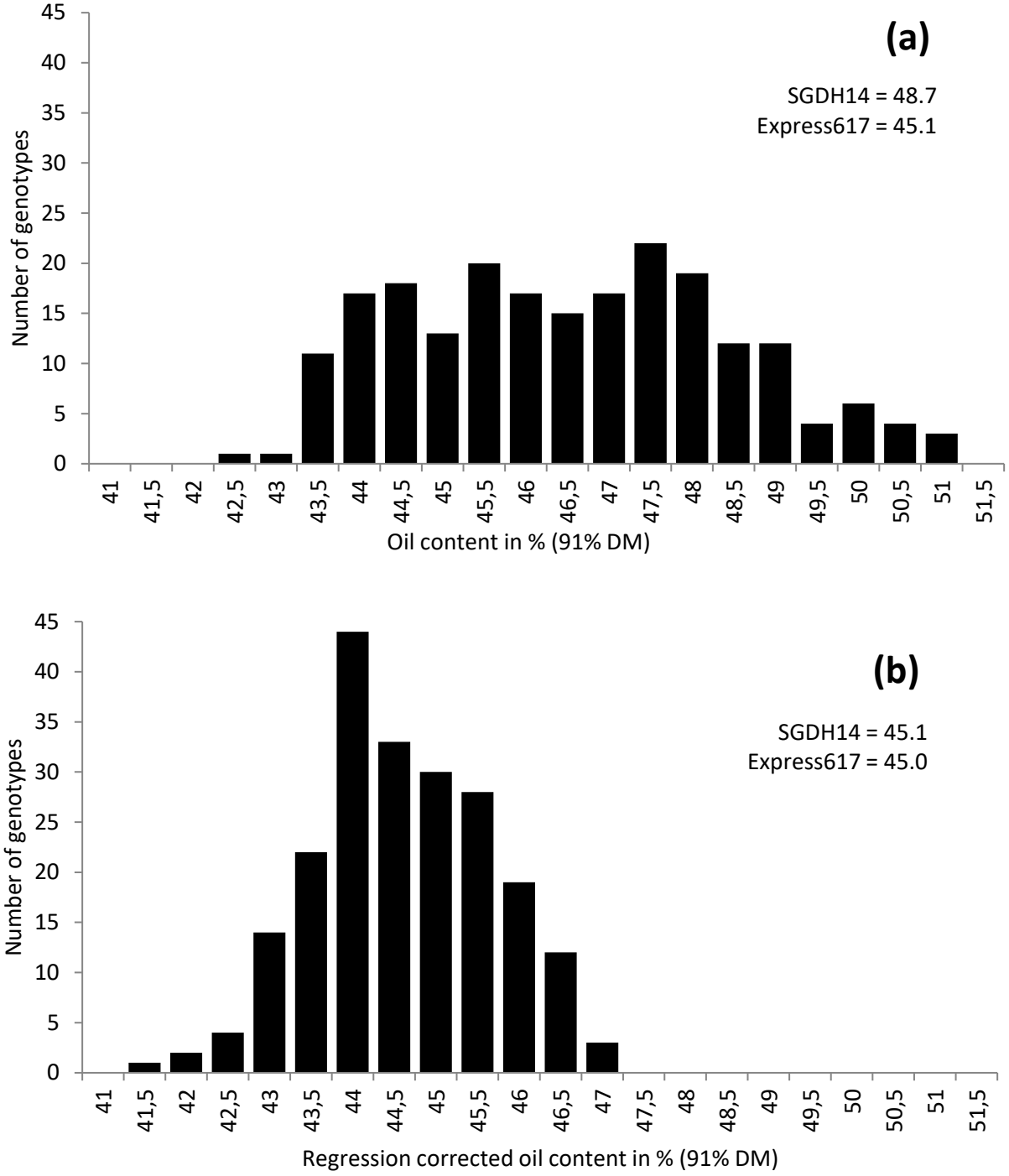

Figure 3.2: Frequency distribution of (a) oil content and (b) oil content corrected for the influence of erucic acid by regression in the SGEDH population; DM = dry matter

Conditional correction of oil contents in the SGEDH population (Figure 3.3d) calculated the smallest correlation coefficient compared to regression and molecular correction. Although conditional correction showed the best results for the elimination of the effect of erucic acid on oil content, it did not allow a direct comparison of oil contents of genotypes containing erucic

acid to the ones without erucic acid on the level of real measurements. As regression and the molecular correction are subtracting the proportion of oil caused by erucic acid and the sum of eicosenoic and erucic acid, respectively, from the oil content measured in total, corrected oil contents of these methods present the theoretical approximation of the erucic acid free oil content of each genotype. Considering the results of regression correction as the second best correction method, the theoretical erucic acid free oil content of SGDH14 was 45.1%. Comparing the oil contents of all genotypes of the SGEDH population applying the regression correction DH line 175 (SGEDH175) and 13 (SGEDH13) were identified as genotypes with highest oil contents, with 47.0% and 46.8% oil, respectively. Since protein content in the defatted meal was as well highly correlated (r_S =0.81) to erucic acid this trait was also regression corrected to calculate its theoretical erucic acid free values. After regression correction still DH line 11 an erucic acid free line showed the highest protein content in the defatted meal with 34.8%. No superior line was identified in the groups with medium or high erucic acid contents. SGDH14 and Express617 showed a regression corrected protein content in the defatted meal of 31.9% and 32.3%, respectively, DH line 195 and 216 showed the lowest erucic acid free protein content in the defatted meal both with 29.5%.

Table 3.4: Spearman's rank correlation for seed oil content and other quality traits

	Oil	Oil-reg_corr	Oil-mol_corr	Oil-cond	Protein	Prot. idM	Prot.idM-reg_corr	Prot.idM-cond	Oil+Protein	GSL	GSLidM
Oil-reg_corr	0.57**										
Oil-mol_corr	0.53**	0.98**									
Oil-cond	0.57**	1.00**	0.98**								
Protein	-0.09	-0.58**	-0.58**	-0.58**							
Prot.idM	0.66**	-0.03	-0.04	-0.03	0.69**						
Prot.idM-reg_corr	-0.03	-0.04	-0.04	-0.04	0.79**	0.58**					
Prot.idM-cond	-0.03	-0.04	-0.04	-0.04	0.79**	0.58**	1.00**				
Oil+Protein	0.94**	0.36**	0.33**	0.36**	0.25**	0.87**	0.24**	0.24**			
GSL	-0.13	-0.06	-0.11	-0.06	-0.01	-0.11	-0.02	-0.02	-0.13		
GSLidM	0.00	0.02	-0.04	0.02	-0.02	-0.02	-0.02	-0.02	0.00	0.99**	
16:0	-0.71**	0.04	0.02	0.04	-0.31**	-0.75**	-0.06	-0.06	-0.79**	0.13	0.04
18:0	-0.76**	-0.07	-0.09	-0.07	-0.25**	-0.74**	-0.06	-0.06	-0.82**	0.17*	0.07
18:1	-0.82**	-0.05	0.03	-0.05	-0.24**	-0.77**	0.01	0.01	-0.88**	0.03	-0.08
18:2	-0.72**	0.01	0.06	0.01	-0.29**	-0.74**	-0.04	-0.04	-0.80**	0.07	-0.03
18:3	-0.38**	0.04	0.11	0.04	-0.11	-0.35**	0.09	0.09	-0.41**	0.11	0.06
20:1	0.49**	0.15*	-0.05	0.15*	0.07	0.39**	-0.02	-0.02	0.50**	0.17*	0.24**
22:1	0.82**	0.00	-0.02	0.00	0.29**	0.81**	0.01	0.01	0.90**	-0.12	-0.02
20:1+22:1	0.82**	0.05	-0.04	0.05	0.25**	0.78**	-0.01	-0.01	0.88**	-0.04	0.07
TKG	0.08	-0.10	-0.06	-0.10	0.22**	0.23**	0.16*	0.16*	0.16*	-0.17*	-0.16*
BOF	0.19**	0.24**	0.16*	0.24**	-0.29**	-0.09	-0.26**	-0.26**	0.09	0.01	0.04
EOF	0.31**	0.26**	0.17*	0.26**	-0.16*	0.10	-0.11	-0.11	0.25**	-0.08	-0.03
FP	-0.09	-0.19**	-0.13	-0.19**	0.31**	0.18**	0.28**	0.28**	0.02	-0.05	-0.07
PH_EOF	0.50**	0.43**	0.36**	0.43**	-0.34**	0.10	-0.27**	-0.27**	0.37**	-0.07	-0.01

*, ** denotes significance at *P* < 5% and 1%

	16:0	18:0	18:1	18:2	18:3	20:1	22:1	20:1+22:1	TKG	BOF	EOF	FT
16:0												
18:0	0.79**											
18:1	0.77**	0.80**										
18:2	0.82**	0.62**	0.84**									
18:3	0.49**	0.21**	0.50**	0.53**								
20:1	-0.27**	-0.27**	-0.73**	-0.54**	-0.44**							
22:1	-0.88**	-0.87**	-0.95**	-0.89**	-0.49**	0.48**						
20:1+22:1	-0.80**	-0.79**	-1.00**	-0.88**	-0.54**	0.71**	0.96**					
TKG	-0.25**	-0.36**	-0.11	0.01	-0.01	-0.11	0.17*	0.10				
BOF	0.08	-0.01	-0.18**	-0.06	-0.15*	0.35**	0.07	0.16*	-0.18**			
EOF	0.00	-0.16*	-0.33**	-0.13	-0.10	0.45**	0.19**	0.30**	-0.13	0.69**		
FP	-0.11	-0.08	0.07	0.01	0.15*	-0.25**	0.03	-0.06	0.19**	-0.94**	-0.44**	
PH_EOF	-0.17*	-0.26**	-0.40**	-0.27**	-0.11	0.43**	0.30**	0.38**	-0.03	0.61**	0.57**	-0.52**

*, ** denotes significance at *P* < 5% and 1%

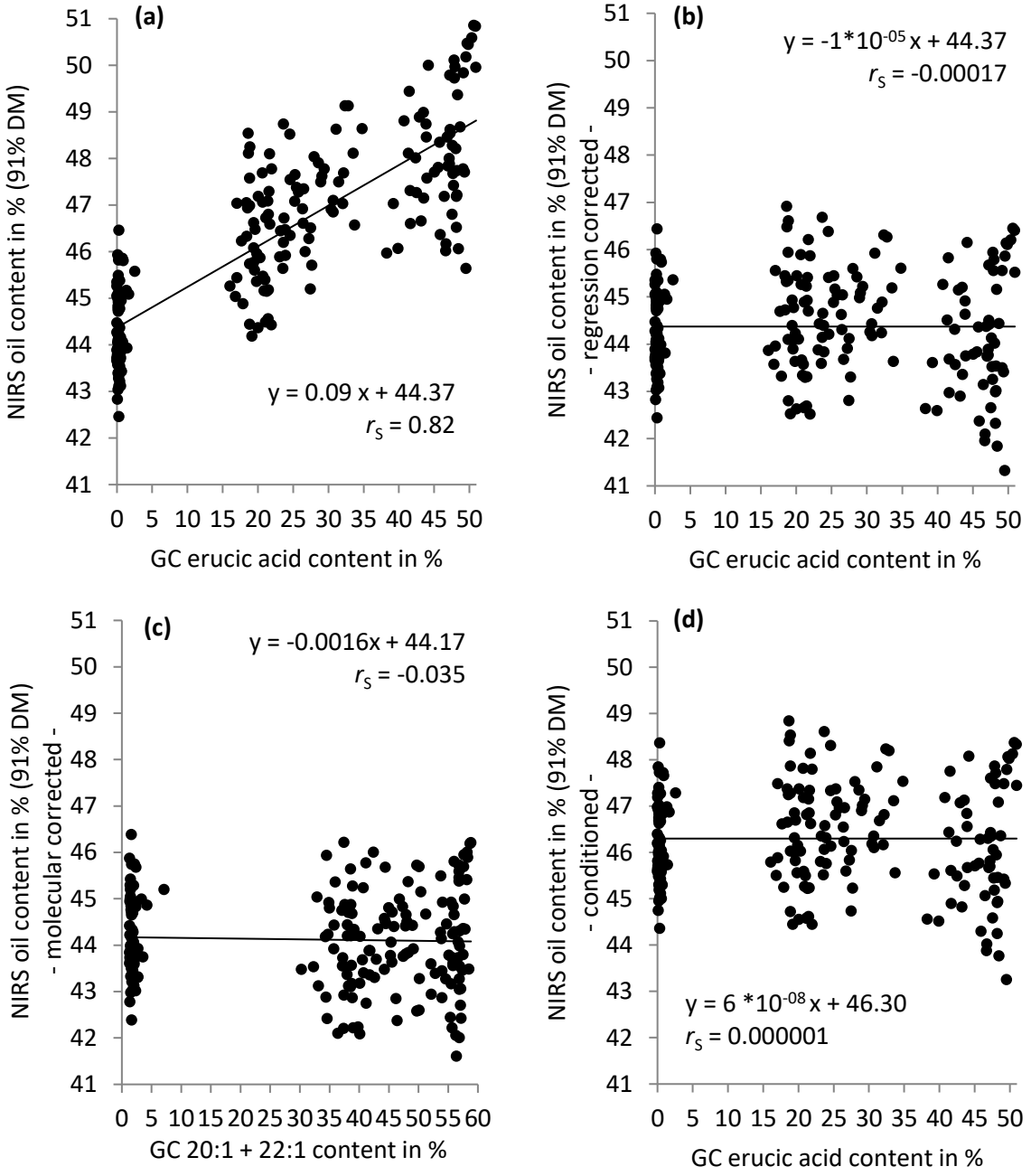

Figure 3.3: Correlation between seed erucic acid content and (a) oil content in the SGEDH population, (b) oil content corrected for the influence of erucic acid by regression (regression corrected oil content = oil content – (0.09 * erucic acid content)), (c) oil content corrected for the influence of erucic acid considering the molecular weight of eicosenoic and erucic acid compared to oleic acid (molecular weight corrected oil content = oil content – (0.09 * (GC eicosenoic acid content * NIRS oil content / 100) + 0.166 *(erucic acid content * oil content / 100))), (d) oil content corrected for the influence of erucic acid by the mixed model approach for the conditional analysis of quantitative traits described by Zhu (1995); The r_S value represents Spearman's rank correlation between seed erucic acid and oil content; NIRS = near infrared reflectance spectroscopy, GC = gas chromatography, DM = dry matter; data represent mean values over 14 locations.

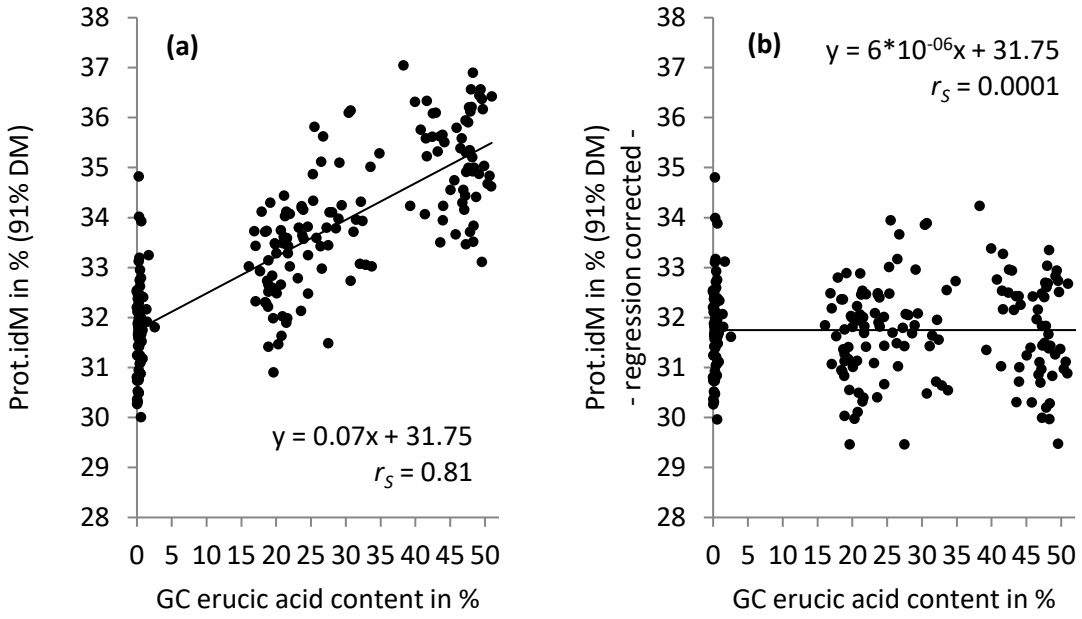

Figure 3.4: seed erucic acid and protein content in defatted meal; NIRS = near infrared reflectance spectroscopy, GC = gas chromatography, DM = dry matter; data represent mean values over 14 locations.

3.4.1.1 Group of erucic acid free genotypes

Since varying erucic acid contents may influence the variation of and the correlation to other traits in the SGEDH population, as e.g. shown by the partial correlation between oil and protein content in the defatted meal (section 3.4.1), statistical analysis was separately performed for the group of seventy erucic acid free genotypes.

Phenotypic analysis

In the group of erucic acid free genotypes of the SGEDH population highly significant effects for the genotype and the environment were found for all traits (Table 3.5). The effects of the genotype were mostly lower than the effect of the environment, while for glucosinolate content as well as for C18 fatty acids and eicosenoic acid the effects of the genotype were higher. Heritabilities were in general high ranging from 0.81 to 0.98, except for eicosenoic acid, erucic acid and end of flowering for which only moderate heritabilities were found.

Table 3.5: Components of variance and heritabilities for contents of seed oil (%), protein (%), protein in defatted meal (Prot.idM in %), glucosinolates (GSL in µmol/g), glucosinolates in defatted meal (GSLidM in µmol/g), fatty acids (%) and thousand kernel weight (TKW in g), begin of flowering (BOF), end of flowering (EOF), flowering period (FP in days) and plant height at end of flowering (PH_EOF in cm) of the 70 erucic acid free genotypes in the SGEDH population

Trait	Variance components			Heritability
	σ^2_g	σ^2_e	σ^2_{ge}	h^2
DF	69	13	861	
Oil	0.62**	5.23**	1.40	0.86
Protein	0.27**	3.50**	0.90	0.81
Prot.idM	0.73**	4.61**	1.44	0.88
Oil+Protein	0.54**	0.50**	0.39	0.95
GSL	95.14**	69.48**	43.06	0.97
GSLidM	314.6**	170.9**	132.7	0.97
16:0	0.08**	0.08**	0.06	0.95
18:0	0.02**	0.03**	0.02	0.93
18:1	5.07**	2.38**	4.92	0.94
18:2	2.80**	0.90**	0.88	0.98
18:3	0.72**	0.64**	0.43	0.96
20:1	0.12**	0.05**	1.01	0.63
22:1	0.09**	0.15**	1.16	0.51
TKW	0.09**	0.24**	0.20	0.87
BOF[a]	6.70**	25.77**	4.85	0.94
EOF[a]	0.58**	47.83**	2.00	0.70
FP	3.85**	36.32**	5.09	0.86
PH_EOF	46.15**	355.01**	78.40	0.88

σ^2_g = genetic variance; σ^2_e = environmental variance; σ^2_{ge} = variance of genotype x environment interaction; DF = degrees of freedom; ** denotes significance at $P < 1\%$
a days counted from 1st of January

The oil content of the erucic acid free genotypes ranged from 42.5 to 46.5%. Interestingly, the maximum oil content of the SGEDH population exceeded the oil content of Express617 by 1.5% and the regression corrected oil content of SGDH14 by 1.4%. And the maximum protein content in defatted meal was 2.5% higher than for Express617 and 2.9% higher than for the regression corrected protein content in defatted meal of SGDH14. For protein, glucosinolate and linolenic acid contents as well as for thousand kernel weight, flowering traits and plant height genotypes exceeding the values of the parental lines were identified, underlining transgressive segregation (Table 3.6), which was already observed in the complete SGEDH population (cf. Table 3.3).

Table 3.6: Minimum, maximum and mean values for contents of seed oil (%), protein (%), protein in defatted meal (Prot.idM in %), glucosinolates (GSL in µmol/g), glucosinolates in defatted meal (GSLidM in µmol/g), fatty acids (%) and for thousand kernel weight (TKW in g), begin of flowering (BOF), end of flowering (EOF), flowering time (FP in days) and plant height at end of flowering (PH_EOF in cm) of the 70 erucic acid free genotypes in the SGEDH population

Traits	Min	Max	Mean	*F*-value	LSD 5%	Parents	
						SGDH14	Express617
Oil	42.5	46.5	44.3	7.2**	0.9	48.7 (45.1)[b]	45.1 (45.0)[b]
Protein	16.6	19.5	17.8	5.2**	0.7	18.1	17.8
Prot.idM	30.0	34.8	31.8	8.1**	0.9	35.1 (31.9)[b]	32.4 (32.3)[b]
Oil+Protein	60.3	63.7	62.1	20.2**	0.5	66.8	62.9
GSL	18.7	69.1	51.1	31.9**	4.9	65.8	26.0
GSLidM	32.8	125.3	91.6	34.2**	8.6	128.0	46.9
16:0	4.2	5.4	4.9	19.2**	0.2	3.6	4.9
18:0	1.2	1.7	1.4	15.3**	0.1	0.8	1.4
18:1	56.4	65.3	60.9	15.4**	1.6	14.7	61.2
18:2	15.7	23.3	19.5	45.3**	0.7	14.0	18.3
18:3	8.0	11.6	9.9	24.3**	0.5	9.4	9.8
20:1	1.2	4.5	1.6	2.7**	0.7	11.7	1.8
22:1	0.0	2.6	0.5	2.0**	0.8	43.3	1.0
TKW	4.1	6.0	5.1	7.7**	0.3	5.3	5.4
BOF[a]	109.0	119.8	113.9	16.2**	1.8	116.1	114.7
EOF[a]	142.1	146.6	144.0	3.3**	1.4	144.5	143.6
FP	25.0	34.1	30.3	7.1**	2.2	29.0	29.3
PH_EOF	115.0	144.2	127.2	8.7**	6.8	138.9	130.0

LSD 5% = least significant difference at $P < 5\%$; ** denotes significance at $P < 1\%$
a days counted from 1st of January
b regression corrected values

Compared to the correlations of the complete SGEDH population, the seventy erucic acid free genotypes showed a highly significant negative correlation between oil and protein content, while neither a significant correlation between oil content and protein in defatted meal, nor with any fatty acid (except for eicosenoic acid) or any flowering trait was identified (Table 3.7). Only a significant positive correlation between oil content and plant height was found in both populations. Protein content was strongly positive correlated with protein content in the defatted meal and negatively correlated with linoleic acid content, begin of flowering, end of flowering and plant height. Protein content in the defatted meal showed negative correlation with linoleic acid content, end of flowering and plant height, but almost no correlation was found with erucic acid (r_s = 0.09) for the erucic acid free genotypes, according to the partial correlation calculated in section 3.4.1 ($r_{s\_partial}$ = -0.013). Stearic and oleic acid content were positively correlated while both were negatively correlated to linoleic and linolenic acid as well as to thousand kernel weight and to end of flowering. In contrast palmitic acid was negatively

correlated with oleic acid but positively correlated with linoleic and linolenic acid as well as with end of flowering. Positive correlation was found between linoleic acid and linolenic acid, thousand kernel weight, end of flowering and plant height. Linolenic acid only showed a significant but low correlation with plant height. A strong positive correlation was identified between eicosenoic and erucic acid content. Begin of flowering and end of flowering showed positive correlation while both were negatively correlated with flowering period and positively correlated to plant height which was found negatively correlated to flowering period.

3.4.2 Marker screening

To create a molecular marker map for the SGEDH population AFLP, DArT and SNP markers were used. 11 AFLP primer combinations out of 12 tested primer pairs showed unambiguously scorable banding patterns and identified 159 putative markers polymorphic for the parental lines of the SGEDH population. 777 out of 3072 available DArT markers (25.3%) were polymorphic within SGEDH population. 231 (29.7%) of these markers included more than 25.1% missing data and therefore were not considered for molecular map construction. Of the remaining 546 (70.3%) DArT markers 323 showed less than 10% missing data. SNP marker screening with the Illumina 60k chip identified 15474/58464 markers (26.5%) polymorphic between SGDH14 and Express617, including 82 markers with more than 10% missing data points.

Table 3.7: Spearman's rank correlation of the 70 erucic acid free genotypes in the SGEDH population for seed oil content and other quality traits

Trait	Oil	Protein	Prot. idM	Oil+ Protein	GSL	GSL idM	16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:1	22:0	22:1	TKW	BOF	EOF	FP
Protein	-0.50**																			
Prot.idM	-0.04	0.89**																		
Oil+Protein	0.75**	0.21	0.63**																	
GSL	0.15	0.09	0.18	0.23																
GSLidM	0.23	0.05	0.17	0.29*	1.00**															
16:0	0.10	-0.14	-0.12	0.00	-0.03	-0.02														
16:1	-0.10	0.11	0.07	-0.03	0.04	0.03	0.31**													
18:0	-0.18	0.14	0.07	-0.10	0.09	0.07	-0.11	0.18												
18:1	-0.22	0.24*	0.17	-0.06	-0.06	-0.07	-0.49**	-0.08	0.69**											
18:2	0.14	-0.31**	-0.30*	-0.08	-0.09	-0.08	0.40**	0.01	-0.74**	-0.87**										
18:3	0.20	-0.03	0.07	0.20	0.17	0.19	0.31**	-0.11	-0.53**	-0.69**	0.46**									
20:0	-0.30*	0.15	0.02	-0.22	0.03	0.01	-0.07	0.11	0.71**	0.47**	-0.56**	-0.42**								
20:1	0.10	0.01	0.08	0.13	0.10	0.10	-0.10	0.13	-0.01	-0.25*	-0.09	-0.08	0.14							
22:0	-0.10	-0.14	-0.21	-0.21	-0.03	-0.04	0.36**	0.01	0.13	-0.11	0.06	0.09	0.25*	-0.13						
22:1	0.19	-0.01	0.09	0.21	0.19	0.20	-0.17	0.17	-0.09	-0.28*	-0.06	-0.02	0.06	0.91**	-0.13					
TKW	-0.06	0.05	0.02	-0.03	-0.15	-0.16	-0.21	-0.08	-0.56**	-0.34**	0.43**	0.12	-0.29*	0.13	-0.09	0.18				
BOF	0.17	-0.27*	-0.21	-0.01	-0.12	-0.10	0.14	0.03	0.04	-0.06	0.14	-0.09	0.00	-0.07	0.07	-0.07	-0.10			
EOF	0.13	-0.26*	-0.24*	-0.06	-0.12	-0.11	0.43**	0.13	-0.24*	-0.43**	0.39**	0.18	-0.15	0.11	0.27*	0.07	-0.16	0.63**		
FP	-0.14	0.22	0.17	0.01	0.10	0.09	0.02	0.00	-0.19	-0.15	0.02	0.23	-0.07	0.15	0.01	0.13	0.08	-0.93**	-0.33**	
PH_EOF	0.29*	-0.34**	-0.24*	0.07	0.02	0.05	0.18	0.00	-0.20	-0.34**	0.38**	0.25*	-0.18	-0.09	0.08	-0.11	0.11	0.54**	0.40**	-0.48**

*, ** denotes significance at $P < 5\%$ and 1%

3.4.3 Genetic map construction of SGEDH population

Genetic map construction for the SGEDH population resulted in a molecular marker map consisting of 15380 SNP, 116 AFLP and 314 DArT markers covering 2650.6cM (Table 3.8). Markers were assigned to 19 linkage groups corresponding to the *B. napus* chromosomes. Remaining markers (94 SNP, 43 AFLP and 232 DArT) previously screened polymorphic within SGEDH population either could not be mapped or were excluded from the map because of too many missing values. The number of markers of individual linkage groups varied between 203 and 1614 markers and the length of the linkage groups ranged from 75.2 to 229.2cM. The 15810 markers included in the genetic full map showed a mean distance of 0.2cM with a marker density of six markers per cM. The A genome was covered with 7843 markers (49.6%) and the C genome comprised 7967 markers (50.4%) resulting in a marker density of 5.5 and 6.5 markers per cM, respectively. Choosing only one representative marker for a group of co-segregating markers reduced the total marker number to 1693 with 979 (57.8%) positions on the A genome and 714 (42.2%) on the C genome. Considering these non-redundant individual marker positions, a mean distance of 1.6cM between adjacent markers was calculated. 7768 of all markers (49%) showed significant deviation ($P < 0.05$) from the expected 1:1 segregation ratio (cf. Appendix 14). 60% of these markers (4642) favoured the Express617 allele. The majority of skewed segregating markers favouring the Express617 allele were clustered on linkage groups A05, A07 and C01, while markers with disturbed segregation favouring the SGDH14 allele were mainly found on linkage groups A02, A03, A09 and A10 (cf. Figure 3.5).

3.4.4 QTL mapping

QTLNetwork 2.1 was used to identify quantitative trait loci within the SGEDH population and to determine epistatic interaction effects of these detected QTL with additive main effects. QTL results were calculated with a framework map consisting of 379 markers selected from the full map (cf. section 3.3.6) and mean values of phenotypic data obtained from 14 environments.

Table 3.8: Marker distribution, size, density and mean distance between markers of each linkage group in the linkage map of the SGEDH population

Linkage group	No. of markers per linkage group				Size (cM)	Marker density (cM^{-1})	Mean distance between markers (cM)	Mean distance btw. non-co-seg. markers (cM)
	SNP	AFLP	DArT	Total				
A01	350	-	7	357	115.6	3.09	0.32	1.58
A02	665	7	21	693	152.8	4.54	0.22	1.74
A03	848	17	25	890	171.4	5.19	0.19	1.49
A04	855	4	29	888	130.3	6.82	0.15	1.33
A05	948	14	18	980	172.7	5.68	0.18	1.74
A06	729	5	19	753	167.9	4.48	0.22	1.37
A07	1282	5	24	1311	122.4	10.71	0.09	1.00
A08	821	4	17	842	92.9	9.06	0.11	1.18
A09	474	6	11	491	184.7	2.66	0.38	2.40
A10	610	7	21	638	122.3	5.22	0.19	1.29
C01	1574	4	36	1614	75.2	21.47	0.05	1.42
C02	697	5	10	712	149.1	4.78	0.21	2.10
C03	1525	8	18	1551	229.2	6.77	0.15	1.34
C04	1389	8	13	1410	164.7	8.56	0.12	1.65
C05	195	1	7	203	100.6	2.02	0.50	2.34
C06	832	5	12	849	133.5	6.36	0.16	1.65
C07	445	3	7	455	122.3	3.72	0.27	1.85
C08	661	4	6	671	125.6	5.34	0.19	2.28
C09	480	9	13	502	117.5	4.27	0.23	1.81
A genome	7582	69	192	7843	1432.9	5.47	0.18	1.48
C genome	7798	47	122	7967	1217.7	6.54	0.15	1.73
Whole genome	15380	116	314	15810	2650.6	5.96	0.17	1.58

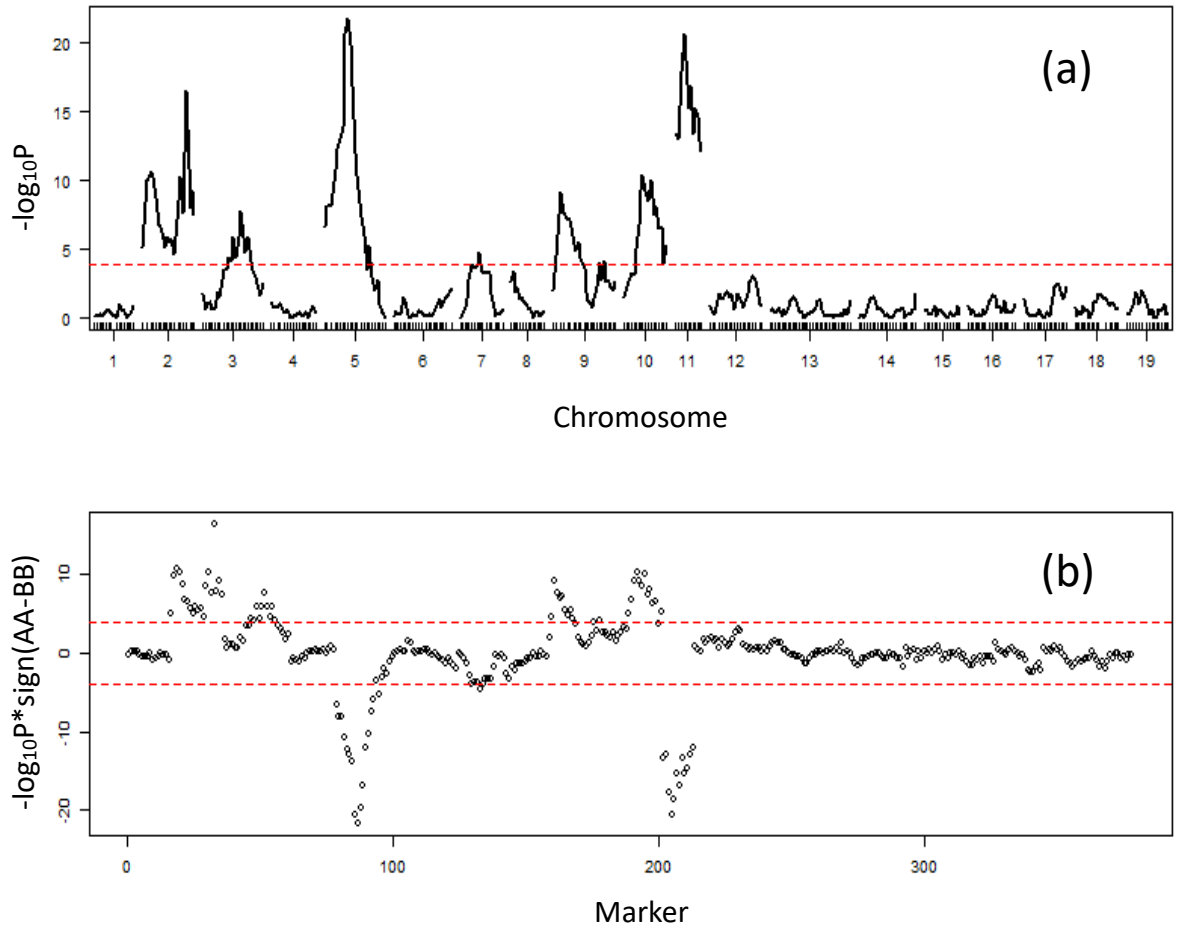

Figure 3.5: Framework map marker segregation in the SGEDH population. (a) Pattern of marker segregation over linkage groups, segregation is represented as $-\log_{10}$ of p-value from Chi-square test for Mendelian segregation, numbers from 1-19 represent linkage groups A01 to C09; (b) marker segregation tendency calculated by directed Chi-square test for Mendelian segregation, positive values represent AA>BB, negative values represent BB>AA, with A = SGDH14 and B = Express617; red lines indicate a Bonferroni corrected threshold at a *P*-value of 5%

3.4.4.1 QTL for oil content using original data

The four QTL for oil content were distributed on the four linkage groups A08, C03, C05 and C07 (Table 3.9). Individual QTL explained between 10 and 50.5% of the phenotypic variance and collectively accounted for 76.2% of the total phenotypic variance. Except QTL E_Oil-4 on linkage group C07 all QTL showed positive additive effects, indicating that the alleles increasing oil content were mainly derived from SGDH14. The QTL E_Oil-1, accounting for the largest phenotypic effect with 50.5%, was located on linkage group A08. E_Oil-2 and E_Oil-3 showed

comparable additive effects, 0.63 and 0.60, respectively, but E_Oil-2 explained 31.2% of the phenotypic variance compared to only 13.8% of E_Oil-3. No epistasis was identified between QTL for oil content.

3.4.4.2 QTL for oil content corrected for the effect of erucic acid content

Corrected oil contents were used in QTL analysis to reveal QTL with small effects that may have remained undetected because of the pleiotropic effect of the erucic acid genes on the oil content. Regression corrected and conditioned oil content each detected four QTL on linkage groups A10, C04, C05 and C07 with identical positions and almost the same confidence intervals of the corresponding QTL (Table 3.9). Additive effects of these QTL pairs also showed identical values and directions and they explained almost the same phenotypic variances. Only E_Oil-cond-3 explained a 0.1% higher phenotypic variance compared to E_Oil-reg_corr-3 on linkage group C05 and the confidence interval of E_Oil-cond-4 on linkage group C07 was 0.6cM larger than the interval of E_Oil-reg_corr-4. Individual QTL of each of these two sets explained between 7.2 and 34.7% of the phenotypic variance and together they explained 54.8% of the total phenotypic variance including epistatic effects. In total five QTL were identified for molecular corrected oil content which were located on A10, C03, C04, C05 and C07. Each QTL of molecular corrected oil content accounted for a phenotypic variance between 1.1 and 33.6% and all QTL together explained 53.4% of the total phenotypic variance. Three of the five QTL for molecular corrected oil content (E_Oil-mol_corr-1, -3 and -4) showed identical positions or overlapping confidence intervals with the QTL identified for regression corrected and conditioned oil content. QTL E_Oil-mol_corr-2 and -5 were only detected following the molecular correction procedure. The QTL of corrected oil contents on linkage groups A10, C03 and C07 all showed negative additive effects, indicating that the alleles increasing oil contents were derived from Express617, whereas QTL on linkage groups C04 and C05 showed positive additive effects. Confidence intervals of QTL for regression corrected, molecular corrected and conditioned oil content on linkage group C07 overlapped with the confidence intervals of QTL E_Oil-4, all showing a negative additive effect, indicating that Express617 is contributing the allele increasing oil content. 18.2cM apart from E_Oil-2 on C03 with a positive additive effect, an additional oil-QTL for molecular correction (E_Oil-mol_corr-2) with a negative additive effect was located.

Table 3.9: QTL detected for seed oil content (%) and corrected oil contents in the SGEDH population

QTL	Linkage Group	Position [cM]	Confidence Interval [cM]	A[a]	R^{2b}	V(A)/V(P)[c]	V(I)/V(P)[d]	V(G)/V(P)[e]
E_Oil-1	A08	24.5	20.5-27.1	1.12	50.5	76.2	-	76.2
E_Oil-2	C03	190.4	187.6-195.4	0.63	31.2			
E_Oil-3	C05	9.3	7.0-13.3	0.60	13.8			
E_Oil-4	C07	49.5	32.7-55.2	-0.39	10			
E_Oil-reg_corr-1	A10	17.8	10.6-24.9	-0.27	11.2	51.5	3.3	54.8
E_Oil-reg_corr-2	C04	51.4	43.3-55.6	0.23	3.2			
E_Oil-reg_corr-3	C05	39.7	35.7-43.7	0.64	34.6			
E_Oil-reg_corr-4	C07	38.9	32.7-44.9	-0.26	7.2			
E_Oil-mol_corr-1	A10	17.8	9.6-24.9	-0.23	10.1	53.4	1.0	54.5
E_Oil-mol_corr-2	C03	208.6	201.2-214.3	-0.21	1.1			
E_Oil-mol_corr-3	C04	32.3	27.8-55.6	0.19	10.1			
E_Oil-mol_corr-4	C05	38.7	34.7-42.7	0.55	33.6			
E_Oil-mol_corr-5	C07	53.2	45.5-67.3	-0.30	10.0			
E_Oil-cond-1	A10	17.8	10.6-24.9	-0.27	11.2	51.5	3.3	54.8
E_Oil-cond-2	C04	51.4	43.3-55.6	0.23	3.2			
E_Oil-cond-3	C05	39.7	35.7-43.7	0.64	34.7			
E_Oil-cond-4	C07	38.9	32.7-45.5	-0.26	7.2			

a additive effect; positive additive effect indicating that the alleles increasing the trait were derived from SGDH14
b percentage of phenotypic variance explained by each QTL
c variance of additive effects/phenotypic variance – total contribution of additive effect QTL in %
d variance of epistatic effects/phenotypic variance in %
e variance of genetic main effects/phenotypic variance in %

3.4.4.3 QTL for seed protein content

Seven QTL for protein content were detected on linkage groups A04, A07, A08, A09, A10, C04 and C06 (Table 3.10). Individual QTL explained between 5.6 to 12.8% of the phenotypic variance. Together these accounted for 56.6% of the total phenotypic variance. QTL on linkage groups A07, A08 and A10 showed positive additive effects, indicating that the alleles increasing protein content were derived from SGDH14, while the QTL on linkage groups A04, A09, C04 and C06 showed negative additive effects. Confidence intervals of E_Protein-5 on A10 and E_Protein-6 on C04 overlapped with those of QTL for corrected oil contents, showing opposite direction of additive effects.

3.4.4.4 QTL for protein content in defatted meal

For protein content in the defatted meal seven QTL were detected on the linkage groups A07 (2), A08, C03, C06 (2) and C08 (Table 3.10). Individual QTL explained between 0.7 to 50.6% of the phenotypic variance and collectively accounted for 71.3% of the total phenotypic variance with additional 5.9% explained by epistatic interactions. QTL E_Prot.idM-3 located on A08 and QTL E_Prot.idM-4 located on C03 were identified as the two major QTL ($R^2 \geq 25\%$) of protein content in the defatted meal explaining 50.6 and 25.6% of the phenotypic variation, respectively. QTL on A07, A08 and C03 showed positive additive effects, indicating that the alleles increasing protein content in defatted meal were derived from SGDH14. On linkage group A08 the confidence interval of QTL E_Prot.idM-3 overlapped with the confidence interval of E_Oil-1, both being major QTL and showing positive additive effects. On linkage group C03 the confidence interval of QTL E_Prot.idM-4 was located 7.8cM apart from the confidence interval of QTL E_Oil-2 for oil content, but again with the same direction of additive effects, indicating that the erucic acid genes in this case did not only increased oil content but also the protein content in the defatted meal. QTL E_Prot.idM-1 on linkage group A07 and QTL E_Prot.idM-6 on linkage group C06 co-localized with QTL E_Protein-2 and E_Protein-7, respectively, both overlapping pairs showing the same directions of additive effects. On A08 E_Prot.idM-3 was located 9.6cM downstream of E_Protein-3, both QTL with positive additive effect.

3.4.4.5 QTL for regression corrected protein content in defatted meal

QTL mapping of regression corrected protein content in defatted meal detected six QTL on linkage groups A07, A09, A10, C05, C06 and C08 (Table 3.10), all representing minor QTL with

individual QTL explaining between 5.0 and 14.6% of the phenotypic variance. Together the six QTL accounted for 50% of the total phenotypic variance. Parental lines are equally contributing positive alleles, SGDH14 is contributing the positive alleles for E_Prot.idM-reg_corr-1, -3 and -4, while Express617 contributed the positive alleles of E_Prot.idM-reg_corr-2, -5 and -6. Confidence intervals of QTL for regression corrected protein content in defatted meal (E_Prot.idM-reg_corr-1, -2, -3 and -5) overlapped with those of QTL for protein content (E_Protein-2, -4, -5 and -7) on A07, A09, A10 and C06 with same directions of additive effects, and on A10 and C05 (E_Prot.idM-reg_corr-3 and -5) with those of corrected oil contents with same direction of additive effects on C05. QTL analysis of protein content in defatted meal corrected for the effect of erucic acid by conditioning applying the method of Zhu (1995) showed exactly same results (data not shown).

3.4.4.6 QTL for the sum of oil and protein content

Four QTL were detected for the sum of oil and protein content located on A08, C03, C05 and C07 (Table 3.10). The major QTL E_Oil+Protein-1 and E_Oil+Protein-2 explained 60.4 and 38.2% of the phenotypic variance, while E_Oil+Protein-3 and E_Oil+Protein-4 explained 20.5 and 3.9%, respectively. All together the QTL accounted for 85.2% of the total phenotypic variance. Only E_Oil+Protein-4 on C07 showed a negative additive effect. Confidence intervals of each QTL for E_Oil+Protein was found co-located for oil content and/or corrected oil contents showing same direction of additive effects. On A08 the confidence interval of E_Oil+Protein-1 overlapped with the one of E_Prot.idM-3, and on C05 the confidence interval of E_Oil+Protein-3 was co-located with that of E_Prot.idM-reg_corr-4.

3.4.4.7 QTL for glucosinolate content

The five QTL identified for glucosinolate content were located on linkage groups A09 (2), C02, C07, and C09 (Table 3.10). Individual QTL explained between 5.4 and 52.4% of the phenotypic variance with E_GSL-1 on A09 and E_GSL-5 on C09 being the major QTL. Together the five QTL accounted for 77.4% of the total phenotypic variance with additional 7.2% explained by epistatic effects. All QTL showed positive additive effects, indicating that the alleles increasing glucosinolate content are as expected derived from the high glucosinolate parent SGDH14. On C07 the confidence interval of QTL E_GSL-4 overlapped with the confidence intervals of E_Oil-4, E_Oil-reg_corr-4 and E_Oil-cond-4, all with additive effects of opposite direction.

3.4.4.8 QTL for glucosinolate content in defatted meal

Four QTL for glucosinolate content in defatted meal were detected on linkage groups A09, C02, C07 and C09 (Table 3.10). Individual QTL explained between 4.2 to 50.6% of the phenotypic variance. Together these accounted for 73.6% of the total phenotypic variance. All four QTL for glucosinolate content in defatted meal were highly comparable to a QTL for glucosinolate content. This proved the high positive correlation between both traits (cf. Table 3.4).

3.4.4.9 QTL for fatty acids

Twenty-six QTL for fatty acids were identified, which were distributed over the entire A genome and over linkage groups C03, C04, C05 and C06 (Table 3.11).

Six QTL for palmitic acid (16:0) were detected on the linkage groups A03, A07 (2), A08, A09 and C03. Individual QTL explained between 1.1 and 55.9% of the phenotypic variance and collectively accounted for 79% of the total phenotypic variance. QTL E_GC16:0-4 on linkage group A08 and QTL E_GC16:0-6 on linkage group C03 were identified as major QTL. These two QTL as well as the minor QTL E_ GC16:0-1 on A03 showed negative additive effects, indicating that the alleles increasing palmitic acid are derived from Express617. The major QTL E_GC16:0-4 on A08 overlapped with the confidence interval of the QTL E_Protein-3 for protein content with opposite sign for the additive effect. On linkage group C03 the major QTL E_GC16:0-6 was co-localized with QTL E_Oil-2, which explained the second highest individual phenotypic variance for oil content. These two QTL showed opposite additive effects. Two QTL were detected for oleic acid content located on A08 and C03 while for linoleic and linolenic acid contents six QTL each were found. The two QTL for oleic acid explained 54.9 and 48.6% of the phenotypic variance, respectively, and together accounted for 80.9% of the total phenotypic variance with additional 8.8% determined by epistatic interactions. Both QTL showed a negative additive effect with -12.72% for QTL E_GC18:1-1 and -9.4% for QTL E_GC18:1-2, respectively, indicating that the alleles increasing oil content were derived from Express617. QTL for linoleic acid were located on linkage groups A05, A08, A10, C03, C05 and C06 and explained between 0.6 and 49.5% of the phenotypic variance, collectively accounting for 81% of the total phenotypic variance and additional 4.3% explained by epistasis. Major QTL were located on linkage group A08 (E_GC18:2-2) and C03 (E_GC18:2-4) showing negative additive effects, like the minor QTL E_GC18:2-1 on A05. The QTL for linolenic acid content were detected on linkage groups A01, A05, A06, A08, C03 and C05. Individual QTL explained between 1.1 and 27.8% of the phenotypic

Table 3.10: QTL detected for contents of seed protein (%), protein in defatted meal (Prot.idM in %), regression corrected protein in defatted meal (Prot.idM-reg_corr in %), the sum of oil and protein (Oil+Protein in %) glucosinolates (GSL in µmol/g) and glucosinolates in defatted meal (GSLidM in µmol/g) in the SGEDH population

QTL	Linkage Group	Position [cM]	Confidence Interval [cM]	A[a]	R[2b]	V(A)/V(P)[c]	V(I)/V(P)[d]	V(G)/V(P)[e]
E_Protein-1	A04	45.9	38.6-50.9	-0.11	8.5			
E_Protein-2	A07	10.7	0.0-25.7	0.24	12.8			
E_Protein-3	A08	1	0.0-6.9	0.17	8.5			
E_Protein-4	A09	55.6	44.5-66.6	-0.2	5.6	56.6	1	57.7
E_Protein-5	A10	19.9	13.8-26.9	0.19	12.4			
E_Protein-6	C04	29.8	21.1-47.4	-0.17	10.1			
E_Protein-7	C06	94.4	87.2-112.6	-0.18	9			
E_Prot.idM-1	A07	12.8	0.0-22.0	0.15	5.2			
E_Prot.idM-2	A07	63.2	57.8-71.2	0.14	0.7			
E_Prot.idM-3	A08	20.5	16.5-25.1	1.03	50.6			
E_Prot.idM-4	C03	176.8	176.2-179.8	0.68	25.6	71.3	5.9	77.2
E_Prot.idM-5	C06	69	64.4-74.0	-0.19	1.3			
E_Prot.idM-6	C06	110.4	84.2-119.6	-0.21	1.5			
E_Prot.idM-7	C08	46.9	31.1-53.1	-0.23	1.5			
E_Prot.idM-reg_corr-1	A07	21.0	8.7-27.7	0.35	14.6			
E_Prot.idM-reg_corr-2	A09	58.6	38.3-68.6	-0.25	5.0			
E_Prot.idM-reg_corr-3	A10	33.6	16.8-46.5	0.19	5.5	50.0	5.7	55.7
E_Prot.idM-reg_corr-4	C05	36.7	29.2-64.8	0.30	5.8			
E_Prot.idM-reg_corr-5	C06	112.4	101.4-112.6	-0.36	7.3			
E_Prot.idM-reg_corr-6	C08	38.8	32.1-47.9	-0.21	9.6			

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Table 3.10: *continued from previous page*

QTL	Linkage Group	Position [cM]	Confidence Interval [cM]	A[a]	R[2b]	V(A)/V(P)[c]	V(I)/V(P)[d]	V(G)/V(P)[e]
E_Oil+Protein-1	A08	23.5	20.5-27.1	1.21	60.4	85.2	1.0	86.15
E_Oil+Protein-2	C03	193.4	190.4-197.2	0.77	38.2			
E_Oil+Protein-3	C05	37.7	33.7-41.7	0.58	20.5			
E_Oil+Protein-4	C07	36.6	25.7-47.5	-0.22	3.9			
E_GSL-1	A09	20.9	13.9-24.1	4.28	27.1	77.4	7.2	84.6
E_GSL-2	A09	95.1	82.5-101.3	0.94	11.2			
E_GSL-3	C02	0	0.0-4.0	3.74	9.8			
E_GSL-4	C07	52.2	44.9-57.2	3.48	5.4			
E_GSL-5	C09	116.7	113.7-116.7	8.63	52.4			
E_GSLidM-1	A09	21.9	13.9-24.1	8.021	25.7	73.6	5.2	78.7
E_GSLidM-2	C02	0	0.0-4.0	6.722	9.92			
E_GSLidM-3	C07	53.2	43.9-64.3	5.026	4.18			
E_GSLidM-4	C09	116.7	113.7-116.7	16.23	50.6			

a additive effect; positive additive effect indicating that the alleles increasing the trait were derived from SGDH14
b percentage of phenotypic variance explained by each QTL
c variance of additive effects/phenotypic variance – total contribution of additive effect QTL in %
d variance of epistatic effects/phenotypic variance in %
e variance of genetic main effects/phenotypic variance in %

variance. All together these six QTL accounted for 67.7% of the total phenotypic variance with additional 6.9% determined by epistatic effects. QTL on A06, A08 and C03 showed negative additive effects, indicating that the alleles increasing linolenic acid were derived from Express617. QTL E_GC18:3-5 on C03 was identified as major QTL for linolenic acid content. Confidence intervals of QTL E_GC18:1-1, E_GC18:2-2 and QTL E_GC18:3-4 on A08 were co-localized and all three QTL showed a negative additive effect. Confidence intervals of these QTL also overlapped with the confidence intervals of QTL E_Oil-1 and E_Prot.idM-3, showing opposite sign of additive effects. On C03 the major QTL E_GC18:3-5 overlapped with confidence intervals of QTL E_GC18:1-2, E_GC18:2-4 and E_Oil-mol_corr-2, showing the same sign of additive effect, as well as QTL E_Oil-2 with opposite sign of additive effect. E_GC18:3-6 on C05 was co-localized with QTL for corrected oil contents, all QTL showing positive additive effects. On A10 the confidence intervals of E_GC18:2-3 overlapped with the confidence intervals of E_Protein-5, both QTL showing a positive additive effect. Whereas on C06 overlapping confidence intervals of E_GC18:2-6, E_Protein-7 and E_Prot.idM-6 showed opposite direction of additive effects.

Two QTL for eicosenoic acid were detected on linkage groups A02 and C03 explaining 5.6 and 22.5% of the phenotypic variance, respectively. Collectively these two QTL explained 32.3% of the total phenotypic variance within the SGEDH population. While epistatic effects additionally accounted for 47.1% of the total phenotypic variance. 46% of the epistatic effects were explained by an epistatic pair of loci corresponding to the QTL for erucic acid, E_GC22:1-2 (A08) and E_GC22:1-3 (C03), respectively. E_GC20:1-2 on C03 explaining the highest phenotypic variance for eicosenoic acid showed a positive additive effect, indicating that the alleles increasing eicosenoic acid content are derived from SGDH14. The confidence interval of E_GC20:1-2 overlapped with the confidence interval of E_Oil-2, showing additive effects of same direction, and with those of E_GC16:0-6, E_GC18:2-4 and E_GC18:3-5, which showed additive effects of opposite direction. For erucic acid content four QTL were detected on A08 (2), C03 and C04. These QTL individually explained between 8 and 64.1% of the phenotypic variance and together accounted for 88.1% of the total phenotypic variance. All QTL except E_GC22:1-4 on C04 were identified as major QTL and showed positive additive effects, as expected indicating the high erucic acid parent SGDH14 to contribute the alleles increasing erucic acid content. On A08 the confidence interval of E_GC22:1-2 overlapped with confidence intervals of E_Oil-1 and E_Prot.idM-3, all three QTL showing a positive additive effect, corresponding to the correlation of these traits found in the phenotypic analysis (Table 3.4). While the confidence intervals of E_GC22:1-2 also overlapped with those of E_GC18:1-1, E_GC18:2-2 and E_GC18:3-4, which showed opposite direction of additive effect. The confidence interval of E_GC22:1-3 on linkage

group C03 overlapped with those of E_GC18:1-2, E_GC18:3-5 and E_Oil-mol_corr-2, all three showing opposite directions of additive effects.

For the sum of eicosenoic and erucic acid three QTL were found on A08, A10 and C03 (Table 3.11). E_20:1+22:1-1 and E_20:1+22:1-3 both were identified as major QTL explaining 56.6 and 49.0% of the phenotypic variance, respectively, and a positive additive effect. E_20:1+22:1-2 on A10 explained only 1.4% of the phenotypic variance and showed a negative additive effect. Confidence intervals of the major QTL for E_20:1+22:1-1 and E_20:1+22:1-3 overlapped with those of E_22:1-2 and E_22:1-3, respectively. While the confidence interval of E_20:1+22:1-1 overlapped with those of E_Oil-1, E_Prto.idM-3 and E_Oil+Protein-1, all showing positive additive effects, the confidence interval of E_20:1+22:1-3 was found co-located with those of E_Oil-mol_corr-2, E_18:1-2 and E_18:3-5, which showed opposite additive effects.

3.4.4.10 Thousand kernel weight

Three QTL for thousand kernel weight were detected on linkage groups A09, A10 and C01 individually explaining 9.3, 7.2 and 13.8% of the phenotypic variance, respectively (Table 3.12). Collectively they accounted for 31.8% of the total phenotypic variance. QTL E_TKW-1 on A09 showed a negative additive effect, whereas QTL E_TKW-2 on A10 and E_TKW-3 on C01 showed positive additive effects. Confidence intervals of QTL E_TKW-1 and QTL E_GC16:0-5 on linkage group A09 overlapped with opposite direction of additive effects.

Table 3.11: QTL detected for seed fatty acid contents (%) in the SGEDH population

QTL	Linkage Group	Position [cM]	Confidence Interval [cM]	A[a]	R[2b]	V(A)/V(P)[c]	V(I)/V(P)[d]	V(G)/V(P)[e]
E_GC16:0-1	A03	58.7	52.4-100.1	-0.09	1.1			
E_GC16:0-2	A07	80.7	74.2-85.7	0.06	6.6			
E_GC16:0-3	A07	120.5	111.5-120.5	0.07	4.6	79.0	0.7	79.7
E_GC16:0-4	A08	4.9	4.0-6.9	-0.34	55.9			
E_GC16:0-5	A09	106	100.3-115.5	0.07	6.8			
E_GC16:0-6	C03	191.4	185.6-195.4	-0.24	30.4			
E_GC18:1-1	A08	23.5	20.5-27.1	-12.72	54.9	80.9	8.8	89.7
E_GC18:1-2	C03	202.2	199.2-206.6	-9.40	48.6			
E_GC18:2-1	A05	111.7	111.7-114.7	-0.47	13.4			
E_GC18:2-2	A08	23.5	19.5-27.1	-1.49	49.5			
E_GC18:2-3	A10	28.6	24.9-34.6	0.37	3.0	81.0	4.3	85.3
E_GC18:2-4	C03	191.4	186.6-195.4	-1.20	38.3			
E_GC18:2-5	C05	57.7	50.7-62.7	0.46	1.6			
E_GC18:2-6	C06	111.4	104.1-118.6	0.28	0.6			
E_GC18:3-1	A01	92.1	74.9-98.3	0.13	7.0			
E_GC18:3-2	A05	92.1	82.7-102.7	0.18	1.1			
E_GC18:3-3	A06	102.3	97.7-112.0	-0.13	8.5	67.7	6.9	74.6
E_GC18:3-4	A08	24.5	17.5-36.0	-0.37	13.3			
E_GC18:3-5	C03	201.2	193.4-206.6	-0.39	27.8			
E_GC18:3-6	C05	33.7	27.2-43.7	0.44	13.1			

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Table 3.11: *continued from previous page*

QTL	Linkage Group	Position [cM]	Confidence Interval [cM]	A[a]	R[2b]	V(A)/V(P)[c]	V(I)/V(P)[d]	V(G)/V(P)[e]
E_GC20:1-1	A02	109	101.0-117.9	-0.58	5.6	32.3	47.1	79.4
E_GC20:1-2	C03	190.4	184.6-197.2	2.12	22.5			
E_GC22:1-1	A08	14.9	13.9-15.5	2.92	53.8	88.1	0.4	88.5
E_GC22:1-2	A08	25.1	23.5-41.6	9.47	64.1			
E_GC22:1-3	C03	201.2	199.2-203.2	9.07	46.9			
E_GC22:1-4	C04	75.6	70.0-87.3	-1.49	8.0			
E_20:1+22:1-1	A08	23.5	20.5-27.1	14.76	56.6	83.2	7.1	90.3
E_20:1+22:1-2	A10	28.6	21.9-35.9	-1.04	1.4			
E_20:1+22:1-3	C03	202.2	199.2-205.6	10.94	49.0			

a additive effect; positive additive effect indicating that the alleles increasing the trait were derived from SGDH14
b percentage of phenotypic variance explained by each QTL
c variance of additive effects/phenotypic variance – total contribution of additive effect QTL in %
d variance of epistatic effects/phenotypic variance in %
e variance of genetic main effects/phenotypic variance in %

3.4.4.11 Phenological traits

The twenty QTL detected for four different phenological traits were located on the nine linkage groups A02, A03, A04, A07, A08, A10, C05, C06 and C08 (Table 3.12). From three to eight QTL were identified for each trait explaining collectively between 47.6 and 70.2% of the total phenotypic variance. A major QTL was found for begin of flowering (E_BOF-3) on linkage group C06, which explained 50.4% of the phenotypic variance with a positive additive effect. A second major QTL was detected for flowering period (E_FP-3) located on linkage group C06, which explained 49.6% of the phenotypic variance with a negative additive effect, indicating that the increasing alleles are derived from Express617. Both major QTL showed the same confidence interval. For end of flowering (EOF) and plant height at end of flowering (PH_EOF) only minor QTL were identified. QTL explaining the highest individual phenotypic variance for end of flowering and plant height were found on C06, E_EOF-4 (17.2%) and E_PH_EOF-7 (20.7%). The confidence intervals of these two QTL overlapped with the confidence interval of the major QTL E_BOF-3 and E_FP-3. The confidence interval of E_BOF-1 on linkage map A04 on the one hand was co-located with the confidence interval of E_Protein-1, both showing a negative additive effect, and on the other hand overlapped with the confidence interval of E_FP-2 showing a positive additive effect. On linkage group A07 E-EOF-1 was co-located with E_PH_EOF-4, showing the same direction of the additive effect, and with E_Protein-2 and E_Prot.idM-1, showing opposite direction of additive effect. The confidence interval of QTL E_BOF-2 on linkage group C05 overlapped with those of QTL E_PH_EOF-6, E_Oil-3 and another QTL for molecular corrected oil content (E_Oil-mol_corr-5), all showing positive additive effects. Whereas the confidence interval of E_EOF-3 on C05 overlapped with the ones of E_GC18:3-6, E_Oil-reg_corr-3, E_Oil-mol_corr-5 and E_Oil-cond-3, all QTL with additive effects. On C06 confidence intervals of E_BOF-3 and E_EOF-4 overlapped with E_PH_EOF-7, all three QTL with positive additive effects, and with the confidence intervals of E_FP-3, E_Protein-7 as well as Prot.idM-6, showing opposite additive effects. On C08 QTL intervals for E_BOF-4, E_EOF-5 and E_PH_EOF-8 were found co-located, all three QTL showing negative additive effects. Additive effects of overlapping QTL for BOF, EOF and PH_EOF showed the same direction, while QTL for FP showed opposite additive effects compared to the other phenological traits. These findings corresponded to the significant positive correlations between BOF, EOF and PH_EOF and the negative correlations of these traits with FP (Table 3.4).

Table 3.12: QTL detected for seed thousand kernel weight (TKW in g), begin of flowering (BOF), end of flowering (EOF), flowering period (FP) and plant height at end of flowering (PH_EOF) in the SGEDH population

QTL	Linkage Group	Position [cM]	Confidence Interval [cM]	A[a]	R^{2b}	V(A)/V(P)[c]	V(I)/V(P)[d]	V(G)/V(P)[e]
E_TKW-1	A09	110.9	104.0-120.7	-0.09	9.3	31.8	2.4	34.2
E_TKW-2	A10	44.5	36.9-49.6	0.15	7.2			
E_TKW-3	C01	49.3	37.6-55.5	0.14	13.8			
E_BOF-1	A04	37.6	32.1-50.9	-0.74	7.8	63.4	6.8	70.2
E_BOF-2	C05	7.3	7.0-14.4	0.51	1.7			
E_BOF-3	C06	93.4	91.4-96.4	2.04	50.4			
E_BOF-4	C08	123.5	119.5-123.5	-0.63	8.7			
E_EOF-1	A07	7.7	0.0-30.7	-0.27	3.2	47.6	2.4	50.0
E_EOF-2	A10	77.2	74.1-82.2	-0.35	15.2			
E_EOF-3	C05	42.7	34.7-43.7	0.26	7.3			
E_EOF-4	C06	97.4	91.4-102.1	0.34	17.2			
E_EOF-5	C08	123.5	116.3-123.5	-0.30	12.0			
E_FP-1	A03	0	0.0-10.8	0.41	1.0	57.1	2.5	59.6
E_FP-2	A04	42.6	30.1-51.9	0.42	7.3			
E_FP-3	C06	94.4	91.4-96.4	-1.64	49.6			

continued on next page

Table 3.12: *continued from previous page*

QTL	Linkage Group	Position [cM]	Confidence Interval [cM]	A[a]	R[2b]	V(A)/V(P)[c]	V(I)/V(P)[d]	V(G)/V(P)[e]
E_PH_EOF-1	A02	24.9	15.7-31.9	-1.72	4.8	56.6	2.4	59.0
E_PH_EOF-2	A02	135.8	124.9-149.2	-2.09	8.1			
E_PH_EOF-3	A04	113.1	108.1-129.4	1.14	5.2			
E_PH_EOF-4	A07	18	4.0-37.4	-1.19	3.5			
E_PH_EOF-5	A08	35	31.8-41.0	2.53	9.0			
E_PH_EOF-6	C05	8.3	7.0-16.4	1.60	5.0			
E_PH_EOF-7	C06	90.4	85.2-96.4	4.41	20.7			
E_PH_EOF-8	C08	123.5	118.5-123.5	-1.99	13.0			

a additive effect; positive additive effect indicating that the alleles increasing the trait were derived from SGDH14
b percentage of phenotypic variance explained by each QTL
c variance of additive effects/phenotypic variance – total contribution of additive effect QTL in %
d variance of epistatic effects/phenotypic variance in %
e variance of genetic main effects/phenotypic variance in %

3.4.4.12 Comparison between QTL detected with erucic acid content determined by gas chromatography and NIRS predicted

Gas chromatography is an accurate technique to determine fatty acid composition but it is destructive and to prepare and analyse samples takes time. In some cases, the investigated material is scarce or the time for analytical approaches is limited. Hence a non-destructive, fast and adequate analytical method is preferred. One of these methods commonly used is near infrared spectroscopy (NIRS). With already well proven calibrations NIRS is used to predict various seed quality traits. However, prediction of erucic acid content is not very accurate and biased. Thus, NIRS predictions for erucic acid were corrected based on gas chromatographic results (cf. section 3.3.3.3). In this section QTL from GC and NIRS data are therefore compared for erucic acid content as well as for regression corrected and conditioned oil content (Table 3.13).

Four QTL were identified for erucic acid content determined by gas chromatography (GC) located on linkage groups A08 (2), C03 and C04. Likewise, four QTL were detected for NIRS predicted erucic acid content, located on the same linkage groups. The confidence intervals of the four QTL as determined by gas chromatography were overlapping with those of the corresponding QTL as determined by NIRS. Additive effects of these four corresponding QTL pairs showed the same direction, and phenotypic variances were similar. The comparison of the total phenotypic variance of GC22:1 QTL (88.5%) and NIRS predicted erucic acid content (89.5%) showed a difference of only 1%. Summarized, QTL based on GC22:1 and NIRS predicted values revealed only small differences. Results of regression correction and conditioning of oil content using GC and NIRS predicted erucic acid contents showed a comparable concordance.

Table 3.13: Comparison between QTL detected with gas chromatographically determined and NIRS predicted erucic acid content (22:1)

	22:1 determined by gas chromatography								22:1 predicted by NIRS							
Trait	QTL	LG[a]	Range [cM]	A[b]	R^{2c}	V(A)/ V(P)[d]	V(I)/ V(P)[e]	V(G)/ V(P)[f]	QTL	LG[a]	Range [cM]	A[b]	R^{2c}	V(A)/ V(P)[d]	V(I)/ V(P)[e]	V(G)/ V(P)[f]
E_22:1	1	A08	13.9-15.5	2.9	53.8	88.1	0.4	88.5	1	A08	13.9-15.5	3.2	55.4	89.0	0.5	89.5
	2	A08	23.5-41.6	9.5	64.1				2	A08	23.5-41.0	9.5	67.3			
	3	C03	199.2-203.2	9.1	46.9				3	C03	198.2-203.2	8.4	45.4			
	4	C04	70.0-87.3	-1.5	8.0				4	C04	69.0-87.3	-1.7	7.2			
E_Oil-reg_corr	1	A10	10.6-24.9	-0.3	11.2	51.5	3.3	54.8	1	A10	9.6-25.9	-0.3	10.8	52.9	2.9	55.9
	2	C04	43.3-55.6	0.2	3.2				2	C04	45.3-55.6	0.2	2.4			
	3	C05	35.7-43.7	0.6	34.6				3	C05	33.7-41.7	0.7	35.8			
	4	C07	32.7-44.9	-0.3	7.2				4	C07	32.7-45.5	-0.3	8.7			
E_Oil-condmap	1	A10	10.6-24.9	-0.3	11.2	51.5	3.3	54.8	1	A10	9.6-25.9	-0.3	10.8	53.0	2.9	55.9
	2	C04	43.3-55.6	0.2	3.2				2	C04	45.3-55.6	0.2	2.4			
	3	C05	35.7-43.7	0.6	34.7				3	C05	33.7-41.7	0.7	35.8			
	4	C07	32.7-45.5	-0.3	7.2				4	C07	32.7-45.5	-0.3	8.7			

a Linkage group
b additive effect; positive additive effect indicating that the alleles increasing the trait were derived from SGDH14
c percentage of phenotypic variation explained by each QTL
d variance of additive effects/phenotypic variance – total contribution of additive effect QTL in %
e variance of epistatic effects/phenotypic variance in %
f variance of genetic main effects/phenotypic variance in %

3.4.5 Physical mapping of the SGEDH population and QTL intervals for oil content

Sequences of 1289 SNP and 94 DArT markers from the set of 1693 individual full map marker positions were blasted against the *B. napus* Darmor-*bzh* reference genome. The marker sequence BLAST hit with the lowest E-value, located on the chromosome corresponding to the linkage group of the marker, was chosen as the physical position of the marker. These physical marker positions were aligned to the genetic position to investigate the congruence of marker orders (Appendix 1 and Appendix 2). Physical marker positions predominantly showed a linear correlation to the genetic position on the linkage groups of the SGEDH population, with linkage group C09 showing the highest number of scattered marker positions.

To identify possible candidate genes influencing oil biosynthesis in the SGEDH population, homologues of *A. thaliana* genes involved in fatty acid synthesis and modification and TAG synthesis (cf. section 2.4; Appendix 4) were searched for on the *B. napus* Darmor-*bzh* reference genome. Through comparison of the physical positions of the candidate genes on the *B. napus* genome with the confidence intervals of oil-QTL identified on the SGEDH population, *FAE1* (AT4G34520) was found within the confidence interval of QTL E_Oil-1 (20.5 - 27.1cM) on A08 (Figure 3.6) and *FAD3* (AT2G29980) was found within the confidence interval of E_Oil-reg_corr-2 (43.3 - 55.6cM) on C04 (Figure 3.8). *FAE1*, which is involved in the elongation of oleic acid to erucic acid, was located approximately 185kbp apart from the closest SNP marker Bn-A08-p12699181 on A08. This marker was also located within the marker intervals of QTL for erucic acid (E_GC22:1-2), C18 unsaturated fatty acids (E_GC18:1-1, E_GC18:2-2, E_GC18:3-4) and protein in defatted meal (E_Prot.idM-3). On C04 *FAD3*, encoding for the enzyme linoleic acid desaturase, was located approximately 1.17Mbp apart from the closest SNP marker Bn-scaff_16804_2-p261726. On linkage group C03 no candidate gene was located within the confidence interval of the oil-QTL E_Oil-2 (187.6 – 195.4cM) (Figure 3.7). But a second *FAE1* copy was found 268kbp apart from the SNP marker Bn-scaff_22466_1-p1371888. This marker with the genetic position 201.4cM was positioned in the confidence interval of the erucic acid-QTL E_22:1-3 (199.2 – 203.2cM).

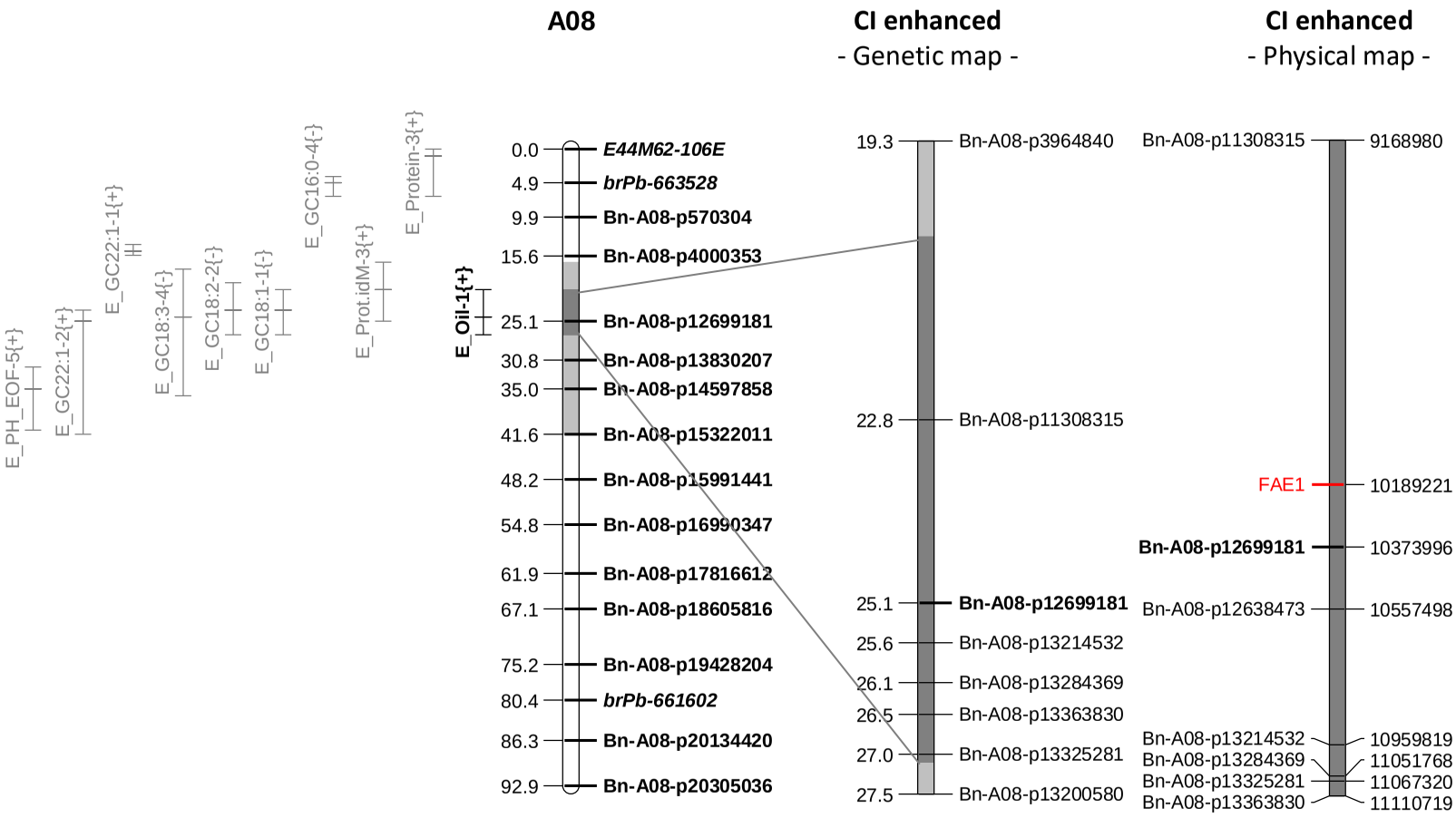

Figure 3.6: Genetic and physical map positions of markers within the QTL genomic region of E_Oil-1 (20.5 – 27.1cM; dark grey bar) on A08. *Left:* Linkage group A08 of SGEDH population, framework map markers with position and mapped QTL; non-overlapping regions of confidence intervals of QTL overlapping E_Oil-1 are represented as light grey bar. *Middle:* Enhanced confidence interval of E_Oil-1 including all individual marker positions from genetic full map of SGEDH population. *Right:* The corresponding physical positions of markers mapped within the E_Oil-1 confidence interval, and the position of the putative candidate gene *fatty acid elongase 1* (*FAE1*; red), a ketoacyl-CoA synthase, in *B. napus* reference genome. Genetic distances are presented in cM, physical positions are presented in base pairs; bold markers are framework map markers, markers in normal fond are markers of individual marker positions in the SGEDH linkage map; CI = confidence interval.

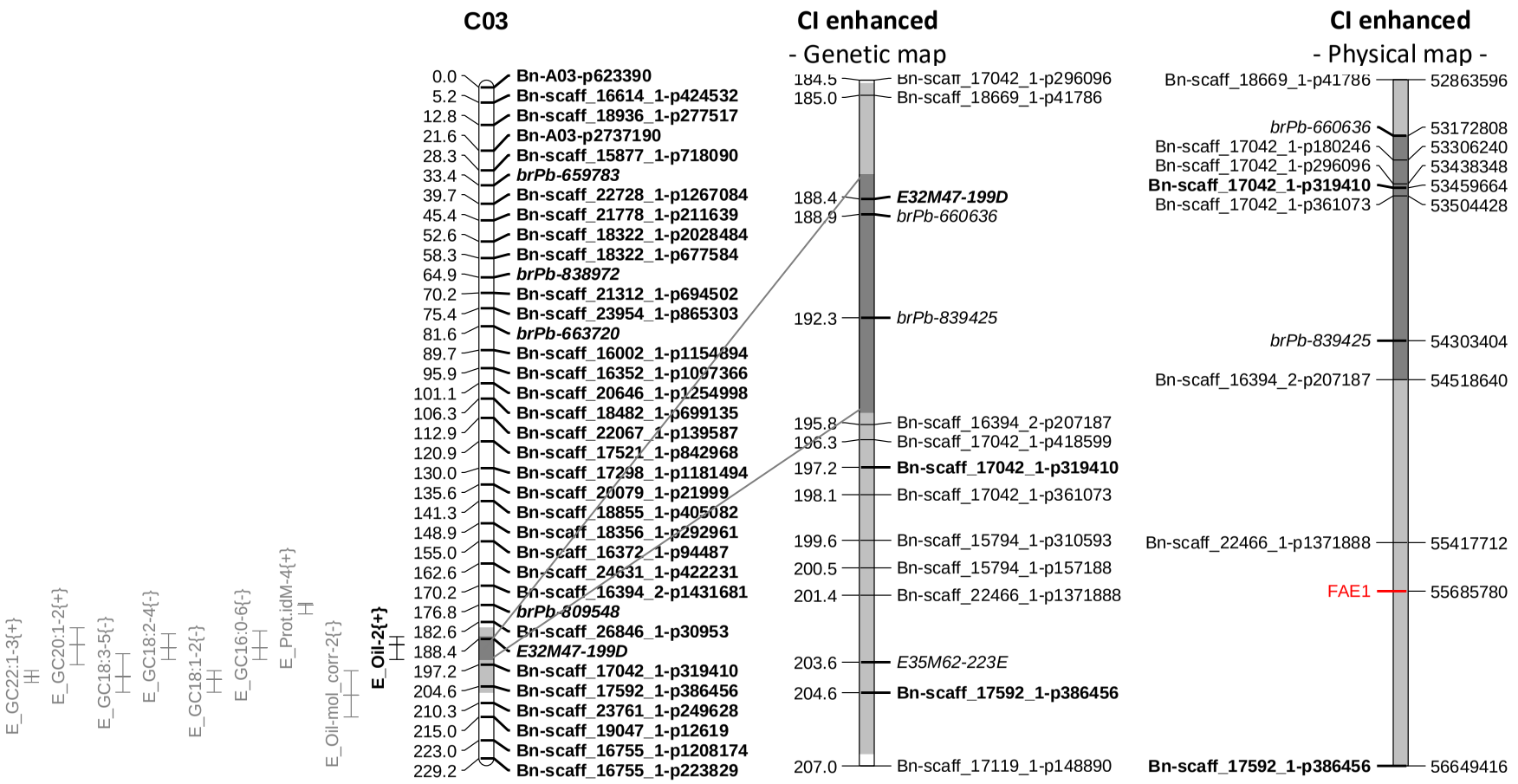

Figure 3.7: Genetic and physical map positions of markers within the QTL genomic region of E_Oil-2 (187.6 – 195.4cM; dark grey bar) on C03. *Left:* Linkage group C03 of SGEDH population, framework map markers with position and mapped QTL; non-overlapping regions of confidence intervals of QTL overlapping E_Oil-2 are represented as light grey bar. *Middle:* Enhanced confidence interval of E_Oil-2 and non-overlapping regions of confidence intervals of QTL overlapping E_Oil-2, including all individual marker positions from genetic full map of SGEDH population. *Right:* The corresponding physical positions of markers mapped within confidence interval of E_Oil-2 and non-overlapping regions of confidence intervals of QTL overlapping E_Oil-2, and the position of the putative candidate gene *fatty acid elongase 1* (*FAE1*; red), a ketoacyl-CoA synthase, in *B. napus* reference genome. Genetic distances are presented in cM, physical positions are presented in base pairs; bold markers are framework map markers, markers in normal fond are markers of individual marker positions in the SGEDH linkage map; CI = confidence interval.

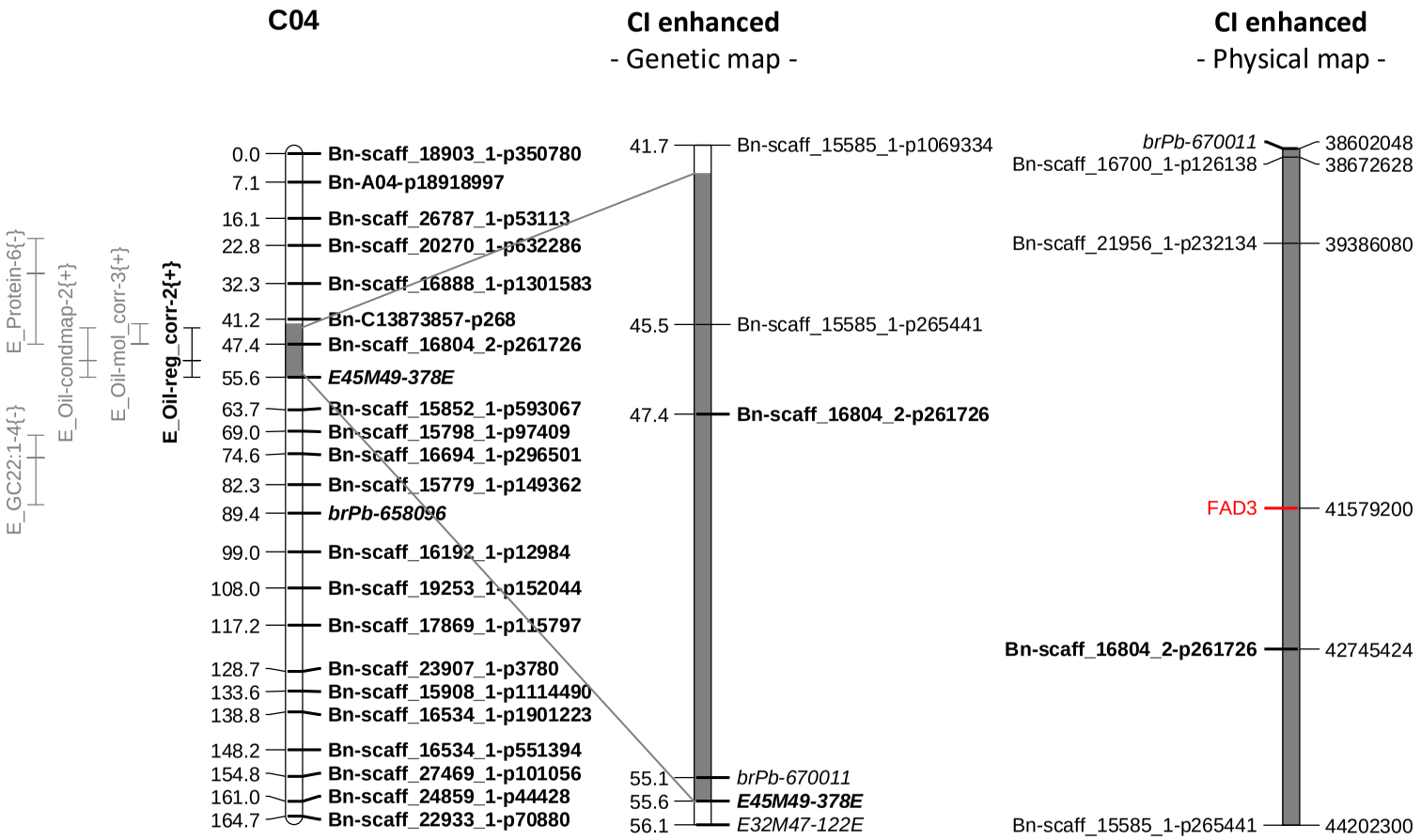

Figure 3.8: Genetic and physical map positions of markers within the genomic region of QTL for corrected oil contents (42.3 - 55.6cM; grey bar) on C04. *Left:* Linkage group C04 of SGEDH population, framework map markers with position and mapped QTL. *Middle:* Enhanced genomic region of QTL for corrected oil contents including all individual marker positions from genetic full map of SGEDH population. *Right:* The corresponding physical positions of markers mapped within the genomic region of QTL for corrected oil contents, and the position of the putative candidate gene *fatty acid desaturase 3* (*FAD3*; red) in *B. napus* reference genome. Genetic distances are presented in cM, physical positions are presented in base pairs; bold markers are framework map markers, markers in normal fond are markers of individual marker positions in the SGEDH linkage map; CI = confidence interval.

3.5 Discussion

3.5.1 Phenotypic variation

Increasing oil content is one of the most important aims of oilseed rape breeding nowadays. The results of the present study show a relatively large phenotypic variation for oil content. With a range from 42.5 to 50.9%, the oil content of the SGEDH population is equivalent to the oil content of commercial double low cultivars, which ranges from 40 to 50%. However, the discovered range was higher compared to a F1 derived DH population segregating for erucic acid, which ranged from 37.0 to 46.4% (Ecke *et al.* 1995). In comparison to observations of the DH population derived from the cross of the two high erucic acid and high glucosinolate cultivars Sollux and Gaoyou, including SGDH14, which showed a range of 37.8 to 57.0% and 40.7 to 56.0% at two German locations (Zhao 2002, Zhao *et al.* 2005), the range of oil content within the present study was lower. Comparing the oil contents of the SGEDH population to those ranging from 41.2 to 48.6% of a double low DH population derived from a cross between the two winter oilseed rape cultivars Sansibar and Oase, which showed contrasting seed oil contents (Teh and Möllers 2015), SGEDH oil contents were higher. Considering the parental lines of the SGEDH population, the oil content of SGDH14 with 48.7% (Table 3.3) was lower compared to the oil contents reported by Zhao (2002) of 56.9 and 56.0%. While the oil content of Express617 at 45.1% (Table 3.3) was comparable to previous observations by Basunanda *et al.* (2009) and Schatzki *et al.* (2013) of 43.6% and 45.7% oil, respectively. Differences in oil content between populations might be caused by different alleles from the different parental lines influencing oil content, which is not only a reason for different ranges of oil content in populations with a common parent but is also a reason for transgressive segregation within one population. Besides, oil content is depending on environmental effects mainly during seed development, which can cause differences in oil contents in different experiments.

In the SGEDH population a very strong positive correlation (r_s = 0.82**) between oil and erucic acid content was observed. This strong interrelation can be explained by the biosynthesis of erucic acid. To produce erucic acid, the plastid derived 18:1 is successively elongated by two C2 molecules at the endoplasmic reticulum. This is leading to a remarkable increase in molecular mass of the erucic acid acyl-chain. Incorporation of erucic acid into TAG consequently leads to the increase of the molecular mass of the storage lipid molecule. Assuming an identical number of storage lipid molecules, this implies an increase in oil content. Erucic acid content in *B. napus* is inherited by two genes which act in an additive manner (Dorrell and Downey 1964, Ecke *et al.* 1995, Sasongko *et al.* 2003). According to the expected segregation of these genes in the SGEDH

population, genotypes could be assigned to three classes with low, medium and high contents of erucic acid (Figure 3.3). As previously described in Ecke *et al.* (1995) and Sasongko *et al.* (2003), in the SGEDH population the class of erucic acid free genotypes was clearly separated from erucic acid containing classes. Separation of the classes containing medium or high erucic acid contents in the SGEDH population was not as clear as for the low erucic acid class. Difficulties in separating these two classes was also reported by Albrecht *et al.* (1995), Ecke *et al.* (1995) and Sasongko *et al.* (2003), and are probably caused by environmental effects on erucic acid content (Harvey and Downey 1964). Since medium and high erucic acid genotypes were not clearly separated, the SGEDH population was partitioned only into two classes, one erucic acid free and erucic acid containing class. Thus, the expected segregation ratio of two genes with equal effects in a DH population is 1:3. With 70 erucic acid free genotypes, more than the expected 53 genotypes were detected, and with 142 erucic acid containing genotype, less genotypes than expected were found, a significant difference of the segregation for erucic acid content within the SGEDH population was detected (χ^2-test: $p_{1/3}$ = 0.007). This indicated a skewed segregation at least for one of the erucic acid genes. Skewed segregation for erucic acid content was also found earlier by Ecke *et al.* (1995). In agreement with this, confidence intervals of the major QTL for erucic acid on linkage groups A08 and C03 showed a high number of markers with disturbed segregation in favour of Express617 and SGDH14, respectively, explaining the deviated number of genotypes within the different classes. The reason for the skewed segregation of the erucic acid content may be the linkage to a genetic factor causing disturbed segregation in this part of the oilseed rape genome (Ecke *et al.* 1995). Additionally, erucic acid content was strongly negatively correlated to oleic acid (r_s = -0.95**) in the SGEDH population, which was previously reported by other authors (e.g. Siebel and Pauls 1989; Sasongko *et al.* 2003). This result is according to the biosynthetic dependency of these two fatty acids, since oleic acid is the direct precursor for the elongation to eicosenoic and erucic acid, and the preferred substrate for the elongation enzyme β-ketoacyl-CoA-synthase (KCS; Millar and Kunst 1997). The close relationship between oleic acid and erucic acid is probably also the reason for the strong negative correlation between oil content and oleic acid content in the SGEDH population. In contrast Dimov and Möllers (2010) as well as Teh (2015) reported a strong positive correlation between oleic acid and oil content. Dimov and Möllers (2010) investigated two sets of double low quality winter oilseed rape cultivars and found correlations of r_s = 0.73** and r_s = 0.66** between oil and oleic acid content, respectively. Teh (2015) detected a correlation of r_s = 0.48** in a DH population derived from a cross between two canola quality lines with contrasting oil contents. Therefore, a separated investigation of the seventy erucic acid free genotypes of the SGEDH population was conducted. But a positive correlation between oil content and oleic acid content for these lines was not detected (r_s = -0.21; Table 3.7). On the other hand, the close

association between oleic acid and erucic acid is probably also the reason for the significant negative correlations between linoleic acid and oil content as well as linolenic acid and oil content. Since the elongation to erucic acid takes up oleic acid, less oleic acid is available to further be desaturated to linoleic and linolenic acid. Thus, the two polyunsaturated C18 fatty acids contribute less to oil content.

The second strongest positive correlation with oil content was observed for protein content in defatted meal (r_s = 0.66**;Table 3.4), indicating a simultaneous increase of oil and protein content in defatted meal. But the calculation of partial correlations for oil, protein in defatted meal and erucic acid content, which showed the highest positive correlation with protein in defatted meal (r_s = 0.81**), identified no direct effect of oil content on protein content in defatted meal. However, the partial correlation indicated that erucic acid content may be causative for the positive correlation between oil and protein content in defatted meal. Using the same SGEDH population, (Suprianto 2014) found a strong negative correlation between oil content and neutral detergent fibre content (NDF; r_s = -0.81**). NDF represents the total cell wall, comprising cellulose, hemicellulose and lignin. These findings suggest that a simultaneous increase in seed oil and defatted meal protein content caused by erucic acid occurs at the expense of fibre content, which was reported before by Si *et al.* (2003). This is also confirming the assumption of Zhao *et al.* (2006) that an increase in oil content is possible without decreasing protein content if there are QTL which are influencing oil content independently from protein content.

The relatively close positive correlation between the phenological traits begin of flowering (BOF) and end of flowering (EOF; rs= 0.69**,Table 3.4) indicates that flowering period (FP) is not much influenced by those two traits. However, the strong negative correlation between BOF, EOF and FP revealed that the later BOF and EOF is, the shorter FP tends to be, and vice versa. With a correlation coefficient of r_s = -0.94** BOF seems to have the highest influence on FP (r_s = -0.44** for EOF). A retarded BOF is also positively related to plant height at EOF (r_s = 0.61), which could be explained by the chronology of plant growth, whereat full plant height is reached at peak of flowering (Kimber and McGregor 1995). So the later the plant starts to flower the longer it can grow.

3.5.2 Molecular marker polymorphisms

AFLP analysis of 11 primer pairs created 159 polymorphic marker loci for the SGEDH population. With an average of 14 to 15 markers per primer pair, the detected number of

polymorphisms within the SGEDH population was lower compared to Radoev (2007), who identified 24 polymorphic markers per primer pair in a DH population derived from a cross between Express617 and the resynthesized line R53. The lower number of polymorphisms detected in the SGEDH population may either be caused by the individual potential of the selected primer pairs or explained by the degree of polymorphism within the population. Since resynthesized oilseed rape generally is a valuable source for broadening the genetic variation (Becker *et al.* 1995), the DH population of Radoev (2007) may have a higher degree of polymorphism compared to the SGEDH population. However, AFLP markers generate only anonymous loci. This is why sequence informative DArT markers were used to supplement the number of polymorphic markers. But only 10% of the screened DArT markers (323/3072) showed an adequate quality for map construction, which might be caused by difficulties in the interpretation of microarray expression data. This was previously reported by Edwards (2012) for *Brassica* and wheat, due to a general inability to distinguish between different homologous copies of specific expressed genes using oligonucleotide-based hybridisation methods. Therefore, a SNP marker screening with the Illumina 60k chip (58464 markers) was conducted additionally, resulting in 15392 polymorphic markers (26.3%) suitable for linkage mapping with less than 10% missing data points.

3.5.3 Linkage map

The linkage analysis of the 212 SGE doubled haploid lines in this study assembled 15380 SNP, 116 AFLP and 314 DArT markers in 19 linkage groups, each representing one of the *B. napus* chromosomes. The linkage map covered a length of 2651cM, which is comparable to a consensus linkage map constructed by Piquemal *et al.* (2005) with 2619cM, but beyond the size of the oilseed rape genome estimated by Lombard and Delourme (2001) also applying a consensus approach with a range from 2127cM to 2480cM. Beside these consensus maps a number of different linkage maps have previously been constructed for *B. napus*. All these maps are smaller compared to the SGEDH full map, although the size of linkage maps in general increased. Parkin *et al.* (1995) and Sharpe *et al.* (1995) e.g. published three maps which ranged from 1606cM to 1741cM which were considered as rather complete (Lombard and Delourme 2001, Radoev 2007). Other linkage maps of oilseed rape were developed by Cheung *et al.* (1997), covering 1955cM, and by Zhao *et al.* (2012) with a length of 1949cM. While a map constructed by Teh and Möllers (2015) reached a length of 2350cM. Nevertheless, a direct comparison of map sizes is difficult, since they are dependent on the degree of genome coverage by marker loci, on the size and the type of the mapping population, on the mapping function applied, and on the

recombination frequencies, which are influenced by the genetic diversity of the parents and/or environmental effects on meiosis (Ferreira *et al.* 1994, Radoev 2007).

With about 50% a large proportion of markers showed a distorted segregation in the SGEDH population. The phenomenon of skewed segregation ratios has often been reported on androgenic plant material in various species (Foisset and Delourme 1996), and maps of microspore derived DH populations of *B. napus* have previously been published (Ecke *et al.* 1995; Teh 2015 and references within). Whereas these populations are ideal to perform mapping analyses because they have no residual heterozygosity, in vitro culture conditions are reducing their genetic variability by selection interfering with androgenesis or the subsequent embryo regeneration in microspore culture (Uzunova *et al.* 1995, Foisset and Delourme 1996, Meksem and Kahl 2006, Ferrie and Möllers 2011, Ecke *et al.* 2015).

Most of the markers polymorphic within the SGEDH population showed redundant information, since the total number of 15810 markers only accounted for 1693 (11%) individual marker positions. Co-segregating markers form haplotype blocks, which prevent genetic mapping of individual markers. The main source of polymorphic markers in this study was the 60k SNP Illumina Infinium chip. To reduce the redundancy of marker information this array has meanwhile been optimized. Today a 15K array developed from four different data sets is available, including different haplotypes from winter oilseed rape, summer oilseed rape, *B. rapa* and *B. oleracea*. The reduced number of polymorphic markers of high quality (meaning a clear cluster separation) still guarantees the construction of a proper map and simultaneously lowers the costs of the analysis (Dr. Jörg Plieske, TraitGenetics GmbH, Gatersleben, Germany, personal communication).

Furthermore, the redundancy of marker information at co-segregating positions is influencing the map coverage and marker density. While the total marker number of the A genome (7843) and the C genome (7967) seems similar (Table 3.8), a larger number of individual markers were mapped on the A genome (979) compared to the C genome (714). A larger number of individual markers on the A compared to the C genome has also been reported before (Teh 2015, and references within). But also, the distribution of the markers is affected when comparing total number of markers to the number of non-co-segregating markers. While there are 2 to 21 markers per cM identified on the different linkage groups, with mean distances ranging from 0.05 to 0.5cM between markers considering all markers (Figure 3.8), there are only 0.4 to 1 markers per cM and mean distances of 1 to 2.4 cM identified for non-co-segregating markers (Appendix 13).

Despite the available high marker density of the SGEDH population, due to computational reasons the number of markers for QTL mapping was reduced. The constructed framework map, which was applied for QTL mapping, consisted of a set of 379 evenly distributed markers with a common distance of 5 to 10cM. According to Visscher *et al.* (1996) a change in marker spacing showed only small effect on the average empirical confidence interval of detected QTL. While it was additionally reported, that the size of an average confidence interval depended heavily on the population size and the effect of the QTL.

3.5.4 Genetic and physical mapping

In this study, QTL analysis identified four main QTL for seed oil content on linkage groups A08, C03, C05 and C07. This number of QTL is comparable to the three distinct QTL detected by Ecke *et al.* (1995), but generally lower compared to other reports in which QTL for oil content were mapped (see section 2.6). With 31.2 and 50.2% of the phenotypic variance, QTL E_Oil-1 and E_Oil-2 on linkage groups A08 and C03, respectively, were identified as the major QTL for seed oil content in the SGEDH population. The major QTL on A08 overlapped with a major QTL for erucic acid (E_GC22:1-2). While the major QTL on C03 was co-located with one of two QTL for eicosenoic acid (E_GC20:1-2) which explained the highest phenotypic variation of this trait. In agreement with this finding the segregation of erucic acid and its intermediate eicosenoic acid in the SGEDH population shows high eicosenoic acid contents for genotypes with medium erucic acid contents carrying one positive erucic acid gene (Figure 3.9). Ranging from 16.1 to 34.8% the variation of the group of genotypes with medium erucic acid contents is relatively large. In this group genotypes are expected to either carry the positive allele for erucic acid content on A08 or C03, which suggests that this group can be divided into two subgroups. In accordance the marker genotype segregation of the DH lines within this group identified two main groups. One group carried the positive allele for erucic acid content on C03 and a negative allele on A08 (Figure 3.9; red dots), while the other group carried the positive allele for erucic acid content on A08 and a negative allele on C03 (Figure 3.9; grey dots). Furthermore, the presence of the major QTL for eicosenoic acid on C03 assumed that the positive erucic acid allele of this locus has a higher affinity to oleoyl-CoA as substrate, producing predominantly eicosenoic acid. Thus, genotypes carrying only the positive allele of C03 showed relatively low erucic acid contents. While the allele of A08 seemed to be less selective for its substrates oleoyl-CoA and eicosenoyl-CoA, which results in high eicosenoic acid and higher erucic acid contents for genotypes carrying only the positive allele on A08.

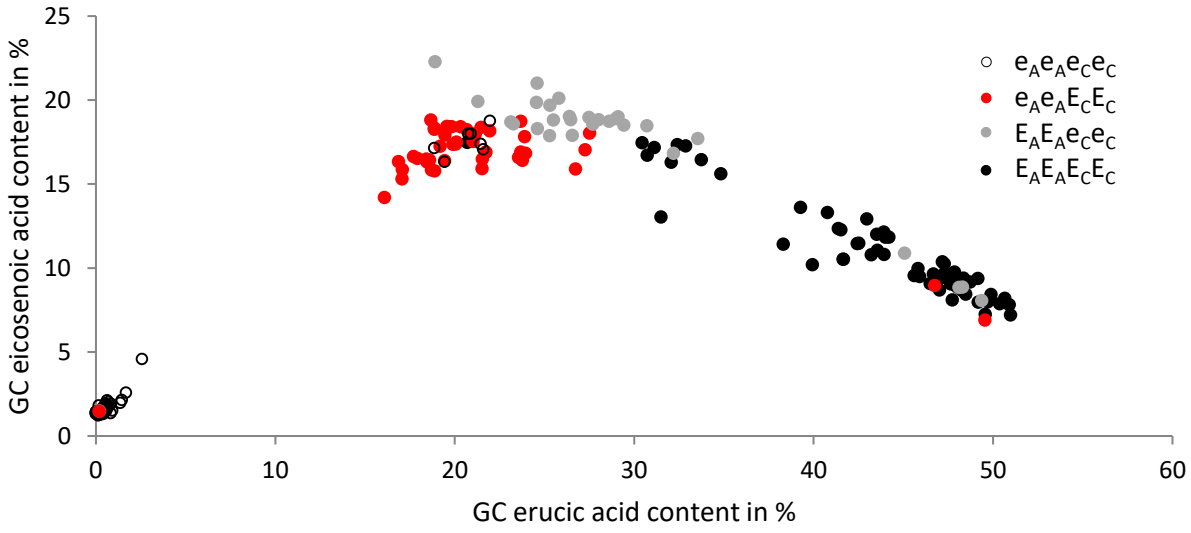

Figure 3.9: xy plot of erucic acid and eicosenoic acid content determined by gas chromatography; dots represent groups of marker genotypes $e_Ae_Ae_Ce_C$ (circles), $e_Ae_AE_CE_C$ (red dots), $E_AE_Ae_Ce_C$ (grey dots) and $E_AE_AE_CE_C$ (black dots); e represents a negative allele for erucic acid, E represents a positive allele for erucic acid content; A indicates the erucic acid locus of the A genome of *B. napus* on A08, C indicates the erucic acid locus of the C genome on C03

In accordance with these results, two of the oil-QTL identified by Ecke *et al.* (1995) showed a strong association to estimated positions of the two erucic acid genes and other subsequent studies, not only identified two major QTL for erucic acid, but also located these QTL on linkage groups N8 (A08) and N13 (C03) (Howell *et al.* 1996, Thormann *et al.* 1996, Qiu *et al.* 2006). For example, Qiu *et al.* (2006) detected two major QTL for erucic acid co-located with QTL for oil content on N8 and N13, investigating a DH population from a cross between a double low and '++'-quality parent independently at four locations. While Radoev (2007), investigating a DH population segregating for only one erucic acid gene, detected major QTL for oil content on N8 and N13, and found one major QTL for erucic acid on linkage group N8 co-located with the oil-QTL on N8. Furthermore, Teh and Möllers (2015) detected a minor QTL for oil content on C03, analysing a DH population of two double low quality cultivars. The SNP marker closest to the peak of this QTL (Bn-scaff_18936_1-p277517) was also found in the SGEDH population, but at the position 12.8cM, while the oil-QTL in the SGEDH population was located at 190.4cM, indicating that the oil-QTL identified in both populations represent different loci. The regulation of erucic acid content in *B. napus* by the additive action of two genes was already reported previously (Harvey and Downey 1964, Stefansson and Hougen 1964, Siebel and Pauls 1989), and matches the established biosynthesis path, in which oleic acid is first elongated to eicosenoic acid, which in a second step is elongated to erucic acid, by subsequent addition of C2 units. Both elongation cycles are catalysed by the cytoplasmic/ER-bound oleoyl-CoA elongation complex,

comprising a four enzyme system analogous to the fatty acid synthase complex of the plastid (section 2.4). The four enzymes include the ketoacyl-CoA synthase (KCS) FAE1 which catalyses the rate-limiting step of oleic acid elongation to erucic acid (Millar and Kunst 1997, Fourmann *et al.* 1998). A comparison of the physical position of the *FAE1* candidate genes and confidence intervals of QTL for oil content and fatty acids showed that *FAE1* was co-located not only with major QTL for erucic acid on linkage groups A08 and C03 (E_GC22:1-2 and E_GC22:1-3), but also with the major oil-QTL on A08, E_Oil-1. On linkage group C03 the confidence interval of the major QTL E_Oil-2 did not overlap with the confidence interval of E_ GC22:1-3 (peak to peak distance of 10.8cM), but with that of E_GC20:1-2, the major QTL for eicosenoic acid, the elongation intermediate, which might be explained by a higher affinity to oleoyl-CoA of the FAE1 on C03 as discussed before (cf. Figure 3.9). However, these findings suggested, that the addition of carbon units to oleic acid to produce erucic acid, and the consequent increase of molecular mass of the fatty acid chain, might explain the association found for seed oil content and erucic acid content. Besides, identifying *FAE1* as the most likely candidate gene, since the number of stored fatty acid chains remains unaffected but the increase in molecular mass is causing an increase of oil content (Ecke *et al.* 1995*)*. Although this effect is most probably caused by a pleiotropic effect of *FAE1*, a close linkage between the erucic acid genes and other independent QTL for seed oil content cannot be excluded (Ecke *et al.* 1995).

Referring to Price (2006) there is evidence that the position of a QTL obtained from a primary mapping population can identify a candidate gene with less than 1cM around the QTL peak for a major QTL, which explains more than 25% of the trait's variation. Since none of the selected lipid related candidate genes (Appendix 4) were identified for the major oil-QTL E_Oil-2 on linkage groups C03 (cf. Table 3.9), a 1cM region around the E_Oil-2 peak (190.4cM) was scanned for *A. thaliana* protein matches on the *B. napus* Darmor-*bzh* reference genome (Chalhoub *et al.* 2014, http://www.genoscope.cns.fr/brassicanapus/). Using the genetic and physical marker information of Figure 3.7, 1cM in proximity of E_Oil-2 equals about 130kbp. As closest marker to the QTL-peak, br-Pb839425 (192.3cM/54303404bp) was used to estimate the +/-1cM-region of E_Oil-2. The scanned region reached from 53910 to 54170kbp on the *B. napus* genome. In this region 27 *A. thaliana* protein matches were found (Appendix 5), but none of the proteins was related to lipid metabolism according to the *A. thaliana* lipid metabolism database (Beisson *et al.* 2003, http://aralip.plantbiology.msu.edu/locations).

QTL E_Oil-3 on C05 indicating SGDH14 as source of the increasing allele, and E_Oil-4 on C07 indicating Express617 as source of the increasing allele, showed only minor effects and the comparison to physical positions of putative candidate genes to their confidence intervals

remained without results. However, Zhao *et al.* (2005) identified QTL for oil content with additive x environment interaction effects at a German location on equal linkage groups in the Sollux x Gaoyou DH population, both indicating the Chinese parent Gaoyou as the source of the increasing allele. Delourme *et al.* (2006) also detected oil-QTL on both linkage groups from field trials conducted in France. While Qiu *et al.* (2006) in contrast detected a QTL for oil content on N17 (C07), but no QTL on N15 (C05). Referring to Price (2006) also for minor QTL an accuracy of less than 3cM around the QTL peak was detected to identify putative candidate genes. If possible, accuracy to identify a candidate gene for minor QTL was even improved by averaging peak positions from different screens or by combining multiple data sets to increase the heritability of the trait of interest (Price 2006). Hence, a scan with a wider scan region might also be used to identify putative candidate genes for the minor QTL E_Oil-3 and E_Oil-4.

The three independent correction approaches applied to eliminate the effect of erucic acid from oil content revealed a consistent major QTL for oil content, represented by E_Oil-reg_corr-3, E_Oil-mol_corr-5 and E_Oil-cond-3, which was located on C05 at 39.7cM (cf. Table 3.9; confidence interval 35.7 – 43.7), about 30cM apart from E_Oil-3. Comparison of the physical positions of lipid related candidate genes with the confidence interval of this QTL for oil content identified no co-localisation. Therefore, as described for E_Oil-2, a scan for *A. thaliana* protein matches on the *B. napus* Darmor-*bzh* reference genome (Chalhoub *et al.* 2014, http://www.genoscope.cns.fr/brassicanapus/) was conducted around the peak of the major QTL for corrected oil contents on C05. The closest marker to the QTL peak (39.7cM) was Bn-A05-p22111286 at 39.9cM with physical position at 39985396bp on C05. 1cM in the confidence interval of the QTL for corrected oil contents equalled around 82kbp, therefore a region between 39.9Mbp to 40.1Mbp on C05 was scanned for suitable *A. thaliana* protein matches, resulting in more than 40 matches. Closest to the position of Bn-A05-p22111286 two protein matches were identified, AT3G10320.1 a Glycosyltransferase family 61 protein (MUCILAGE-RELATED21) ranging from 39982337 to 39983989bp, and AT3G10310.1 a P-loop nucleoside triphosphate hydrolases superfamily protein with CH (Calponin Homology) domain ranging from 39984486 to 39988842bp. For both proteins an overlapping *B. napus* gene prediction was identified, BnaC05g42790D alias GSBRNA2T00110924001 and BnaC05g42800D alias GSBRNA2T00110923001, respectively. While AT3G10320.1 is involved in the mucilage biosynthetic process in seed coat development of *A. thaliana*, AT3G10310 is involved in microtubule-based movement. None of these genes seemed to be directly related to seed oil biosynthesis. Both genes were also not listed in the Arabidopsis acyl-lipid metabolism database (Beisson *et al.* 2003). More distant to the QTL peak of the major QTL for corrected oil contents, two protein matches were found with entry in the Arabidopsis acyl-lipid metabolism database

(Beisson *et al.* 2003), AT3G10370.1 the FAD-dependent oxidoreductase family protein (SDP6) ranging from 39950760 to 39953567bp, and AT3G14270.1 a phosphatidylinositol-4-phosphate 5-kinase family protein (FAB1B) ranging from 39908061 to 39910664bp. For both proteins an overlapping *B. napus* gene prediction was identified, BnaC05g42730D alias GSBRNA2T00110930001 and BnaC05g42630D alias GSBRNA2T00110943001, respectively. AT3G10370.1 is involved in the mitochondrial phospholipid synthesis and AT3G14270.1 in phospholipid signalling. Thus, a direct relationship to seed oil biosynthesis could not be observed. Regarding position and function no other suitable protein matches were identified.

In total this study identified eight QTL for oil content in the SGEDH population using original and corrected oil contents. This number in general is compared to the number of oil-QTL of previous studies (Zhao *et al.* 2005, Qiu *et al.* 2006, Zhao *et al.* 2012, Teh and Möllers 2015), identifying six to nine QTL. Comparison of QTL for oil content in the SGEDH population to QTL results from other populations indeed identified QTL on same linkage groups, but due to lack of common markers between populations or the use of none sequence-informative markers in previous studies, in most cases it is still unclear if QTL on same linkage groups are identical or not. Physical mapping in general showed a good correspondence of genetic and physical positions. Nevertheless, a number of markers showed an incorrect genetic order according to their physical position. This lack of consensus might be one reason for relatively large confidence intervals of some QTL or might even cause errors in QTL detection. Thus, it is recommended to reorder or delete markers with extremely deviating order in the genetic and physical map. Likewise, sequence informative markers without or with no suitable physical position were detected. To improve the information content of the SGEDH map of individual marker positions and to improve QTL mapping and the search for candidate genes, these markers should also be replaced if a co-segregating marker is available with matching sequence information regarding the physical position. On the other hand using the *B. napus* reference genome to physically map marker positions as well as candidate genes, it needs to be considered that the reference genome order is not yet fixed, especially the position and orientation of scaffolds only represents a most likely order/anchoring due to the high complexity of the allotetraploid *B. napus* genome, including multiple homoeologous gene copies, chromosomal rearrangements and amplification of repetitive DNA (Edwards *et al.* 2012).

To test if the putative candidate genes identified are responsible for changes in oil content in a next step expression analyses could be used to investigate transcriptional differences of the parental lines and other genotypes, or the allelic diversity could be tested by sequencing. Furthermore, to demonstrate a functional relationship between a candidate gene and its

corresponding QTL a knock-down or knock-out mutants could be produced. Alternatively, a transgenic approach to overexpress a candidate gene or an antisense RNA approach to inhibit or silence a candidate gene post-transcriptionally could shed light on the role of this candidate gene to influence oil content.

3.5.5 Correction of oil content for the effect of erucic acid

Due to the relation between oil and erucic acid content, genotypes with erucic acid content showed higher oil contents compared to genotypes without erucic acid. To be able to compare oil contents of genotypes of the SGEDH population, two different methods of considering the effects of erucic acid on oil content were applied. One method was using the regression equation based on oil and erucic acid contents of the SGEDH population. The other method based on the molecular relationship of the main fatty acids of *B. napus*, oleic and erucic acid. During the elongation of oleic acid to erucic acid the molecular mass of the individual fatty acid molecule is increased by about 19%, assuming that all other parameters of fatty acid synthesis and storage, especially the number of stored fatty acid chains, remain unaffected by the elongation process (Ecke *et al.* 1995). This 19% difference was used to adjust the oil content of erucic acid containing genotypes of the SGEDH population. Since both correction methods aimed at the elimination of the effect of erucic acid, no correlation between oil content and erucic acid content was to be expected. Comparison of both correction methods (Figure 3.3b and c) showed that the correlation coefficient of the regression correction was smaller than that of the molecular correction, and the slope of the regression correction equation was closer to zero than the slope of the molecular correction equation. These results are indicating that regression correction is more accurate than molecular correction. The negative slope of the molecular correction method seems to overestimate the effect of erucic acid on oil content. This might be explained by the intermediate product of the elongation from oleic acid to erucic acid, eicosenoic acid (20:1), which on average accounts for 10% of the fatty acid composition in genotypes with erucic acid. Applying the molecular correction method in the present study, the effect of eicosenoic acid content on oil content was not considered. Therefore, it is suggested to modify the molecular correction method including the effect of eicosenoic acid content, and compare the results of the recalculated molecular correction to the results of regression correction again. But the correction of oil content was not only conducted to make oil contents of genotypes with varying erucic acid contents comparable, but also to perform QTL mapping for oil content uncoupled form the effect of erucic acid to identify additional minor QTL shaded by the effect of erucic acid. For this purpose a third correction method for oil content was included in this study,

the conditional correction, which was previously used by Zhao *et al.* (2006). The conditional corrected oil content showed the lowest correlation coefficient and a slope closest to zero compared to the other two correction methods, indicating the highest accuracy of correction, but the conditional correction is not based on zero erucic acid content. It is based instead on a method very similar to the estimation of adjusted values in a covariance analysis using the mean oil content of the SGEDH population as fixed point. Hence, the oil content values shown in Figure 3.3d are higher compared to those of Figure 3.3b+c. A parallel shift of the conditioned values by changing the y-intercept to the mean oil content of the erucic acid free genotypes might enable the comparison of all three correction methods. Nevertheless, QTL results of regression and conditional corrected oil contents were identical, except for a difference of 0.6cM of the confidence intervals of QTL on C07. In contrast, QTL for molecular correction showed differences not only in positions and confidence intervals of QTL comparable to these of regression and conditional correction, but also two additional QTL were identified with molecular correction. In comparison, these findings repeatedly identified the highest deviation for molecular correction. This shows that QTL results for molecular correction should also be recalculated and compared again after improving the method. From these results it is suggested to use the regression correction method for further experiments to have a reliable correction and to be able to compare oil contents of genotypes with and without erucic acid contents on the basis of zero erucic acid content. For QTL mapping regression or conditional correction can equally be used, since the results from both methods suggest that they are appropriate to calculate identical QTL. Another advantage of the regression and conditional correction is that these approaches can easily be applied to other traits.

3.5.6 Comparison of NIRS prediction and gas chromatography to determine erucic acid content

Near-infrared reflectance spectroscopy (NIRS) is a technique that uses the radiation absorbed by a set of samples to determine its properties. This technique has several advantages when compared to chemical-based analysis because it is non-destructive, rapid, and cost effective, does not require labour intensive sample processing, is environmentally safe and allows the simultaneous estimation of several traits in one sample (Stuth *et al.* 2003, Suprianto 2014). Comparing NIRS predictions and GC measures of erucic acid content within the SGEDH population a conspicuous underestimation of erucic acids contents of NIRS data was identified (Figure 3.1a). Especially the occurrence of negative values for potentially erucic acid free genotypes was striking, since negative values for erucic acid are not possible. Therefore, NIRS

predictions were adjusted as described in section 3.3.3.3. This was done to test if QTL results from adjusted NIRS data were comparable to results from GC for erucic acid content as well as for regression corrected and conditioned oil content (Table 3.13). Since with adjusted NIRS data the same QTL were detected as with the GC method, NIRS predicted values can be used for QTL mapping instead of using results from the more laborious and cost intensive GC analysis.

The quality of NIRS prediction depends on the quality of its calibration equation. To develop a significant calibration equation a representative set of samples needs to be analysed by precise reference laboratory methods. Although well proven calibrations for NIRS are used to predict various seed quality traits like oil or protein content, the calibration used in this study should be improved for erucic acid prediction. In a previous study (Radoev 2007) also analysed erucic acid content by NIRS in a DH population derived from a cross of a low and medium erucic acid containing parent, and did not observe negative values for potentially erucic acid free genotypes. Concluding that although results achieved with adjusted NIRS predictions of erucic acid were matching GC results, in the long term an improvement of the calibration for erucic acid containing material seems appropriate.

3.5.7 Application in practical breeding

In practical breeding individual QTL controlling a trait of interest can be used for marker assisted selection. Different loci from different material or different genomic regions in this way can be pyramided into an elite germplasm. This process is a time consuming challenge in plant breeding, therefore pleiotropic QTL regions controlling different traits of interest simultaneously in a favourable manner are of high value.

In the present study four QTL controlling oil content were identified on A08, C03, C05 and C07. Following correction of oil content for the effect of erucic acid using three different approaches three additional loci influencing oil content on A10, C04 and C05 were consistently identified. Major QTL for original oil content were located on A08 and C03 within QTL hot spot regions, either overlapping with or in close proximity to QTL for fatty acids and protein traits. In both hot spot regions a *FAE1* gene was identified as underlying candidate gene, causing a decrease of palmitic acid and C18 fatty acids, while increasing erucic acid and oil content. These regions were additionally showing a simultaneous increase in protein and or protein content in defatted meal. This suggests that these regions can be utilized in practical breeding to increase oil and protein content in defatted meal at once, which is meeting the increasing demand of oil as

foodstuff and renewable resource, and oilseed meal as feedstuff for animal diets. Due to the close linkage and positive correlation between QTL for oil and erucic acid content at these loci and the fact that current oilseed rape breeding focuses on canola quality breeding, their use is restricted to high erucic acid breeding programs. Therefore, original oil-QTL on C05 and C07 might be of higher interest. E_Oil-4 on C07, which was approved not to be linked to erucic acid since QTL for corrected oil content were found co-located, might also be a promising candidate locus to further increase oil content in current breeding material by marker assisted selection. Since this locus not only increases oil content but simultaneously seems to decrease unfavourable glucosinolate content. E_Oil-3 on C05 was found linked to QTL simultaneously increasing plant height delaying begin of flowering. This relation might be explained by the hypothesis that the later the plant starts to flower the longer it can grow, and the longer the growing period the taller the plant resulting in a higher biomass which provides a higher biosynthetic activity enabling the increased production of seed storage lipids, and might be useful for further breeding approaches. Besides, a second QTL was identified for corrected oil content about 30cM apart from this original oil-QTL, explaining the highest amount of the phenotypic variation. This QTL overlapped with QTL for linolenic acid and end of flowering, which should be considered when breeding for low linolenic acid content. Corrected oil-QTL on A10 and C04 overlapped with QTL for protein content with additive effects of opposite directions, thus increasing oil content at the expense of protein content.

To increase seed protein content in current breeding material individual QTL for seed protein content on A04 and A09 might be used for marker assisted selection. But since the protein content in oilseed meal after oil extraction is of higher interest compared to the protein content in total seed, marker assisted selection might preferably take advantage of QTL individually controlling protein content in defatted meal on linkage groups A07, C06 and C08 or QTL controlling both protein traits as found on A07 (co-located with QTL for end of flowering and plant height with opposite additive effects) and C06 (overlapping phenological traits). If deciding on the locus on C06 to increase protein content in defatted meal, it needs to be considered that due to an overlapping QTL linoleic acid content might be decreased. In general, in the SGEDH population a significant positive correlation between seed protein and protein content in defatted meal (r_s = 069**) indicates, that a selection for one or the other trait will be positive for both protein traits. Whereas this correlation also shows that both traits are sharing only 50% (R^2) of their genetic basis, which is confirming previous results of (Zhao 2002), and shows the potential to increase both traits independently.

In contrast, breeding in favour of increased linoleic acid content might cause a decrease in protein content of defatted meal, when utilizing the locus identified for these traits on C06. Thus, the individual QTL identified for linoleic acid on A05 might be used preferably. To influence linolenic acid individual QTL on A01, A05 and A06 might be of interest for marker assisted selection. And palmitic acid might be modified by using the individual QTL identified on A03.

Beside increasing oil content, the improvement of yield performance is a major breeding aim in oilseed rape. Seed yield is a complex trait that includes various components, including number of pods per plant, the number of seeds per pod and the individual seed weight as the most important once (Diepenbrock 2000). Seed weight was determined as thousand kernel weight in the SGEDH population, and identified a QTL explaining the highest phenotypic variation (13.8%) on linkage group C01, indicating that the alleles increasing oil content were derived from SGDH14. A previous study of Basunanda *et al.* (2009) also identified a QTL for thousand seed weight on C01 in a DH population derived from a cross between Express617 x R53. For this QTL alleles increasing oil content were derived from Express617. Assuming that the locus identified in both populations is the same, the reported results indicate that although the Express617 allele was identified as the better allele in the cross Express617 x R53, the SGDH14 allele was identified to be even better in the SGEDH population. These findings illustrate a general weakness of QTL analysis, which enables to identify the parental line contributing the better alleles within the population analysed, but this need not be the better allele compared to other material.

QTL for phenological traits were in most cases linked to QTL for oil, fatty acid and or protein content, or to each other. But individual QTL were found for end of flowering on A10, flowering period on A03, and plant height on A02 (2) and A04, which might be of interest for further breeding approaches.

3.6 Conclusion

The present study identified eight QTL for seed oil content. Previously detected major QTL on A08 and C03 for erucic acid content were confirmed, and a promising major QTL for oil content was found on C05, revealing SGDH14 as source of positive alleles. Furthermore, physical mapping utilizing the *B. napus* Darmor-*bzh* reference genome not only enabled to determine the physical positions of sequence-informative SNP and DArT markers, but also the screening of putative candidate genes of oil biosynthesis within the confidence intervals of oil-QTL. While the

genomic regions of major QTL on A08 and C03 showed co-localisation with alleles of the candidate gene *FAE1*, no candidate gene was identified within the genomic region of the major oil-QTL on C05 so far. However, the candidate gene *FAD3* was found co-located with the minor QTL on C04. Further investigations are necessary to identify potential candidate genes for the remaining five oil-QTL.

4 Genetic variation and inheritance of oil content and relevant seed quality traits of the SGEDH population cultivated in East China

4.1 Abstract

Oilseed rape (*Brassica napus* L.) is one of the most important oil cops in the world. Therefore, increasing oil content represents a major breeding aim. Chinese and European cultivars of *B. napus* represent two distinct gene pools, providing independent sources of variation to each other. In previous work, the old German cultivar Sollux was crossed to the Chinese cultivar Gaoyou. Both cultivars are known for their high oil content but also contain high contents of erucic acid and glucosinolates ('++'-quality). From this cross a doubled haploid (DH) population was developed and tested in field experiments. Following field testing under north-western German growing conditions DH line SGDH14 had the highest oil content and a combination of all QTL alleles increasing oil content from both parents. The present study further investigates the inheritance of seed oil content and other seed quality traits in a new DH population derived from the cross of SGDH14 and the inbred line 617 of the German cultivar Express ('00'- or canola quality), which also shows a high oil content. This population (n=212) was tested during two consecutive years at one location in replicated field trials in East China. Seed quality traits as well as phenological traits were determined. A framework map consisting of 19 linkage groups with 379 SNP, DArT and AFLP markers was developed, covering 2651cM. Mean trait values were used to map QTL. Analysis of variance revealed highly significant genotypic effects for most traits and heritabilities varied from 0.55 to 0.96. QTL mapping identified six QTL for oil content including four QTL for oil content not linked to erucic acid content on A06, A07, A10 and C05. Although the comparison of physical positions of QTL for oil content to the *B. napus* Darmor-*bzh* reference genome enabled the screening for potential candidate genes, only one genomic region co-located with a lipid related candidate gene. Three of the oil-QTL detected in China corresponded to QTL previously found in European field trials. Identifying environment-stable and environment-sensitive QTL for oil content by comparison of European and Chinese experiment, the current study discloses new potential to further increase oil content in both mega-environments by marker assisted selection.

4.2 Introduction

Increasing oil content is one of the most important breeding aims of oilseed rape (*Brassica napus* L.). Originally comprising high levels of erucic acid, which in animal experiments appeared to have a negative effect on the heart, oilseed rape oil was primarily used for industrial purposes. This changed with the development of the so called canola- or double low quality oilseed rape in

the 1970s. In the seeds of double low quality oilseed rape not only erucic acid was nearly completely eliminated, but also another anti-nutritional seed component was remarkably reduced, the glucosinolates. Thus, oil as well as protein quality in oilseed rape seeds were improved at once, and gained an important role for food and feed supply. Oilseed rape is an important oil crop in Europe as well as in China. Therefore, breeding independently focused on the increase of seed oil content. Due to different growing conditions, different oilseed rape varieties are grown in Europe and in China. In Europe, predominantly the winter or biennial form of oilseed rape is grown, whereas in China, both winter and spring varieties are grown depending on the climatic conditions. On average daily temperature from sowing to flowering is about 1 to 4°C and from flowering to maturity 3 to 4°C lower in Europe, and the total growth period is about 84 d longer including a 14 to 17 days longer growth period from flowering to maturity (Zhao *et al.* 2005). Because of a longer growing period and lower mean temperatures winter type oilseed rape out-yields the summer type (Kramer and Sauer 1983). Selection for high oil content in both environments already showed success, and investigations of the genetic diversity detected an increase from 1950 to 1980, but then it remained at a similar level in China and Europe (Wang *et al.* 2014). Today, the demand for plant oil as food and renewable resource is worldwide growing, which makes the further increase of seed oil content in oilseed rape necessary. And the limited genetic variation of the relatively young crop *B. napus*, initiated the search for new genetic resources. One approach to broaden the genetic basis in oilseed rape was using the combination of two cultivars from the distinct European and Chinese gene pools (Zhao *et al.* 2005). A cross of the old German cultivar Sollux to the Chinese cultivar Gaoyou was conducted. Both cultivars have high oil contents, but also a high erucic acid and glucosinolate content. A DH population was derived from this cross and investigated in Germany and China to test whether these gene pools contain different alleles for high seed oil content. By QTL mapping eight QTL for oil content were detected with additive main effects, five of these QTL showed significant additive x environment effects. Positive alleles were found in both parental lines. Alleles of Sollux and Gaoyou were more often positive in their respective environment. However, at few loci alleles from the Chinese parent were positive in the German environment. The results of Zhao *et al.* (2005) indicated that a combination of positive alleles of Chinese and European material is a promising approach for increasing oil content in both mega-environments. The present study continues these investigations. In the experiments with the Sollux x Gaoyou DH population of Zhao *et al.* (2005), DH line SGDH14 was identified as one of the lines with the highest oil content and a combination of all positive QTL alleles under north-western German growing conditions. This line SGDH14 was crossed to the inbred line 617 of the German winter oilseed rape cultivar Express, and a new DH population termed SGEDH was developed. This population was tested in field experiments under north-western European conditions and QTL

for oil content and other traits were mapped (cf. chapter 3). The objective of this study was to investigate the inheritance of seed oil content and other seed quality traits of the SGEDH population in field experiments performed in the Hangzhou area of the Zhejiang region in the PR of China and to compare the results with previous ones obtained from European trials.

4.3 Materials and methods

4.3.1 Plant material

Plant material was the SGEDH population described previously in section 3.3.1.

4.3.2 Field experiments

DH lines were tested by Prof. Jianyi Zhao (Zhejiang Academy of Agricultural Sciences, Hangzhou) in the two consecutive years 2010/11 and 2011/12 at Hangzhou located in East China. Field experiments were conducted as group wise randomized block design in small plots with two replications. The three different groups within the experiment were defined as described in section 3.3.2. For each genotype seed samples were harvested at maturity from the main raceme of ten open pollinated plants. Seeds were bulked and seed quality traits were analysed by near-infrared reflectance spectroscopy (NIRS). During growing season begin of flowering (BOF) and end of flowering (EOF) were scored, plant height at end of flowering (PH_EOF) was measured and flowering period (FP) was calculated. Phenological traits were determined as described in section 3.3.2.

4.3.3 Phenotypic analysis

4.3.3.1 Near-Infrared Reflectance Spectroscopy

NIRS was performed as described in section 3.3.3.1. Since NIRS calibration is not predicting oleic and linolenic acid contents accurately for seed samples containing erucic acid only erucic acid content was analysed for Chinese field trials.

4.3.3.2 Adjustment of NIRS predicted erucic acid contents

NIRS was performed as described in section 3.3.3.1. NIRS predicted erucic acid contents were adjusted corresponding to section 3.3.3.3. Erucic acid free genotypes were corrected by subtracting the group specific correction factor of -6.35%. Genotypes with medium and high NIRS erucic acid content were adjusted using the regression equation of this group, y = 1.50x – 5.05 (Figure 3.1b). The comparison of NIRS predicted erucic acid contents with adjusted erucic acid contents for Chinese experiments showed a high coefficient of determination with $R^2 = 0.96$.

4.3.3.3 Other traits

Thousand kernel weight (TKW), protein content in the defatted meal (Prot.idM), glucosinolate content in defatted meal (GSLidM) and the correction of oil content considering erucic acid content were determined according to section 3.3.3.

4.3.4 Statistical analysis

PLABSTAT software version 3A (Utz 2011) was used to perform analysis of variance (ANOVA) applying the following general model:

$$Y_{ij} = \mu + g_i + e_j + r_{jk} + ge_{ij} + \varepsilon_{ijk}$$

where Y_{ij} is the trait value of genotype *i* in environment *j* in replication *k*; μ is the general mean; g_i is the effect of *i*th genotype, e_j is the effect of *j*th environment, r_{jk} is the effect of replicate k in the environment j; ge_{ij} is the interaction between *i*th genotype and *j*th environment; and ε_{ijk} is the within environment error associated with genotype *i*, environment *j* and replicate *k*. Genotypes were considered fixed in the analysis, whereas environment and replicate were treated as random variables. Years were treated as environments. The data were tested for outliers by a modification of the Anscombe and Tukey method (1963) based on the detection of extreme residuals. After examining the list of detected outliers the measured values of the outliers with highest standardized residual were checked for errors and the ANOVA was repeated considering missing values for extreme outliers. The adjusted results were used in the subsequent analyses.

Heritability (h^2) was calculated as:

$$h^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_{ge}^2}{E} + \frac{\sigma_\varepsilon^2}{ER}}$$

where σ^2_g, σ^2_{ge} and σ^2_ε are variance components for g, e and ε, respectively. E and R refer to number of environments and number of replicates (Hill and Weir 1988). Spearman's rank correlation coefficients between traits mean values of the genotypes across the environments were calculated using PLABSTAT's BASIC command.

4.3.5 QTL mapping

QTL detection was performed as described in section 3.3.7.

4.3.6 Physical mapping

Physical mapping was conducted according to section 3.3.8.

4.4 Results

4.4.1 Phenotypic analysis

Variance analysis revealed highly significant effects of the genotypes for all traits investigated, except for flowering period (Table 4.1). In contrast, a highly significant effect of the environment was observed only for FP and for end of flowering. Glucosinolate and erucic acid content also showed significant effects of the environment. Genotype x environment interactions were significant for all traits except for protein content in the defatted meal. Effects of the genotypes showed high values for glucosinolate and erucic acid contents while highest effects of the environment were observed for end of flowering and flowering period. The highest residual error was detected for plant height. Heritability of flowering period was low with only 0.1 and heritability of end of flowering was moderate (0.55), while all other traits showed high heritabilities ranging from 0.70 for protein content to 0.99 for erucic acid content.

Table 4.1: Components of variance and heritabilities for contents of seed oil (%), protein (%), protein in defatted meal (Prot.idM in %), glucosinolates (GSL in µmol/g), glucosinolates in defatted meal (GSLidM in µmol/g), erucic acids (determined by NIRS; %) and begin of flowering (BOF), end of flowering (EOF), flowering period (FP) and plant height at end of flowering (PH_EOF in cm) in the SGEDH population

Trait	Variance components				Heritability
	σ^2_g	σ^2_e	σ^2_{ge}	σ^2_ε	h^2
DF	211	1	210	413	
Oil	3.83**	0.04	0.16**	0.73	0.94
Protein	0.43**	-0.02	0.07*	0.61	0.70
Prot.idM	2.31**	-0.02	0.08	1.11	0.88
GSL	211.64**	16.50*	10.78**	14.81	0.96
GSLidM	689.14**	60.79*	6.05**	3.52	0.96
NIRS22:1	266.26**	2.53*	2.88**	5.72	0.99
BOF[a]	4.48**	0.00	0.73**	1.36	0.86
EOF[a]	1.93**	33.20**	1.76**	2.72	0.55
FP	0.20	35.10**	2.05**	2.90	0.10
PH_EOF	89.50**	-0.03	8.23*	64.39	0.82

σ^2_g = genetic variance; σ^2_e = variance of the environment; σ^2_{ge} = variance of genotype x environment interaction; σ^2_ε = residual error; DF = degrees of freedom; *, ** denotes significance at $P < 5\%$ and 1%
a days counted from 1st of January

Transgressive segregation was observed for all traits (Table 4.2). Oil content ranged from 39.4 to 49.8%, with a mean value of 44.5%. For contents of oil, glucosinolates, glucosinolates in defatted meal and erucic acid as well as plant height SGDH14 showed higher values compared to Express617, while for protein, protein in defatted meal, end of flowering and flowering period Express617 exceeded SGDH14. The parental lines showed similar values for begin of flowering, differing only 0.2 days.

Table 4.2: Minimum, maximum and mean values for contents of seed oil (%), protein (%), protein in defatted meal (Prot.idM in %), glucosinolates (GSL in µmol/g), glucosinolates in defatted meal (GSLidM in µmol/g), fatty acids (determined by NIRS; %) and begin of flowering (BOF), end of flowering (EOF), flowering period (FP) and plant height at end of flowering (PH_EOF in cm) in the SGEDH population

Trait	Min	Max	Mean	*F*-value	LSD 5%	Parents	
						SGDH14	Express617
Oil	39.4	49.8	44.5	15.7**	1.4	47.8	42.5
Protein	17.2	22.0	19.0	3.3**	1.2	17.9	20.3
Prot.idM	30.5	38.4	34.3	8.4**	1.6	34.4	35.3
GSL	12.2	75.4	46.3	24.3**	8.4	61.9	20.6
GSLidM	22.1	135.9	83.3	24.2**	15.2	118.6	35.8
C22:1	-5.8	46.1	17.3	93.7**	4.7	37.4	-0.5
BOF	89.5	100.5	95.5	7.3**	2.4	96.1	95.9
EOF	113.3	124.8	120.4	2.2**	3.5	119.3	121.5
FP	20.3	29.3	24.9	1.1	3.7	23.1	25.6
PH_EOF	136.0	189.0	167.0	5.4**	12.5	177.8	166.3

LSD 5% = least significant difference at $P < 5\%$; ** denotes significance at $P < 1\%$

Oil content showed highly significant positive correlation to erucic acid (0.57) and protein in defatted meal (0.54) as well as to plant height (0.37) and end of flowering (0.28) (Table 4.3). A highly significant negative correlation was found for oil content and protein content. Protein content was positively correlated to protein content in defatted meal, while protein content in defatted meal was positively correlated to erucic acid content. A close correlation was also observed between begin of flowering and end of flowering as well as plant height, but a negative correlation was found with the flowering period. End of flowering and plant height showed highly significant positive correlation.

Table 4.3: Spearman's rank correlation for seed oil content and other quality traits

Traits	Oil	Protein	Prot.idM	GSL	GSLidM	C22:1	BOF	EOF	FP
Protein	-0.26**								
Prot.idM	0.54**	0.67**							
GSL	-0.12	-0.12	-0.20**						
GSLidM	-0.02	-0.15*	-0.15*	0.99**					
C22:1	0.57**	0.17*	0.55**	-0.20*	-0.14				
BOF	0.18*	0.00	0.13	0.06	0.08	-0.18*			
EOF	0.28**	0.06	0.27**	-0.08	-0.05	-0.09	0.79**		
FP	0.09	0.09	0.15*	-0.20**	-0.20**	0.19*	-0.57**	0.05	
PH_EOF	0.37**	-0.17*	0.13	0.04	0.08	-0.06	0.53**	0.56**	-0.12

*, ** denotes significance at $P < 5\%$ and 1%

The linear relation between oil content and erucic acid content showed a segregation of the DH population for erucic acid (Figure 4.1a). One group of low erucic acid content comprising 70 genotypes showed erucic acid contents ranging from -5.8 to 2.3%, and oil contents of 39.4 to 45.4%. Medium and high erucic acid genotypes did not show a clear separation. According to the grouping of the European trials (section 3.4.1) the group of genotypes (84) with medium erucic acid content showed erucic acid contents between 6.8 to 29.3%, and had oil contents between 42.7 and 48.1%. The group of genotypes (58) with high erucic acid content showed erucic acid contents between 30.2 to 46.1% erucic acid in which oil content ranged from 41.9 to 49.8%. Within the group of low erucic acid genotypes SGEDH172 was detected as the genotype with highest oil content (45.4%). Regression correction of oil contents (Figure 4.1b) nearly eliminated the effect of erucic acid on oil content, reducing the coefficient of determination to 0.0000002. Regression correction calculated the theoretical erucic acid free oil content of SGDH14 to 44.1%. Comparing the oil contents of all genotypes of the SGEDH population applying the regression correction DH line 210 (SGEDH210) and 145 (SGEDH145) were identified as genotypes with highest oil contents, with 45.9% and 45.8% oil, respectively.

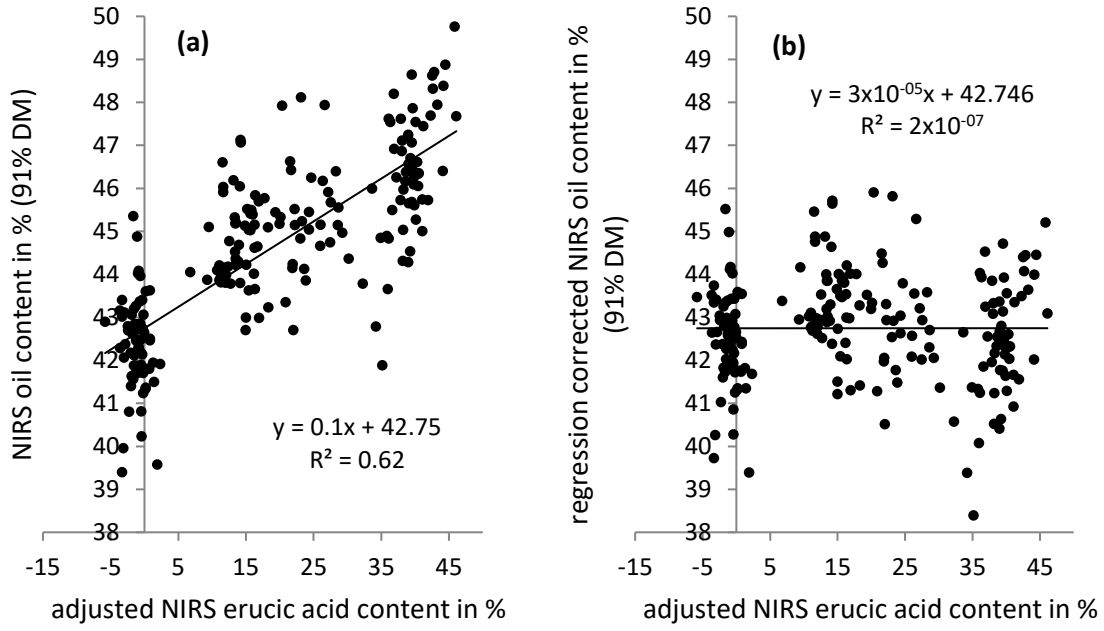

Figure 4.1: Correlation between corrected NIRS predicted seed erucic acid content and (a) NIRS predicted oil content, and (b) NIRS predicted oil content corrected for the influence of erucic acid by regression information (regression corrected oil content = oil content – (0.1 * erucic acid content)) in the SGEDH population; NIRS = near infrared reflectance spectroscopy; DM = dry matter

4.4.2 QTL mapping

4.4.2.1 QTL for oil content using original data

QTL mapping identified six QTL for oil content on the linkage groups A06, A07, A08, A10, C03 and C05. These QTL individually explained between 3.3 and 46.5% of the phenotypic variance and collectively accounted for 75.8% of the total phenotypic variance. The major QTL C_Oil-3 on A08 and C_Oil-5 on C03 explained 46.5 and 29.4% of the phenotypic variance, respectively. Except C_Oil-4 on linkage group A10, all QTL showed positive additive effects, indicating that the alleles increasing oil content are derived from SGDH14. On linkage group A06 C_Oil-1 overlapped with C_PH_EOF-2 both showing a positive additive effect according to their positive correlation. On linkage group A08 major QTL C_NIRS22:1-2 and C_Prot.idM-2 overlapped with C_Oil-3. All three QTL showed positive additive effects, indicating that alleles derived from SGDH14 increased oil content by 1.06%, erucic acid content by 8.7% and protein content in the defatted meal by 0.91%, respectively. C_Oil-5 on C03, the second major QTL for oil content was co-located with the major QTL C_NIRS22:1-3 for erucic acid content, both showing a positive additive effect, indicating alleles derived from SGDH14 were increasing oil content by 0.73% and erucic acid by 7.58%, respectively. C_Oil-4 located on A10 showed an individual position.

4.4.2.2 QTL for oil content corrected for the effect of erucic acid content

Three QTL were detected for regression corrected and for conditioned oil content located on linkage groups A06, A07 and C05 with identical positions and almost identical confidence intervals. Individual QTL explained 6.7% (A06), 12.7% (A07) and 17.2% (C05) of the phenotypic variance and all three QTL accounted for 33.9% of the total phenotypic variance. All three QTL showed positive additive effects, indicating that alleles derived from SGDH14 were increasing corrected oil contents by 0.32%, 0.43% and 0.46%, respectively. QTL for corrected oil contents all overlapped with QTL for oil content on respective linkage groups.

4.4.2.3 QTL for seed protein content

Two QTL for protein content were detected on the linkage groups A09 and C08 explaining 9.6% and 7.2% of the phenotypic variance respectively and together accounting for 16.5% of the total phenotypic variance. Both QTL showed negative additive effects, indicating that alleles derived from Express617 were increasing protein content.

4.4.2.4 QTL for protein content in defatted meal

The five QTL for seed protein content in the defatted meal were detected on linkage groups A07, A08, C01, C03 and C04. Individual QTL explained between 0.7 and 39.8% of the phenotypic variance and collectively explained 56% of the total phenotypic variance. 4.9% of the total phenotypic variance (60.9%) was explained by epistatic interactions. C_Prot.idM-3 and C_Prot.idM-5 showed negative additive effects, indicating that alleles increasing protein content in the defatted meal were derived from Express617. While the other three QTL showed positive additive effects including the major QTL C_Prot.idM-2, which increased protein content in defatted meal by 0.91%.

4.4.2.5 QTL for glucosinolate content

Five QTL for glucosinolate content were detected on linkage groups A09, C02 (2), C07 and C09, each explaining between 0 and 58% of the phenotypic variance. All QTL together accounted for 81% of the total phenotypic variance. QTL C_GSL-5 was identified as the major QTL explaining 58% of the phenotypic variance. The additive effect of C_GSL-5 was positive, indicating that an allele derived from SGDH14 was increasing glucosinolate content by 11.4µmol/g. QTL C_GSL-1, C_GSL-2 and C_GSL-4 also showed positive additive effects. Only C_GSL-3 on C02 explaining 0% of the phenotypic variance showed a negative additive effect.

4.4.2.6 QTL for glucosinolate content in defatted meal

Seven QTL for glucosinolate content were detected on linkage groups A04, A09, C02 (2), C07 and C09 (2) each explaining between 0 and 55.6% of the phenotypic variance. All QTL together accounted for 81% of the total phenotypic variance. Additional 7.3% of the total phenotypic variance was explained by epistatic interactions. Among the seven QTL, C_GSLidM-6 and C_GSLidM-7 were identified as major QTL explaining 55.2% and 55.6% of the phenotypic variance, respectively. The additive effect of C_GSLidM-1 and C_GSLidM-4 were negative, indicating that an allele derived from Express617 was increasing glucosinolate content by 2.4µmol/g and 3.6µmol/g, respectively. Except C_GSLidM-1 on A04 and C_GSLidM-6 on C09, all other QTL for glucosinolate content in defatted meal were co-located with QTL for glucosinolate content.

4.4.2.7 QTL for erucic acid

For erucic acid content four QTL were located on the linkage groups A08 (2), C03 and C04. C_NIRS22:1-4 on C04 explained only 7.6% of the phenotypic variance, the other three QTL however showed major effects explaining between 44.6 and 67.2% of the phenotypic variance. All four QTL together accounted for 89.2% of the total phenotypic variance. Major QTL showed positive additive effects, indicating that the alleles increasing erucic acid content were derived from SGDH14, while C_NIRS22:1-4 on C04 showed a negative additive effect.

4.4.2.8 Phenological traits

Nineteen QTL were detected for the four phenological traits begin of flowering (BOF), end of flowering (EOF), flowering period (FP) and plant height at end of flowering (PH_EOF) which were located on the linkage groups A02, A03, A04, A06, C02, C03, C06 and C08. Five QTL each were detected for BOF, EOF and PH_EOF while four QTL were detected for FP. These QTL collectively explained between 33.7 and 60.9% of the total phenotypic variance. Major QTL were identified for BOF (C_BOF-4) explaining 39.8% of the phenotypic variance and for EOF (C_EOF-5) which explained 29.9% of the traits phenotypic variance. For FP the QTL which had the largest effect (21.8% of the phenotypic variance) was C_FP-3 found on C06. The QTL explaining the largest effect (20.2% of the phenotypic variance) for plant height was C_PH_EOF-4 which was located on C06. QTL for BOF all showed negative additive effects except the major QTL C_BOF-4 on linkage group C06 which showed a positive additive effect. QTL for plant height instead all showed positive additive effects except C_PH_EOF-5 which showed a negative additive effect. QTL for phenological traits were mostly found either as separate individual QTL or overlapping with other phenological QTL. On C06 C_BOF-4, C_EOF-4 and C_FP-3 were found co-located, with C_BOF-4 and C_EOF-4 both having a positive and C_FP-3 having a negative additive effect. On linkage group C08 QTL for all four phenological traits overlapped (C_BOF-5, C_EOF-5, C_FP-4 and C_PH_EOF-5), all showing negative additive effects (although there was a negative correlation found between BOF and FP). Overlapping with these QTL for EOF and FP and close to the QTL for BOF and PH_EOF with only 3.2 and 1.2cM distance, respectively, the QTL C_Protein-2 with negative additive effect was located.

Table 4.4: QTL detected for contents of seed oil (%) and corrected seed oil (%), protein (%), protein in defatted meal (Prot.idM in %), glucosinolates (GSL in µmol/g), glucosinolates in defatted meal (GSLidM in µmol/g), erucic acid (determined by NIRS; %) and begin of flowering (BOF), end of flowering (EOF), flowering period (FP) and plant height at end of flowering (PH_EOF in cm) in the SGEDH population

QTL	Linkage Group	Position [cM]	Range [cM]	A[a]	R^{2b}	V(A)/V(P)[c]	V(I)/V(P)[d]	V(G)/V(P)[e]
C_Oil-1	A06	60.3	51.1-79.8	0.33	8.0			
C_Oil-2	A07	95.2	88.7-102.7	0.39	3.3			
C_Oil-3	A08	24.5	19.5-27.1	1.06	46.5	75.8	1.1	76.9
C_Oil-4	A10	28.6	27.9-36.9	-0.34	5.3			
C_Oil-5	C03	188.4	185.6-194.4	0.73	29.4			
C_Oil-6	C05	7.3	7.0-12.3	0.41	11.4			
C_Oil-reg_corr_NIRS-1	A06	69.2	56.3-80.5	0.32	6.7			
C_Oil-reg_corr_NIRS-2	A07	89.2	83.7-96.2	0.43	12.7	33.9	-	33.9
C_Oil-reg_corr_NIRS-3	C05	8.3	3.0-13.3	0.46	17.2			
C_Oil-cond_NIRS-1	A06	69.2	56.3-80.5	0.32	6.7			
C_Oil-cond_NIRS-2	A07	89.2	83.7-96.2	0.43	12.7	33.9	-	33.9
C_Oil-cond_NIRS-3	C05	8.3	3.0-13.3	0.46	17.2			
C_Protein-1	A09	54.6	33.1-65.6	-0.26	9.6	16.5	-	16.5
C_Protein-2	C08	111.7	106.2-117.3	-0.21	7.2			
C_Prot.idM-1	A07	52.1	46.3-57.1	0.23	0.9			
C_Prot.idM-2	A08	25.1	20.5-27.1	0.91	39.8			
C_Prot.idM-3	C01	67.2	54.5-74.4	-0.33	0.7	56.0	4.9	60.9
C_Prot.idM-4	C03	176.8	176.2-179.8	0.31	14.2			
C_Prot.idM-5	C04	78.6	71.0-86.3	-0.43	11.3			

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Table 4.4: *continued from previous page*

QTL	Linkage Group	Position [cM]	Range [cM]	A[a]	R^{2b}	V(A)/V(P)[c]	V(I)/V(P)[d]	V(G)/V(P)[e]
C_GSL-1	A09	57.6	51.6-65.6	4.73	20.3			
C_GSL-2	C02	0	0.0-4.0	5.51	9.7			
C_GSL-3	C02	61.7	45.9-61.9	-1.52	0.0	81.0	6.8	87.8
C_GSL-4	C07	68.1	59.3-71.1	2.53	6.1			
C_GSL-5	C09	116.7	113.7-116.7	11.40	58.0			
C_GSLidM-1	A04	129.4	122.4-129.4	-2.43	2.5			
C_GSLidM-2	A09	57.6	44.5-70.0	7.11	20.1			
C_GSLidM-3	C02	0	0.0-3.0	9.56	10.0			
C_GSLidM-4	C02	60.7	43.9-61.9	-3.58	0.0	80.7	7.3	88.0
C_GSLidM-5	C07	68.1	61.3-71.1	4.44	5.5			
C_GSLidM-6	C09	100.6	100.0-103.6	8.48	55.2			
C_GSLidM-7	C09	114.7	112.8-116.7	11.23	55.6			
C_NIRS22:1-1	A08	4.9	4.0-6.9	2.98	55.9			
C_NIRS22:1-2	A08	25.1	23.5-41.6	8.74	67.2	89.2	0.5	89.7
C_NIRS22:1-3	C03	193.4	190.4-195.4	7.51	44.6			
C_NIRS22:1-4	C04	74.6	70.0-83.3	-1.28	7.6			
C_BOF-1	A02	118.9	113.0-132.8	-0.39	5.8			
C_BOF-2	A04	45.9	35.1-51.9	-0.40	7.6			
C_BOF-3	C02	111.3	107.7-127.9	-0.42	6.0	60.9	3.8	64.7
C_BOF-4	C06	93.4	90.4-96.4	1.33	39.8			
C_BOF-5	C08	123.5	120.5-123.5	-0.57	13.9			

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Table 4.4: *continued from previous page*

QTL	Linkage Group	Position [cM]	Range [cM]	A[a]	R²[b]	V(A)/V(P)[c]	V(I)/V(P)[d]	V(G)/V(P)[e]
C_EOF-1	A02	40.8	25.9-59.7	-0.29	6.7			
C_EOF-2	A02	116	86.5-123.9	-0.35	5.3			
C_EOF-3	C03	200.2	196.4-211.3	0.51	7.3	55.2	-	55.2
C_EOF-4	C06	96.4	90.4-101.4	0.61	17.1			
C_EOF-5	C08	121.5	115.3-123.5	-0.94	29.9			
C_FP-1	A03	9	0.0-14.8	0.24	2.3			
C_FP-2	A06	16.3	2.0-25.1	0.25	1.1	33.7	-	33.7
C_FP-3	C06	98.4	92.4-102.1	-0.80	21.8			
C_FP-4	C08	118.5	113.3-122.5	-0.27	2.8			
C_PH_EOF-1	A04	129.4	118.4-129.4	1.91	3.3			
C_PH_EOF-2	A06	70.2	61.3-75.8	1.93	4.5			
C_PH_EOF-3	C03	197.2	196.4-208.6	3.55	15.4	45.2	5.6	50.8
C_PH_EOF-4	C06	80.6	69.0-86.2	4.10	20.2			
C_PH_EOF-5	C08	123.5	118.5-123.5	-2.81	10.1			

a additive effect; positive additive effect indicating that the alleles increasing the trait were derived from SGDH14
b percentage of phenotypic variation explained by each QTL
c variance of additive effects/phenotypic variance – total contribution of additive effect QTL in %
d variance of epistatic effects/phenotypic variance in %
e variance of genetic main effects/phenotypic variance in %

4.4.3 Physical mapping of QTL intervals for oil content

Physical confidence interval regions of oil-QTL were compared to the physical positions of selected homologues of *A. thaliana* genes involved in fatty acid synthesis and modification and TAG synthesis to identify putative candidate genes related to oil-QTL in the SGEDH population (cf. section 2.4 and 3.4.5). The alignment of oil-QTL confidence interval regions and selected gene homologues of *A. thaliana* identified two matches on linkage groups A06 and A08. On linkage group A06 *ketoacyl-ACP reductase* (*KAR*; AT1G62610) was detected within the confidence interval of C_Oil-1 (51.1 - 79.8cM) (Figure 4.2). *KAR*, which is part of the fatty acid synthase complex and catalyses the first reduction of the repeated reaction cycle needed to produce fatty acids, was located approximately 196kbp apart from the SNP marker Bn-A06-p16362555 (59.9cM), which is the closest marker to the QTL peak of C_Oil-1 at 60.3cM. On A08 *fatty acid elongase 1* (*FAE1*; AT4G34520) was found within the confidence interval of QTL C_Oil-3 (19.5 - 27.1cM) (Figure 4.3). FAE1, which is involved in the elongation of oleic acid to erucic acid, was located approximately 185kbp apart from the SNP marker Bn-A08-p12699181 which represents the closest marker to the QTL peak of C_Oil-3 at 24.5cM. This marker was also located within the marker intervals of C_Prot.idM-2 and C_NIRS22:1-2.

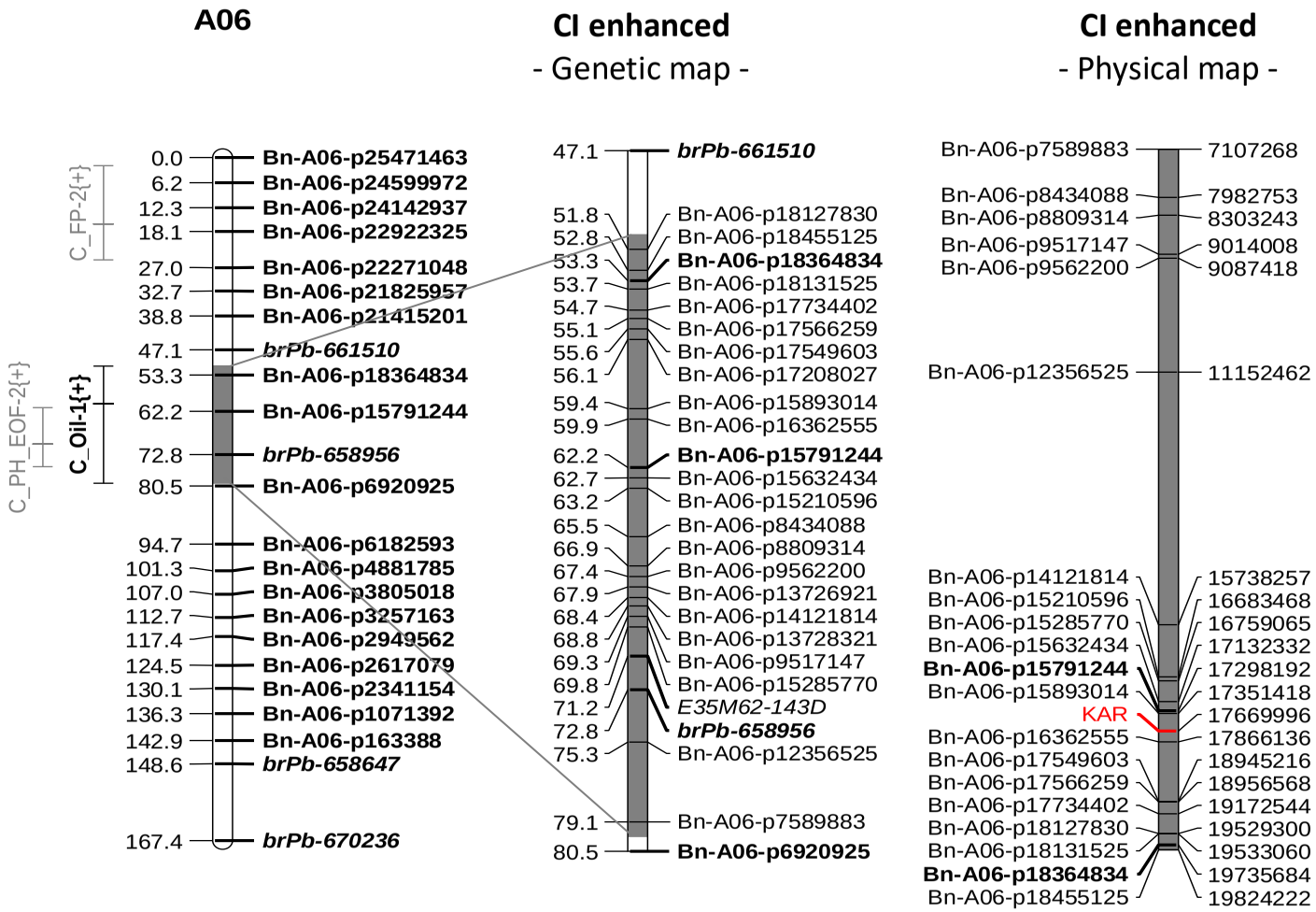

Figure 4.2: Genetic and physical map positions of markers within the QTL genomic region of C_Oil-1 (51.1 – 79.8cM; grey bar) on A06. *Left:* Linkage group A06 of SGEDH population, framework map markers with position and mapped QTL. *Middle:* Enhanced confidence interval of C_Oil-1 including all individual marker positions from genetic full map of SGEDH population. *Right:* The corresponding physical positions of markers mapped within the C_Oil-1 confidence interval, and the position of the putative candidate gene *ketoacyl-ACP reductase* (*KAR*; red) in *B. napus* reference genome. Genetic distances are presented in cM, physical positions are presented in base pairs; bold markers are framework map markers, markers in normal fond are markers of individual marker positions in the SGEDH linkage map; CI = confidence interval.

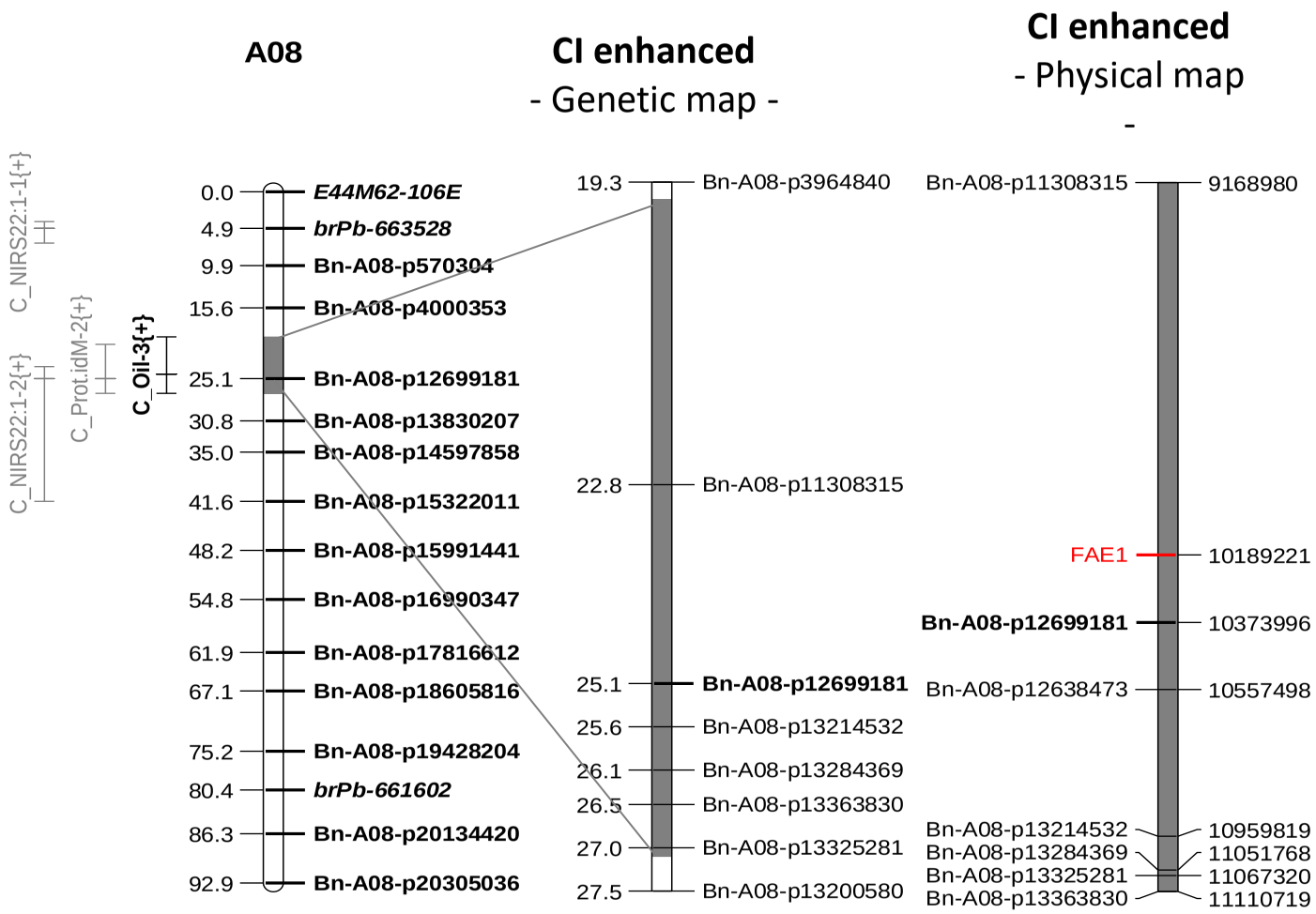

Figure 4.3: Genetic and physical map positions of markers within the QTL genomic region of C_Oil-3 (19.5 – 27.1cM; grey bar) on A08. *Left:* Linkage group A08 of SGEDH population, framework map markers with position and mapped QTL. *Middle:* Enhanced confidence interval of C_Oil-3 including all individual marker positions from genetic full map of SGEDH population. *Right:* The corresponding physical positions of markers mapped within the C_Oil-3 confidence interval, and the position of the putative candidate gene *fatty acid elongase 1* (*FAE1*; red), a ketoacyl-CoA synthase, in *B. napus* reference genome. Genetic distances are presented in cM, physical positions are presented in base pairs; bold markers are framework map markers, markers in normal fond are markers of individual marker positions in the SGEDH linkage map; CI = confidence interval.

4.5 Discussion

4.5.1 Phenotypic analysis

In comparison to the previous results from European trials (EU trials), oil content of the SGEDH population showed a larger variation in Chinese trials ranging from 39.4 to 49.8%, but the range of the EU trials was higher with oil contents between 42.5 to 50.9%. Experiments of Zhao *et al.* (2005) tested the DH population derived from a cross of the '++'-quality cultivars Sollux x Gaoyou, including SGDH14, in Germany and China at two locations each. Ranges for oil content of Zhao *et al.* (2005) at German locations were higher compared to the SGEDH population (section 3.5.1). While oil contents of Chinese locations ranging from 40.4 to 49.2% (Xiang; West China) and from 38.3 to 49.5% (Hangzhou; East China), were comparable to the oil contents of the SGEDH population in Chinese trials. In comparison, a study of Teh (2015) investigating a DH population derived from a cross of the double low quality cultivars Sansibar and Oase in Europe and in China, identified lower oil contents in both environments with ranges from 41.2 to 48.6% and 39.9 to 45.8%, respectively.

Oil content in Chinese trials showed highly significant positive correlation with erucic acid (r_s = 0.57**) and protein in defatted meal (r_s = 0.54**). These results confirmed the correlations previously found in EU trials, although correlations in EU trials were higher which might be explained by the higher number of experiments in EU trials. Besides, a highly significant positive correlation between erucic acid and protein content in defatted meal was found in Chinese trials with r_s = 0.55**, comparatively lower than in EU trials (r_s = 0.81**). Calculation of partial correlations for oil, protein in defatted meal and erucic acid content reduced the effect of oil content on protein content in defatted meal, but still showed a positive correlation coefficient (r_s = 0.33). For the correlation between oil content and protein content in defatted meal contrasting results were reported in previous studies. Suprianto (2014) found a significant negative correlation (r_s = -0.53**) in the DH population derived from Sollux x Gaoyou (Zhao *et al.* 2005), but no correlation (r_s = -0.04) in a DH population derived from a cross between Express617 and the resynthesized line R53 (Radoev 2007). Teh and Möllers (2015) also found a significant negative correlation between oil and protein content in defatted meal (r_s = -0.43**). Oil and protein share the basic sources of energy in the metabolic pathway, which usually results in a significant negative correlation between oil and protein content in seeds (Stefansson and Kondra 1975, Grami and Stefansson 1977, Röbbelen and Thies 1980, Zhao 2002). The positive

correlation found in the SGEDH population seems to differ from this explanation, suggesting the influence of other factors on oil and protein content in the SGEDH population.

Heritabilities of oil, glucosinolate and erucic acid content in Chinese trials with 0.94, 0.96 and 0.99 remained comparable to heritabilities determined in EU trials with 0.96, 0.97 and 1.00, respectively, although C trails were conducted at only one location in only two years, while EU trials included three years of field testing at several different locations. Usually the heritability stays low if field testing is conducted at only one location, even if experiments include replications. Furthermore, it seems that two years of field trails for oil, glucosinolate and erucic acid content are sufficient to achieve almost the maximum attainable heritability. Comparable results were observed previously by Teh (2015), who identified a heritability of 0.83 for oil content and 0.80 for protein content in defatted meal by investigating European field data of different locations over three years, and heritabilities of 0.7 and 0.82, respectively, by Chinese field trials at on location over two years. Heritabilities for protein, protein content in defatted meal, begin of flowering and plant height remained high in the SGEDH population, but decreased by 0.07 to 0.15. In contrast, the reduction of locations and years showed the highest effect on heritabilities for end of flowering and for flowering period, which were reduced by 0.21 and 0.78, respectively.

4.5.2 Genetic and physical mapping

In Chinese trials a total number of 54 QTL were detected for 12 traits (Table 4.4). The comparison of these QTL to the 59 QTL detected in EU trials (section 3.4.4) identified 23 common QTL for the same traits across both experiments (Table 4.5). While 16 environment-stable QTL showed comparable peak positions, either with same positions or differing only 1 to 2cM, seven of the stable QTL identified QTL peaks that differed 4 to 16.3cM. In Chinese trials six QTL for oil content were found, whereas only four QTL were detected in EU trials. Three of these oil-QTL were identified in both trials and therefore were considered as environmentally stable. The consistently detected oil-QTL were located on linkage groups A08, C03 and C05 and explained the highest individual phenotypic variances in both mega-environments. For all three QTL SGDH14 was identified as source for the allele increasing oil content. Furthermore, on A08 physical mapping identified a *fatty acid elongase 1* (*FAE1*) gene within the confidence interval of the oil-QTL. In both trials QTL for erucic acid and protein content in defatted meal were found overlapping with the consistent oil-QTL, matching the significant positive correlations between oil content, erucic acid content and protein content in defatted meal. The environment-stable oil-

QTL on C03 was co-located with a QTL for erucic acid content only in Chinese trials. However, in the EU trials the confidence interval of the stable oil-QTL on C03 overlapped with a QTL for eicosenoic acid content. In EU trials a QTL for erucic acid content was found 9.8cM apart from the stable oil-QTL on C03. However, no candidate gene was detected within the confidence interval of the stable QTL for oil content on C03 in both environments. But in the EU trials a *FAE1* gene was detected within the confidence interval of the adjacent QTL for erucic acid content. Teh (2015) also found an environmentally stable QTL for oil content on C03 in the '00'-DH population derived from the cross of the two winter cultivars Sansibar x Oase, which co-located with a stable QTL for protein content in defatted meal, both showing additive effects of same direction. These QTL were located at another position on linkage group C03, not linked to a QTL for erucic acid, therefore this QTL might be of even higher interest to increase oil and protein content in defatted meal simultaneously. Since the loci of these QTL do not show differences in the SGEDH population, thus detecting no QTL, it needs to be tested if the loci in Sansibar x Oase and SGEDH population are the same or not, and if they differ which one carries the more favourable allele. No lipid related candidate gene was found to be co-located with the environmentally stable major oil-QTL on C05. However, using the same SGEDH population and the seed quality data from the same Chinese field experiments, but a marker map based on lipid related candidate genes, two QTL for oil content on C05 were identified, each explaining 21% of the phenotypic variance (Prof. Jianyi Zhao, Zhejiang Academy of Agricultural Sciences, Hangzhou, PR China, personal communication). A second QTL position for oil content on C05 was also identified in EU trials of this study using oil contents corrected for the effect of erucic acid. This QTL position explained about 35% of the phenotypic variance. This demonstrates the relevance of oil-QTL on C05, but results need to be further investigated and approved. In Chinese trials three environment-specific minor QTL for oil content were found on linkage groups A06 (C_Oil-1), A07 (C_Oil-2) and A10 (C_Oil-4) which explained 8.0, 3.3 and 5.3% of the phenotypic variance, respectively. Whereas the minor QTL E_Oil-4 on linkage group C07 was only identified in EU trials. In both environments only one QTL for oil content was identified showing that alleles increasing oil content were derived from the double low quality parent Express617, E_Oil-4 on C07 and C_Oil-4 on A10, respectively. Regression correction and conditioning of oil content was applied in both environments to eliminate the effect of erucic acid and thereby to identify additional minor QTL for oil content independent of the influence of erucic acid. Regression corrected and conditioned oil content allowed to identify four QTL on A10, C04, C05 and C07 in EU trials, and three QTL on A06, A07 and C05 in Chinese trials. Both, the regression and conditional correction led to the identification of identical QTL in both environments. This shows that both methods are appropriate. However, none of the QTL for corrected oil contents was stable over the two mega-environments. This might be explained due to the fact that almost

all QTL for oil content represented only minor QTL, and major QTL are more likely to be stable across different environments (Tanksley 1993, Teh 2015). On the other hand, oil content is a complex quantitative trait which is known to be strongly influenced by environmental effects (Zhao *et al.* 2006, Delourme *et al.* 2006, Teh 2015), which may result in the detection of environment specific minor QTL. However, the major QTL for corrected oil content on C05 was only detected in EU trials and hence to be regarded as environment specific. All three QTL for corrected oil contents in Chinese trials corresponded to QTL for oil content without correction.

EU trials identified seven QTL for protein content. Four of these QTL showed that the positive alleles were derived from Express617. In contrast, in Chinese trials only two QTL were detected for protein content, both showing that the positive alleles were derived from SGDH14. Neither in EU nor in Chinese trials a major QTL for protein content was detected, reflecting the quantitative character of protein content. However, one QTL for protein content was identified in both environments, which was located on A09 showing a negative additive effect. This QTL might be of interest for breeding programs that aim at increasing protein content independently of other seed quality traits. In Chinese trials the stable protein-QTL overlapped with a QTL for glucosinolates showing opposite additive effects. This could even increase the value of this QTL for high protein breeding at least in China, since by increasing protein content anti-nutritional glucosinolates would be decreased in the seed and in the defatted meal. In comparison, protein content in defatted meal detected seven QTL in EU trials, three of these QTL showing a negative additive effect. While five QTL for protein content in defatted meal were identified in Chinese trials, with two QTL showing negative additive effects. QTL detected on linkage groups A08 and C03 were found in both environments showing positive additive effects and explaining the highest phenotypic variances for protein content in defatted meal in both trials.

For glucosinolate content in EU as well as in Chinese trials five QTL were detected. All of these QTL showed that the alleles increasing glucosinolate content were derived from SGDH14, except QTL C_GSL-3 on C02 which showed a negative additive effect, but accounted for a not measurable phenotypic variation in this trait. Three QTL in each environment showed individual positions, while two QTL were commonly detected in both trials. Individual QTL of EU trials were located on A09 (2) and C07, and individual QTL of Chinese trials were located on A09, C02 and C07. Environment-stable QTL for glucosinolates were located on C02 and C09. The stable QTL on C09 was also detected as the major QTL for glucosinolate content in both trials. In comparison, QTL mapping identified four QTL for glucosinolate content in defatted meal in EU trials and seven in Chinese trials. In both trials each QTL for glucosinolate content in defatted meal overlapped with a QTL for glucosinolate content. QTL E_GSLidM-1 on A09 was identified

environment-specific in EU trials, and QTL on A04, A09, C02 and C09 were specific in Chinese trials. For glucosinolate content in defatted meal QTL on C02, C07 and C09 showed stable positions. Except C_ GSLidM-1 on A04 and C_ GSLidM-4 on C02, all QTL identified SGDH14 as source for increasing alleles.

Erucic acid content in both trials identified four QTL on A08 (2), C03 and C04. QTL on A08 and C04 were identified stable in both trials with identical QTL positions and only small differences in confidence intervals, additive effects and phenotypic variance. Peaks of the additionally identified QTL on linkage group A08 were located 10cM apart from each other showing no overlap of confidence intervals, and QTL on C03 of EU and Chinese trials showed a difference of 7.8cM between QTL peaks and likewise did not have overlapping confidence intervals. All QTL for erucic acid were identified as major QTL and showed positive additive effects, except QTL on C04 which only showed minor contribution to the phenotypic variance and had negative additive effects.

In EU trial three to eight QTL were detected for phenological traits, while four to five QTL were found in Chinese trials. Begin of flowering identified three environment-stable QTL on A04, C06 and C08, end of flowering identified two on C06 and C08, flowering period two on A03 and C06 and plant height at end of flowering three on A04, C06 and C08. Confidence intervals of stable phenological QTL on C06 and C08 not only overlapped across environments within one trait, but also overlapped among traits for QTL on same linkage groups. The stable QTL on C06 was identified as the major QTL for begin of flowering and flowering period, and likewise QTL on C06 of the other phenological traits showed the highest contribution to the phenotypic variance. According to the correlations found for phenological traits within the SGEDH population flowering period was generally showing opposite additive effect, while all other phenological traits showed same direction of additive effects when overlapping. Except on C08 the QTL flowering period showed same direction of additive effect compared to overlapping QTL of the other phenological traits, showing an exceptional high phenotypic variance for end of flowering at this locus.

Table 4.5: QTL repeatedly identified on the same linkage groups in both environments, Europe (E) and China (C)

		Europe			China		
Trait	LG[a]	QTL	Range [cM]	A[b]	QTL	Range [cM]	A[b]
Oil	A08	E_Oil-1	20.5-27.1	1.12	C_Oil-3	19.5-27.1	1.06
	C03	E_Oil-2	187.6-195.4	0.63	C_Oil-5	185.6-194.4	0.73
	C05	E_Oil-3	7.0-13.3	0.60	C_Oil-6	7.0-12.3	0.41
Protein	A09	E_Protein-4	44.5-66.6	-0.20	C_Protein-1	33.1-65.6	-0.26
Prot.idM	A08	E_Prot.idM-3	16.5-25.1	1.03	C_Prot.idM-2	20.5-27.1	0.91
	C03	E_Prot.idM-4	176.2-179.8	0.68	C_Prot.idM-4	176.2-179.8	0.31
GSL	C02	E_GSL-3	0.0-4.0	3.74	C_GSL-2	0.0-4.0	5.51
	C09	E_GSL-5	113.7-116.7	8.63	C_GSL-5	113.7-116.7	11.40
GSLidM	C02	E_GSLidM-2	0.0-4.0	6.72	C_GSLidM-3	0.0-3.0	9.56
	C07	E_GSLidM-3	43.9-64.3	5.03	C_GSLidM-5	61.3-71.1	4.44
	C09	E_GSLidM-4	113.7-116.7	16.23	C_GSLidM-7	112.8-116.7	11.23
C22:1	A08	E_GC22:1-2	23.5-41.6	9.47	C_NIRS22:1-2	23.5-41.6	8.74
	C04	E_GC22:1-4	70.0-87.3	-1.49	C_NIRS22:1-4	70.0-83.3	-1.28
BOF	A04	E_BOF-1	32.1-50.9	-0.74	C_BOF-2	35.1-51.9	-0.40
	C06	E_BOF-3	91.4-96.4	2.04	C_BOF-4	90.4-96.4	1.33
	C08	E_BOF-4	119.5-123.5	-0.63	C_BOF-5	120.5-123.5	-0.57
EOF	C06	E_EOF-4	91.4-102.1	0.34	C_EOF-4	90.4-101.4	0.61
	C08	E_EOF-5	116.3-123.5	-0.30	C_EOF-5	115.3-123.5	-0.94
FP	A03	E_FP-1	0.0-10.8	0.41	C_FP-1	0.0-14.8	0.24
	C06	E_FP-3	91.4-96.4	-1.64	C_FP-3	92.4-102.1	-0.80
PH_EOF	A04	E_PH_EOF-3	108.1-129.4	1.14	C_PH_EOF-1	118.4-129.4	1.91
	C06	E_PH_EOF-7	85.2-96.4	4.41	C_PH_EOF-4	69.0-86.2	4.10
	C08	E_PH_EOF-8	118.5-123.5	-1.99	C_PH_EOF-5	118.5-123.5	-2.81

a Linkage group

b additive effect; positive additive effect indicating that the alleles increasing the trait were derived from SGDH14

4.5.2.1 Applications in breeding programs

Generally, environmental stable QTL are of higher value to breeding programs, since those QTL are effective in many different environments, enabling their broader use in breeding material. Almost half of the QTL were consistently identified in Chinese and EU trials. In the present study three environment-stable main QTL for oil content were found within the SGEDH population. But two of these oil-QTL on A08 and C03 showed close linkage to QTL for erucic acid content. These loci are of little interest, since breeding is focused on '00'-quality. However, an environmentally stable QTL not linked to the erucic acid genes was identified on C05. In EU trials this QTL co-located with two QTL, one simultaneously delaying begin of flowering and the other increasing plant height. Thus, this stable QTL might be used in marker assisted selection to further increase the oil content in current breeding material in both environments. Beside this environmentally stable QTL also the mega-environment-specific QTL might be of interest to further increase oil content in current breeding material in the respective region. In Europe E_Oil-4 on C07, and a second QTL on C05 detected for corrected oil content might be of interest (compare section 3.5.6). Whereas in China the oil-QTL on A06, which was co-located with a *ketoacyl-ACP reductase* (*KAR*) gene, encoding an important enzyme of the fatty acid synthase complex, and a QTL for plant height with additive effect of same direction, might be considered in local breeding programs. In addition, individual QTL on A07, which was found 40cM apart from a QTL increasing protein content in defatted meal, and A10 might be used in marker assisted selection to increase oil content in oilseed rape in China.

5 General discussion

5.1 Comparison of QTL mapping results calculated by QTLNetwork and WinQTL Cartographer

To detect QTL different mapping programs are available. Due to different significance thresholds and mapping approaches QTL mapping programs may lead to the detection of different QTL (McElroy *et al.* 2006, Mackay and Powell 2007). Hence, the stability of QTL detection might be enhanced by using more than one mapping software (Ravi *et al.* 2011). Two of the frequently used programs are QTLNetwork (Yang *et al.* 2008) and WinQTL Cartographer (Wang *et al.* 2012a). Thus, WinQTL Cartographer was applied additionally in this study, to verify the QTL detected within the SGEDH population by QTLNetwork. The same framework map (cf. section 3.3.6, Appendix 14) was used in both QTL mapping programs.

Applying composite interval mapping (CIM) QTLNetwork detected seven individual QTL positions influencing oil content (considering both, original and regression corrected data; Table 3.9) in European experiments. Meanwhile, the multiple interval mapping (MIM) approach of WinQTL Cartographer identified eight individual oil-QTL (Appendix 6, Appendix 7). In comparison, six oil-QTL positions were consistently detected with both programs on linkage groups A08, A10, C03, C05 (2) and C07. However, positions of QTL differed between 0 to 10.6cM, and confidence intervals were overlapping but showed variation in ranges. Additive effects of the matching QTL pairs had same direction, while the fraction of explained phenotypic variance of WinQTL Cartographer were generally lower than those of QTLNetwork, indicating that either QTLNetwork tends to overestimate or WinQTL Cartographer tends to underestimate the fraction of explained phenotypic variance. When comparing the size of the additive effect with its fraction of explained phenotypic variance in both programs, it was observed that in QTLNetwork these were often not collinear, while in WinQTL Cartographer a better collinearity was found. Beside common QTL positions, QTLNetwork additionally identified one individual minor QTL for oil content on C04, while WinQTL Cartographer detected two additional minor QTL on C03 and C04, although the LOD threshold value of WinQTL Cartographer was 0.2 higher compared to QTLNetwork. QTL obtained from both programs analysing Chinese data also revealed comparable results (Appendix 10). Both programs detected five QTL controlling oil content in common, while QTLNetwork found one more individual minor QTL on C05 and WinQTL Cartographer found two more on A02 and C05. Similar observations

were found comparing QTL results for protein as well as GC determined erucic acid content of both programs (Appendix 8, Appendix 9, Appendix 11). This suggests that consistently detected QTL can be considered as reliable, while minor QTL that were detected individually by one or the other program might need additional validation by other approaches, like association studies. In general these results were in accordance with observations of Ravi *et al.* (2011) who used both programs to prove the reliability of QTL for drought tolerance identified in groundnut. In conclusion the application of an additional QTL program seems to complement QTL data detected with only one program. Therefore, it is recommended to use a second QTL program to increase the reliability of QTL results. And within the programs different configurations should be considered since a change of the testing window size from 10 (default) to 5cM in QTLNetwork for example resulted in the detection of one QTL for erucic acid content on A08 (Appendix 12) instead of two adjacent ones which in this case would be more likely because there is only one *FAE1* gene reported on A08 regulating erucic acid synthesis (Fourmann *et al.* 1998). This suggests one of the two erucic acid QTL detected in both programs under default settings could be a false positive QTL.

5.2 Marker genotypes of best SGEDH lines and comparison to established breeding material

Current breeding programs to increase oil content in oilseed rape, which are mainly focused on canola-quality, are seeking new resources. As discussed previously (section 3.5 and 4.5) individual loci controlling oil content identified in this study represent promising candidates and might be of use for marker assisted selection. Of highest interest in this case are genotypes combining as many positive alleles influencing oil content as possible. Phenotypic analyses of the SGEDH population in Europe and in China both identified DH lines exceeding the oil content of both parental genotypes. In the group of erucic acid free lines, which are of highest interest for practical breeding, SGEDH172 was identified with highest oil content in both environments. In addition, the application of a regression correction of oil content to eliminate the effect of erucic acid on oil content enabled the comparison of oil contents between erucic acid free and erucic acid containing genotypes in the SGEDH population. This correction identified SGEDH175 and SGEDH13 in European experiments, and SGEDH210 and SGEDH145 in Chinese experiments, with highest oil contents of all lines.

To confirm the potential of the lines with highest oil content as basic material for further increasing oil content in their respective environment, the marker genotypes of these lines were investigated at the positions of oil-QTL (Table 5.1 and Table 5.2). In Europe SGEDH175 exhibited five (71%) favourable marker alleles out of seven, while SGEDH13 and SGEDH172 included four (57%) out of the seven (Table 5.1). As expected, at the two oil-QTL positions linked to erucic acid content on A08 and C03, SGEDH172 exhibited no favourable marker allele, since it does not contain erucic acid, while SGEDH13 and SGEDH175 both showed the favourable marker allele for the oil-QTL on C03. At the remaining five positions which did not show linkage to erucic acid, SGEDH172 exhibited a non-favourable marker allele at the oil-locus on C07, and SGEDH175 lacked a favourable allele at the oil-locus on A10, indicating that the higher oil content of SGEDH175 was mainly caused by its favourable allele on C03. Whereas SGEDH13 missed two favourable alleles of the remaining five oil-QTL positions, explaining the lower oil content compared to SGEDH175. However, none of the top oil genotypes incorporated all favourable marker alleles of the five oil-loci not related to erucic acid content. These results show the potential to further increasing the oil content in these lines incorporating the missing favourable alleles. Additionally, Sollux and Gaoyou, the parental lines of SGDH14, were genotyped together with the SGEDH population. Comparing the favourable marker allele constitution of the oil-QTL loci identified in the SGEDH population to the marker alleles occurring in Sollux and Gaoyou, indicated that none of the favourable alleles identified for oil-QTL in the SGEDH population in European experiments was due to an allele contributed by Gaoyou, although most of the favourable marker alleles were contributed by SGDH14. Instead, Sollux was identified as the original contributor of the favourable marker allele of the oil-locus at C04, and showed the favourable allele of A10 in common with Express617, which was not present in SGDH14 and Gaoyou.

In Chinese trials comparable results were found (Table 5.2). Regarding the two oil-QTL loci associated with erucic acid, SGEDH172 carried no favourable allele, but SGEDH210 and SGEDH145 did. But SGEDH172 and SGEDH210 both showed the favourable marker genotypes at all four remaining oil-loci independent of erucic acid content. Nevertheless, due to the relatively small effect of these QTL, in SGEDH145 the favourable allele of the oil-locus on C03 compensated the absence of two minor favourable alleles, leading to a higher total oil content of this line compared to SGEDH172.

Table 5.1: Marker genotypes of the parental lines of the SGEDH population, SGE DH lines with highest oil content (SGEDH172) and highest regression corrected oil content (SGEDH175, SGEDH13) in European trials, and the parental lines of SGDH14, Sollux and Gaoyou, for oil-QTL of European trials (environment-stable QTL are presented in bold; grey label indicates oil-QTL linked to erucic acid; favourable marker alleles are represented as capital letters)

			LG	**A08**	A10	**C03**	C04	**C05**	C05	C07
			QTL	**E_Oil-1**	E_Oil-reg_corr-1	**E_Oil-2**	E_Oil-reg_corr-2	**E_Oil-3**	E_Oil-reg_corr-3	E_Oil-4
			Additive effect	**1.12**	-0.27	**0.63**	0.23	**0.6**	0.64	-0.39
			R^2	**50.5**	11.2	**31.2**	3.2	**13.8**	34.6	10
			QTL position [cM]	**24.5**	17.8	**190.4**	51.4	**9.3**	39.7	49.5
			Marker position [cM]	**25.1**	17.5	**195.8**	47.4	**7.3**	39.9	50.2
Genotype	Oil	Oil-reg_corr	Marker locus	**Bn-A08-p12699181**	Bn-A10-p15442975	**Bn-scaff_16394_2-p207187**	Bn-scaff_16804_2-p261726	**Bn-scaff_21634_1-p62623**	Bn-A05-p22111286	Bn-scaff_15705_1-p2504173
SGDH14	48.7	45.1		A	a	A	A	A	A	a
Express617	45.1	45		b	B	b	b	b	b	B
		Favourable marker alleles		**A**	**B**	**A**	**A**	**A**	**A**	**B**
SGEDH172	46.5	46.4		b	B	b	A	A	A	a
SGEDH175	48.5	46.9		b	a	A	A	A	A	B
SGEDH13	48.7	46.7		b	a	A	b	A	A	B
Sollux	48.6	44.9		A	B	A	A	A	A	a
Gaoyou	46	42.8		A	a	A	b	A	A	a

Table 5.2: Marker genotypes of the parental lines of the SGEDH population, SGE DH lines with highest oil content (SGEDH172) and highest regression corrected oil content (SGEDH210, SGEDH145) in Chinese trials, and the parental lines of SGDH14, Sollux and Gaoyou, for oil-QTL of Chinese trials (environment-stable QTL are represented in bold; grey label indicates oil-QTL linked to erucic acid; favourable marker alleles are represented as capital letters)

			LG	A06	A07	**A08**	A10	**C03**	**C05**
			QTL	C_Oil-1	C_Oil-2	**C_Oil-3**	C_Oil-4	**C_Oil-5**	**C_Oil-6**
			Additive effect	0.33	0.39	**1.06**	-0.34	**0.73**	**0.41**
			R^2	8	3.3	**46.5**	5.3	**29.4**	**11.4**
			QTL position [cM]	60.3	95.2	**24.5**	28.6	**190.4**	9.3
			Marker position [cM]	62.2	94.9	**25.1**	33.5	**195.8**	7.3
Genotype	Oil	Oil-reg_corr	Marker locus	Bn-A06-p15791244	Bn-A07-p18201301	**Bn-A08-p12699181**	Bn-A10-p16285282	**Bn-scaff_16394_2-p207187**	**Bn-scaff_21634_1-p62623**
SGDH14	47.8	44.4		A	A	A	a	A	A
Express617	42.5	42.6		b	b	b	B	b	b
			Favourable marker alleles	**A**	**A**	**A**	**B**	**A**	**A**
SGEDH172	45.4	45.5		A	A	b	B	b	A
SGEDH210	47.9	45.9		A	A	A	B	b	A
SGEDH145	48.1	45.8		A	A	A	a	b	b
Sollux	46.4	43.1		A	b	A	a	A	A
Gaoyou	43.8	42.1		A	A	A	a	A	A

Additionally, the lines identified with highest oil content in European and Chinese experiments were compared to a set of established and well-known cultivars mainly of European origin, but also including the Chinese cultivar Gaoyou. This comparison showed that the oil content of SGEDH172 not only exceeded the oil content of its parental line Express617 in Europe and in China, but also the oil content of most of the simultaneously tested cultivars. Only Oase showed 0.3% higher oil content in European trials, and NKBeauty showed 0.1% higher oil content in Chinese trials. Protein content and protein content in defatted meal were comparable to those of tested cultivars, but glucosinolate content was higher. While cultivars showed glucosinolate contents ranging from 15.9 to 26µmol/g in Europe and from 12.9 to 20.6µmol/g in China, SGEDH172 showed more than 60µmol/g in both environments. And also the thousand kernel weight of SGEDH172 was 0.4 to 1.4g lower compared to the cultivars. Whereas, the lines with highest corrected oil content exceeded the oil contents of all cultivars in their respective environment, and not only showed comparable contents of protein and protein in defatted meal, but also showed equal thousand kernel weights. Even more convincing except of SGEDH145 with 58µmol/g, the lines of highest corrected oil contents showed substantially lower glucosinolate contents. While SGEDH175 showed around 45µmol/g and SGEDH210 40µmol/g, SGEDH13 even reached a comparable amount of only 24µmol/g. However, due to the production of erucic acid in these lines their oleic acid content is drastically reduced. In further attempts towards increasing oil content in oilseed rape at the Department of Crop Sciences at the University of Göttingen line SGEDH13, due to its relatively high corrected oil content and low glucosinolate content, was used as parent in a cross with the high oil double low cultivar Adriana. From this cross a new DH population was developed which is currently characterized in field experiments.

In parallel to the development and investigation of the SGEDH population in Europe, a sister DH population was developed and is currently analysed in China, derived from a cross of a sister line of SGDH14 which performed best under Chinese conditions, and a Chinese high oil cultivar (Prof. Jianyi Zhao, Zhejiang Academy of Agricultural Sciences, Hangzhou, PR China, personal communication). Comparison of the SGEDH population and its sister population might confirm the present results, and allow further insights into the control and regulation of oil content.

Table 5.3: Mean values of EU trials for contents of seed oil (%), regression corrected seed oil (%), protein (%), protein in defatted meal (Prot.idM in %), glucosinolates (GSL in µmol/g), fatty acids (%) and for thousand kernel weight (TKW in g), begin of flowering (BOF), end of flowering (EOF), flowering period (FP in days) and plant height at end of flowering (PH_EOF in cm) of SGE DH lines with highest oil content (SGEDH172) and highest regression corrected oil content (SGEDH175, SGEDH13), SGEDH parental lines and cultivars

	Genotype	Oil	Oil-reg_corr	Protein	Prot. idM	GSL	16:0	18:1	18:2	18:3	20:1	22:1	TKW	BOF	EOF	FT	PH_EOF
without 22:1	SGEDH172	46.5	46.4	17.2	32.1	61.7	5.0	60.1	19.7	10.9	1.3	0.3	4.6	116.7	144.9	28.1	134.6
	Express																
	617	45.1	45.0	17.8	32.4	26.0	4.9	61.2	18.3	9.8	1.8	1.0	5.4	114.7	143.6	29.3	130.0
	Oase	46.8	46.8	16.2	30.3	19.5	4.3	64.2	18.3	8.5	1.5	0.2	5.5	113.8	143.9	30.9	130.6
	NKBeauty	46.4	46.4	16.7	31.1	15.9	4.5	62.3	19.7	9.5	1.2	0.1	5.0	114.9	144.3	29.4	136.5
	Adriana	46.3	46.3	16.3	30.2	17.9	4.7	63.8	19.2	9.0	1.4	0.0	6.0	113.9	139.5	25.7	135.2
	Billy	45.7	45.7	17.4	32.0	19.7	4.6	63.0	18.9	8.9	1.5	0.3	5.6	114.0	144.1	30.6	126.5
	Komando	45.7	45.7	16.5	30.4	19.6	4.6	63.9	18.9	9.2	1.4	0.0	5.8	115.1	142.6	27.9	131.9
	Express	45.1	45.1	17.8	32.3	23.8	4.9	61.5	19.3	9.8	1.5	0.2	5.3	113.7	143.5	30.2	127.7
	Krypton	45.1	45.0	17.2	31.1	21.7	4.6	61.7	19.8	9.9	1.4	0.1	5.7	115.9	144.4	29.1	131.2
	Favorite	43.8	43.8	18.2	32.4	17.2	4.8	61.3	19.1	10.1	1.6	0.4	5.6	114.7	142.8	28.6	130.8
with 22:1	SGEDH175	48.5	46.9	17.4	33.7	45.5	4.4	29.4	16.8	9.1	18.8	18.7	5.1	117.6	146.3	28.6	148.9
	SGEDH13	48.7	46.7	17.6	34.2	24.1	4.8	25.3	14.8	10.0	18.8	23.7	5.0	118.3	146.5	28.6	143.9
	SG DH14	48.7	45.1	18.1	35.1	65.8	3.6	14.7	14.0	9.4	11.7	43.3	5.3	116.1	144.5	29.0	138.9
	Sollux	48.6	44.9	18.2	35.4	77.8	3.6	14.0	14.6	8.8	11.3	44.5	5.3	119.7	145.4	25.9	143.5
	Gaoyou	46.0	42.8	17.7	32.6	60.8	3.6	19.7	13.2	9.1	13.0	37.7	5.5	113.7	143.6	30.1	139.6

Table 5.4: Mean values of Chinese trials for contents of seed oil (%), regression corrected seed oil (%), protein (%), protein in defatted meal (Prot.idM in %), glucosinolates (GSL in µmol/g), erucic acid (%) and begin of flowering (BOF), end of flowering (EOF), flowering period (FP in days) and plant height at end of flowering (PH_EOF in cm) of SGE DH lines with highest oil content (SGEDH172) and highest regression corrected oil content (SGEDH210, SGEDH145), SGEDH parental lines and cultivars

	Genotype	Oil	Oil-reg_corr	Protein	Prot.idM	GSL	NIRS22:1	BOF	EOF	FP	PH_EOF
without 22:1	SGEDH172	45.4	45.5	18.4	33.7	60.3	-1.6	142.8	165.8	23.0	173.3
	Express 617	42.5	42.6	20.3	35.3	20.6	-0.5	141.9	167.5	25.6	166.3
	NKBeauty	44.5	45.0	18.1	32.5	12.9	-4.7	140.0	165.3	25.3	166.0
	Krypton	43.8	44.3	18.6	33.1	17.1	-5.0	143.0	167.3	24.3	166.5
	Billy	43.8	43.9	18.0	32.0	13.6	-1.1	140.8	165.3	24.5	164.0
	Komando	43.2	43.6	18.6	32.7	17.6	-3.4	142.8	168.3	25.5	170.3
	Oase	42.7	43.2	18.8	32.8	16.5	-4.7	140.8	167.3	26.5	154.8
	Adriana	42.6	43.1	18.3	31.9	15.5	-4.6	141.3	165.0	23.8	161.0
	Favorite	42.7	43.0	19.5	34.0	13.6	-2.8	141.8	166.5	24.8	155.8
	Express	42.5	42.5	19.8	34.4	17.9	-0.3	140.3	166.3	25.9	158.6
with 22:1	SGEDH210	47.9	45.9	18.1	34.8	40.2	20.3	144.0	168.8	24.8	175.3
	SGEDH145	48.1	45.8	18.2	35.1	58.0	23.2	138.3	164.3	26.0	153.5
	SGDH14	47.8	44.4	17.9	34.4	61.9	34.7	142.1	165.3	23.1	177.8
	Sollux	46.4	43.1	19.6	36.6	69.6	33.6	148.3	170.3	22.0	158.0
	Gaoyou	43.8	42.1	17.9	31.9	56.4	17.6	140.6	166.3	25.6	165.4

5.3 Oil-QTL on C05, fibre content and candidate genes

A subset of SGEDH population data was previously investigated by Suprianto (2014), analysing fibre content and seed hull proportion. The observed trait data of fibre content and seed hull proportion by Suprianto (2014) were used to recalculate QTL of these traits using the optimized framework map of the SGEDH population described in this study. Results of the recalculated QTL for fibre content and seed hull proportion are presented in Table 3.14. Compared to the results of Suprianto (2014) noticeable differences were observed for minor QTL positions of each trait, but major QTL remained in comparable marker intervals. Corresponding to the previous results of Suprianto (2014) a common major QTL for fibre content and seed hull proportion, was detected on linkage group C05. Major QTL for hemicellulose and cellulose showed positive additive effects, while major QTL of all other fibre contents and seed hull showed negative additive effects. All these QTL showed overlapping confidence intervals with the major QTL for corrected oil contents (E_Oil-reg_corr-3, E_Oil-mol_corr-5 and E_Oil-cond-3; confidence interval 35.7 - 43.7cM) as well as with a QTL for linolenic acid (E_GC18:3-6; confidence interval 27.2 - 43.7) content and EOF (E_EOF-3; confidence interval 34.7 - 43.7). The overlapping QTL for corrected oil contents, linolenic acid and EOF all showed positive additive effects. The QTL results were in accordance with the negative correlation between oil and fibre content (Suprianto 2014). Thus, an additional investigation of candidate genes for fibre content was conducted for the major QTL on linkage group C05. Individual peak positions of the major QTL for fibre content and seed hull varied between 32.7 and 36.7cM, therefore a physical region ranging from 40000 to 40320kbp was scanned, which was approximately covering the peaks' region. Within this region about 90 protein matches were found. The screening of this region identified an interesting *A. thaliana* protein match, related to storage lipid biosynthesis. This match, the AT3G09560.2, represented the lipin family protein phosphatidate phosphohydrolase 1 (PAH1), a Mg^{2+}-dependent phosphatidic acid phosphatase (PAP), ranging from 40279609 to 40282919bp. The gene prediction for BnaC05g43390D alias GSBRNA2T00073250001 was overlapping with the PAH1 *A. thaliana* protein match. By catalysing the conversion of phosphatidic acid (PA) to diacylglycerol (DAG) this enzyme plays a central role in lipid metabolism by governing the supply of substrates not only for storage but also for membrane lipids (Han *et al.* 2006, Carman and Han 2009). Eastmond *et al.* (2011) reported that PAH1 is capable of repressing phospholipid biosynthesis at the endoplasmic reticulum in *A. thaliana*. These results suggest that an increase in oil content might be explained by a tendency of PAH1 to channel the DAG supply in favour of the storage lipid production by repressing phospholipid synthesis. This limitation of phospholipids might indirectly have a negative influence on fibre components and seed hull. The QTL for EOF also co-localised with PAH1, showed same direction

of the additive effect with the overlapping major QTL for corrected oil content, indicating the later the EOF the higher the oil content. This might indicate some time dependency, suggesting a relation to a growth stage which generally might regulate the selective activity of PAH1 and in parallel influence the production of fibre components and seed hull. But the precise mechanism of the PAH1 repression is not yet known (Eastmond *et al.* 2011) and the proposed relation between oil content and fibre content and seed hull need to be further investigated.

Table 5.5: QTL detected for NDF, ADF, ADL, hemicellulose (HC) and cellulose (C) content (%) on the basis of defatted meal (suffix m) and seeds (suffix s) in the SGEDH population

QTL	Linkage Group	Position [cM]	Confidence Interval [cM]	A[a]	R^{2b}	V(A)/ V(P)[c]	V(I)/ V(P)[d]	V(G)/ V(P)[e]
E_ NDFm-1	A08	4.9	0.0-7.9	-0.39	9			
E_NDFm-2	A09	113.9	106.0-123.7	-0.23	1.6			
E_NDFm-3	C03	23.6	0.0-28.3	0.3	1.3	62.1	5.5	67.6
E_NDFm-4	C05	35.7	32.2-38.7	-0.55	45.9			
E_NDFm-5	C05	52.7	49.7-56.7	-0.42	45.7			
E_ADFm-1	A04	98.9	96.2-100.9	0.21	3.4			
E_ADFm-2	A07	120.5	93.2-120.5	0.16	0			
E_ADFm-3	A08	4.9	1.0-7.9	-0.2	5.9			
E_ADFm-4	A10	25.9	11.6-35.9	-0.26	1	81.7	-	81.7
E_ADFm-5	C02	71	66.9-75.0	-0.21	3.1			
E_ADFm-6	C05	33.7	31.2-35.7	-1.61	71.8			
E_ADFm-7	C08	34.8	32.1-40.8	0.2	4.7			
E_ADLm-1	A02	84.5	80.3-91.5	0.24	1.3			
E_ADLm-2	A04	63.8	58.4-68.8	0.18	0.6			
E_ADLm-3	A10	0	0.0-4.0	-0.24	0	84.6	0.3	84.9
E_ADLm-4	C02	71	66.9-75.0	-0.25	2.6			
E_ADLm-5	C05	34.7	32.2-36.7	-1.95	78.2			
E_ADLm-6	C08	34.8	32.1-41.8	0.14	3			
E_HCm-1	A04	98.9	94.2-101.9	-0.17	3.4			
E_HCm-2	C03	212.3	201.2-222.0	-0.18	3.8	69.2	0.6	69.8
E_HCm-3	C05	32.7	31.2-35.7	0.73	63			
E_Cm-1	A05	155.9	151.1-163.9	-0.07	9.7			
E_Cm-2	A07	0	0.0-5.7	-0.08	8.1			
E_Cm-3	C02	0	0.0-5.0	-0.1	2.4	63.5	10.5	74
E_Cm-4	C03	188.4	175.2-195.4	-0.04	1.6			
E_Cm-5	C05	33.7	31.2-37.7	0.31	41.1			
E_Cm-6	C09	86.7	80.5-105.6	-0.09	5.2			
E_Hull-1	A08	19.5	14.9-33.8	-0.34	13.1			
E_Hull-2	C05	33.7	31.2-36.7	-0.76	55.7	61.9	3.3	65.2
E_Hull-3	C05	51.7	48.7-55.7	-0.33	47.1			

a additive effect; positive additive effect indicating that the alleles increasing the trait were derived from SGDH14

b percentage of phenotypic variation explained by each QTL

c variance of additive effects/phenotypic variance – total contribution of additive effect QTL in %

d variance of epistatic effects/phenotypic variance in %

e variance of genetic main effects/phenotypic variance in %

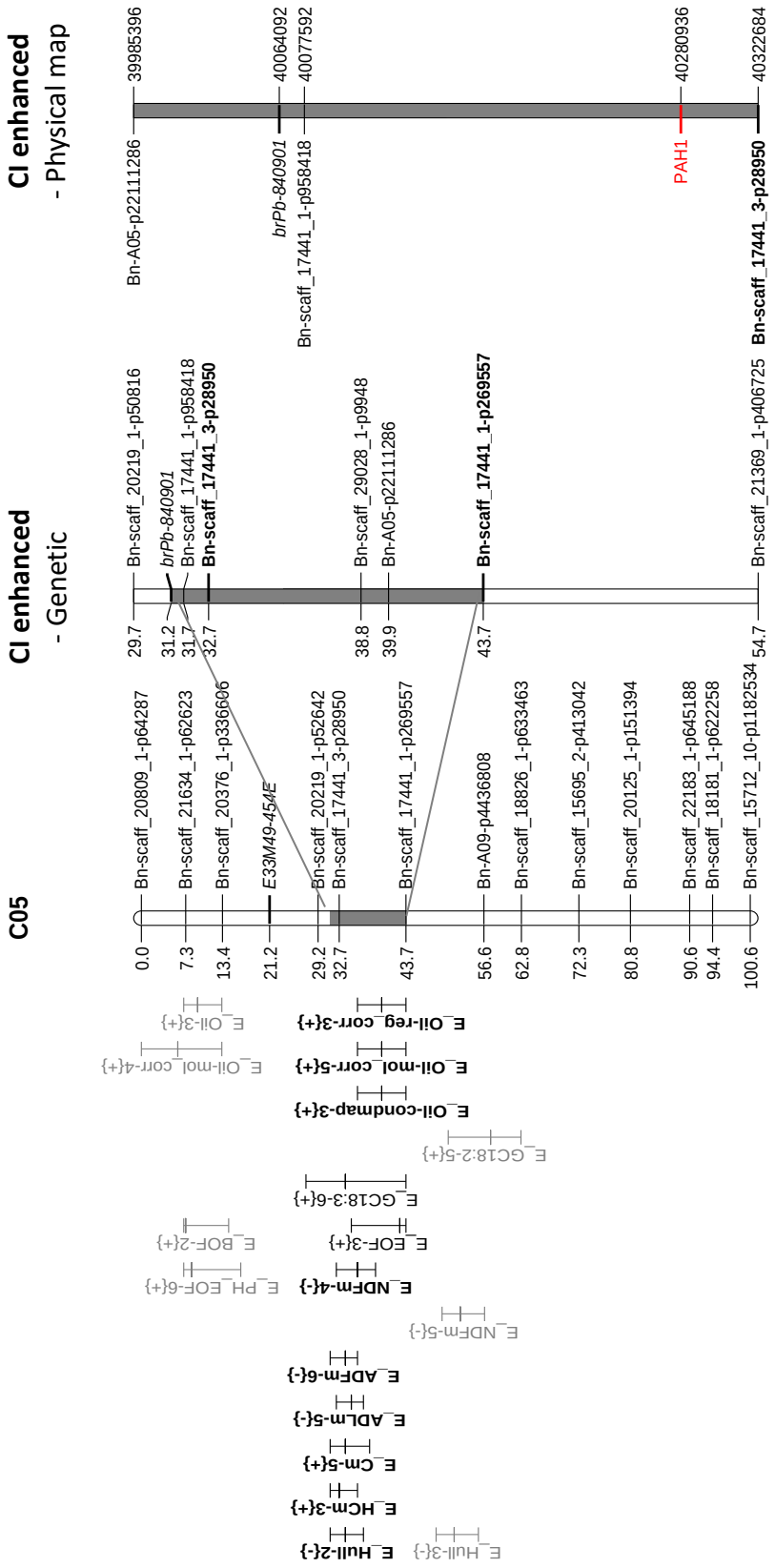

Figure 5.1: Genetic and physical map positions of markers within the QTL genomic region of corrected oil contents, fibre content and seed hull (31.2 – 43.7cM; grey bar) on C05. *Left:* Linkage group C05 of SGEDH population, framework map markers with position and mapped QTL. *Middle:* Enhanced QTL genomic region of corrected oil contents, fibre content and seed hull including all individual marker positions from genetic full map of SGEDH population. *Right:* The corresponding physical positions of markers mapped within the QTL genomic region of corrected oil contents, fibre content and seed hull, and the position of the putative candidate gene *phosphatidate phosphohydrolase 1* (*PAH1*; red), a Mg^{2+}-dependent phosphatidic acid phosphatase, in *Brassica napus* reference genome. Genetic distances are presented in cM, physical positions are presented in base pairs; bold markers are framework map markers, markers in normal fond are markers of individual marker positions in the SGEDH linkage map; CI = confidence interval.

5.4 Comparison of oil-QTL in different populations

In a previous study, Radoev (2007) was investigating a DH population derived from a cross of Express617 and the resynthesized line R53. Radoev (2007) identified one major QTL for glucosinolate content on linkage group N19 (C09) explaining 23.5% of the phenotypic variance. Co-located in the same marker interval of this QTL, a minor QTL for oil content was detected. To confirm these results and to compare QTL positions of the Express617 x R53 population (ExR) to the SGEDH population, QTL of the ExR population were recalculated using a set of 725 DArT markers mapped in a GABI-OIL project (data provided by Dr. W. Ecke, Universität Göttingen, Göttingen, Germany). QTL were calculated applying composite interval mapping of QTLNetwork. QTL results from recalculated data (Table 5.6) showed a reduced number of QTL for all traits. While original data showed between two to nine QTL per trait, the recalculation only identified one to four QTL. Besides, linkage groups of detected QTL in some cases differed from previous results. Since R53 has medium erucic acid content, one QTL for erucic acid is expected within the ExR population, representing one of the erucic acid genes. Radoev (2007) identified a major QTL for erucic acid on N08 (A08), while with recalculated data a major QTL was found on N13 (C03). Nevertheless, as previously reported a major QTL for glucosinolate content ExR_GSL-3 was detected on N19 (C09) explaining 62.4% of the phenotypic variance, but a co-located QTL for oil content was not detected anymore. Physical mapping for the ExR_GSL-3 was conducted using the closest marker to the QTL peak (34.3cM), brPb660902 at 31.6cM. The physical position of the marker was identified at 1958690bp on *B. napus* Darmor-*bzh* reference genome, and a region from 1.9 to 2.5Mbp was scanned for glucosinolate candidate gene matches, resulting in no suitable matches. In comparison, within the SGEDH population the major QTL GSL-5 on C09 which was detected stable in Europe and in China, was found at position 116.7cM (confidence interval 113.7 - 116.7cM). As closest marker to the QTL peak the SNP Bn-A09-p2730673 of position 117.5cM was identified. The physical position of this marker was found at 2894200bp. Due to a striking marker disorder comparing genetic and physical marker positions on C09 a region from 2.5 to 3.1Mbp around Bn-A09-p2730673 was scanned for glucosinolate candidate gene matches. This screen identified 115 *A. thaliana* protein matches including four matches involved in the regulation of the glucosinolate biosynthetic process. 32.7kbp apart from Bn-A09-p2730673, AT1G18570.1 the myb domain protein 51 (MYB51; 2926900-2927542bp) and AT5G60890.1 the myb domain protein 34 (MYB34; 2926900 - 2927977bp) were found, which overlapped the *B. napus* gene prediction BnaC09g05060D alias GSBRNA2T00146117001. About 205kbp apart from Bn-A09-p2730673 in the region from 3099235-3100634bp, AT5G07700.1 the myb domain protein 76 (MYB76) and AT5G61420.2 the myb domain protein 28 (MYB28)

were identified, overlapping gene predictions BnaC09g05290D alias GSBRNA2T00146147001 and BnaC09g05300D alias GSBRNA2T00146148001, respectively.

Most of the increasing alleles for oil content in the SGEDH population are contributed by SGDH14 which was derived from a cross between the German cultivar Sollux and the Chinese cultivar Gaoyou both showing high oil contents, but also high erucic acid and glucosinolate contents (Zhao *et al.* 2005). Five out of seven QTL for oil content in EU trials and five out of six QTL in Chinese trials identified SGDH14 to contribute the increasing alleles for oil content. In the Sollux x Gaoyou DH population Zhao *et al.* (2005) previously detected eight oil-QTL on A01, A07, A09, C01, C02, C04, C08 and C09. Comparison of these oil-QTL to those of the SGEDH population showed the detection of oil-QTL on A07 and C04 (cf. section 5.2) in both populations. QTL positions on these linkage groups were supposed to be the same, although no common marker or sequence information was available to prove this assumption. Since no more oil-QTL were detected on common linkage groups, it was suggested that alleles of Express617 and SGDH14 showed equal effects at the other QTL positions previously detected in the Sollux x Gaoyou DH population, thus detecting no significant differences at these positions anymore in the SGEDH population. But Zhao *et al.* (2006) not only investigated the genetic basis of oil content, but also evaluated the genetic interrelationship between oil content and phenological traits, specifically begin of flowering and flowering period, by applying conditional mapping. Results of the evaluation of a possible genetic relation between oil content and phenological traits indicated that although begin of flowering and flowering period still showed significant genetic correlations to oil content, only three of the eight oil-QTL of the Sollux x Gaoyou DH population failed to show significant effects after conditioning on phenological traits, but these QTL showed the smallest effects. Therefore, it was assumed, that most of the variation in oil content occurred independent from the variation in phenological traits (Zhao *et al.* 2006). Nevertheless, in the SGEDH population significant positive correlations between oil content and begin of flowering (r_s = 0.2**), end of flowering (r_s = 0.3**), as well as plant height at end of flowering (r_s = 0.5** EU/ r_s = 0.4** China) were observed. Furthermore, in EU experiments the oil-QTL E_Oil-3 on C05, which explained 13.8% of the phenotypic variance for oil content, was found co-located with QTL for phenological traits with same sign for the additive effects, however indicating a genetic interrelation of oil content and phenological traits.

Table 5.6: QTL detected for contents of seed oil (%), protein (%), glucosinolates (GSL in µmol/g) and erucic acid (%), and for thousand kernel weight (TKW in g) and plant height at end of flowering (PH_EOF) in the Express617 x R53 population (ExR)

QTL	Linkage Group	Position [cM]	Confidence Interval [cM]	A[a]	R^{2b}	V(A)/V(P)[c]	V(I)/V(P)[d]	V(G)/V(P)[e]
ExR_Oil-1	C02	90.3	88.4-94.5	0.48	9.9	36.4	-	36.4
ExR_Oil-2	C03	4.0	0.0-10.0	-0.85	18.1			
ExR_Oil-3	C03	124.4	109.4-138.3	0.51	7.4			
ExR_Protein-1	A02	50.0	38.6-67.6	0.30	9.1	9.1	-	9.1
ExR_GSL-1	A02	48.0	36.6-58.6	2.43	5.4	69.6	6.3	75.9
ExR_GSL-2	C02	88.8	88.6-90.3	-2.79	8.4			
ExR_GSL-3	C09	34.3	30.3-38.3	-12.00	62.4			
ExR_NIRS22:1-1	C03	4.0	0.0-7.0	-8.63	67.8	67.8	-	67.8
ExR_TKW-1	A05	32.0	28.7-55.1	0.07	5.7	33.0	-	33.0
ExR_TKW-2	A07	89.0	79.3-96.3	-0.10	8.5			
ExR_TKW-3	C01	89.4	87.2-89.5	0.10	9.5			
ExR_TKW-4	C02	81.0	79.7-88.4	-0.10	10.7			
ExR_PH_EOF-1	C03	137.9	137.9-137.9	3.06	12.2	19.9	-	19.9
ExR_PH_EOF-2	C05	26.0	16.0-33.4	2.44	7.7			

a additive effect; positive additive effect indicating that the alleles increasing the trait were derived from SGDH14

b percentage of phenotypic variation explained by each QTL

c variance of additive effects/phenotypic variance – total contribution of additive effect QTL in %

d variance of epistatic effects/phenotypic variance in %

e variance of genetic main effects/phenotypic variance in %

5.5 Future perspectives of breeding for increased oil content

Classical breeding efforts of the past 50 years facilitated an impressive steady improvement of increasing oil content in *B. napus*. And through its unique variety of oil qualities, especially the launch of double low quality, oilseed rape became one of the world's leading oil crops.

Nevertheless, today classical approaches to further increase the already high oil content in oilseed rape suffers from the relatively narrow genetic basis of the comparably young crop, which is leading to the search for new genetic resources. One attempt is to use foreign germplasm to broaden the genetic basis, as presented in this study. While another attempt focuses on the utilisation of the wide variation of the *B. napus* progenitors *B. rapa* and *B. oleracea* by investigating exotic and resynthesized germplasm (Girke *et al.* 2011, Jesske *et al.* 2011, Weis 2014). And also induced mutagenesis was already successfully used to create new variability (Xu *et al.* 2012). Promising new resources to further increase oil content in oilseed rape, like the quantitative locus on C05 identified in this study (E-Oil_reg_corr-3), can subsequently be transferred into elite material (cf. section 3.5.7 and section 4.5.2.1). However, conventional breeding for increased seed oil content in *B. napus* necessarily involves generation and detailed phenotyping of large populations in which only few individuals can be expected to carry all positive allelic combinations for the large number of loci contributing to all desired traits (Becker 2011, Snowdon and Iniguez Luy 2012). This makes breeding for high oil content time-consuming and labour intensive due to the quantitative nature of oil content, and the complexity of the allopolyploid structure of oilseed rape.

Enormous progress in new molecular technologies during the last decades developed new prospects to substantially improve breeding processes in oilseed rape. High-throughput technologies are available which provide large numbers of sequence informative markers, thus improving QTL mapping. Substantial improvement of sequencing technologies enabled the decoding of the *B. napus* genome (Chalhoub *et al.* 2014), a mile stone for *B. napus* breeding. Using both high-throughput marker technologies and the *B. napus* reference genome information in this study proved the potential of these new prospects. Furthermore, new tools for genome editing are promising to allow fast and targeted genome modifications by the use of engineered nucleases (Puchta and Fauser 2013) or targeting induced local lesions in genomes (TILLING) (McCallum *et al.* 2000). But these technologies still need to be established in *B. napus*, and it is not yet clear if the organisms produced by these methods will be considered genetically modified or not.

Breeding for high oil content in oilseed rape additionally might benefit from experiences from animal breeding which demonstrated the possibility to replace expensive and time-consuming phenotyping in breeding populations through implementation of statistical models to calculate genomic estimated breeding values (GEBVs). The GEBV can directly be used to predict the expected performance of a non-phenotyped individual (Meuwissen *et al.* 2001). Besides, continuously decreasing costs for genotyping and sequencing, genomic selection, which is also playing an important role in animal breeding (Hayes *et al.* 2009, Bagnato and Rosati 2012), will most likely as well gain importance in plant breeding (Jannink *et al.* 2010, Snowdon and Iniguez Luy 2012).

Although traditional and new breeding approaches will facilitate the further increase of seed oil content in *B. napus*, still time and effort will be needed to reveal and understand the complex regulation of oil biosynthesis.

6 Summary

Oilseed rape (*Brassica napus* L.) is one of the world's most important oil crops, and due to a growing demand of vegetable oil for nutritional as well as industrial purposes increasing seed oil content is a major aim for oilseed rape breeding.

Compared to other field crops oilseed rape is a rather young species and therefore its genetic variation is limited. However, breeding efforts of the last decades increased seed oil content substantially in European breeding material. But the intensive selection process further decreased genetic diversity, thus the need for a new source of genetic variation rose. As a candidate of high potential to lead to a rapid breeding success, Chinese breeding material, which independently underwent an intensive selection for high yield and oil content during the last decades, was chosen in a previous study. Within this, a quantitative genetic analysis was conducted on a DH population derived by a cross between the Chinese cultivar Gaoyou and the European cultivar Sollux, both with high oil content, but also high erucic acid and glucosinolate content. Thereby, the DH line 14 (SGDH14) was identified as best performing line under European conditions with highest oil contents and a combination of all favourable QTL alleles for oil content from both parental cultivars. Thereupon SGDH14 was crossed to Express617, an inbred line of Express, a European high oil cultivar of canola quality, since erucic acid and glucosinolates are undesirable traits in modern breeding material. And a F1 derived DH population (SGEDH) consisting of 212 genotypes was developed segregating for erucic acid and glucosinolate content.

In the present study, the SGEDH population was analysed to find out more about the genetic variation and inheritance of seed oil content, the underlying fatty acid composition and other seed quality traits, and to identify new QTL responsible to further increase oil content. Thus, field experiments of the SGEDH population were conducted in the two mega-environments, Europe (North Germany and South Sweden; EU trials) in three consecutive growing seasons 2009/10, 2010/11 and 2011/12, and East China (Hangzhou; Chinese trials) in two consecutive growing seasons 2010/11 and 2011/12. And a genetic map was constructed including 15380 SNP, 314 DArT and 116 AFLP markers, organized in 19 linkage groups and covering 2651cM. This map comprised 1693 individual marker positions. QTL mapping was conducted using a framework map consisting of a subset of 379 markers selected form the full map, and applying the composite interval mapping (CIM) approach of QTLNetwork software version 2.1. Mega-environments were investigated separately, and results subsequently compared.

Analysis of variances of the SGEDH population revealed highly significant genotypic effects for all traits in both environments, except for flowering period in the Chinese trials. The phenotypic variation was moderate to high depending on the trait considered. Seed oil content in the SGEDH population ranged from42.5 to 50.9% in the EU trials, and from 39.4 to 49.8% in the Chinese trials, showing a slightly broader range for seed oil in the Chinese trials, but in total higher oil contents in the EU trials. Heritability for oil content was high as well for the EU trials with 0.96 as for the Chinese trials with 0.94. Heritabilities for all other traits investigated in the EU tails were high ranging from 0.76 to 1.00. High heritabilities ranging from 0.70 to 0.99 were also found for most other traits in the Chinese tails, except for end of flowering (0.55) and flowering period (0.1). High significant positive correlations (P = 0.01) were consistently observed in both trials between oil content and erucic acid content, protein content in defatted meal and plant height.

Since a strong association between oil content and erucic acid was reported previously and confirmed by a high positive correlation between these two traits in this study, corrections of oil content were conducted to eliminate the effect of erucic acid on oil content. This enabled a comparison of the oil contents of genotypes with varying erucic acid contents, and identified SGEDH175 and 13, originally of medium erucic acid content, as the genotypes with highest oil contents in the EU trials, and SGEDH210 and 145 in Chinese trials. Corrected oil contents were subsequently used to identify additional QTL for oil content independent of erucic acid content. In the group of erucic acid free genotypes SGEDH172 was identified with highest oil content in both mega-environments.

QTL mapping applying the CIM method identified four QTL for oil content in the EU trials and six in the Chinese trials. Individual QTL explained between 10 and 50.5% of the phenotypic variance in the EU trials and between 3.3 and 46.5% in the Chinese trials. Four additional oil-QTL were found for corrected oil contents in the EU trials. Comparison of all QTL results for oil content from both mega-environments, including QTL for corrected oil contents, revealed that three QTL were constantly detected. These three environmentally stable QTL were located in overlapping genetic regions on linkage groups A08, C03 and C05. Furthermore, four environmental-specific QTL for oil content were identified in EU trials, located on A10, C03, C04 and C05, while three individual oil-QTL were found in Chinese trials on A06, A07 and A10. For other traits, between two and eight QTL were identified in the EU trials, and between two to five QTL were identified in the Chinese trials. Individual QTL explained between 0.6 and 64.1% of the phenotypic variance in the EU trials and 0.0 and 63.8% in the Chinese trials. Comparison of QTL results

revealed that between one and three QTL were repeatedly identified for other traits across the two trials.

BLAST search of SNP and DArT marker sequences to the *B. napus* Darmor-*bzh* reference genome enabled the identification of the physical position of 1289 SNP and 94 DArT markers from the set of 1693 individual full map marker positions. Alignment of genetic and physical map positions of markers showed a good collinearity for all linkage groups, except for linkage group C09. Physical positions of putative candidate genes involved in storage oil biosynthesis were compared to the physical positions of markers within QTL confidence intervals for seed oil content. This identified two genes co-located with QTL for oil content in the EU trials and two genes co-located in the Chinese trials. *FAD3*, encoding the linoleic acid desaturase, was located within the confidence interval of the oil-QTL on C04 in EU trials, and *KAR*, encoding the ketoacyl-ACP reductase an enzyme of the fatty acid synthase complex, was located within the confidence interval of the oil-QTL on A06 in Chinese trials. In both trials *FAE1*, encoding a ketoacyl-CoA synthase involved in the elongation of oleic acid to erucic acid, was found co-located with the stable oil-QTL identified on A08. Of highest interest for further breeding the major oil-QTL for corrected oil content E_Oil-reg_corr-3 on C05 was identified in the present study.

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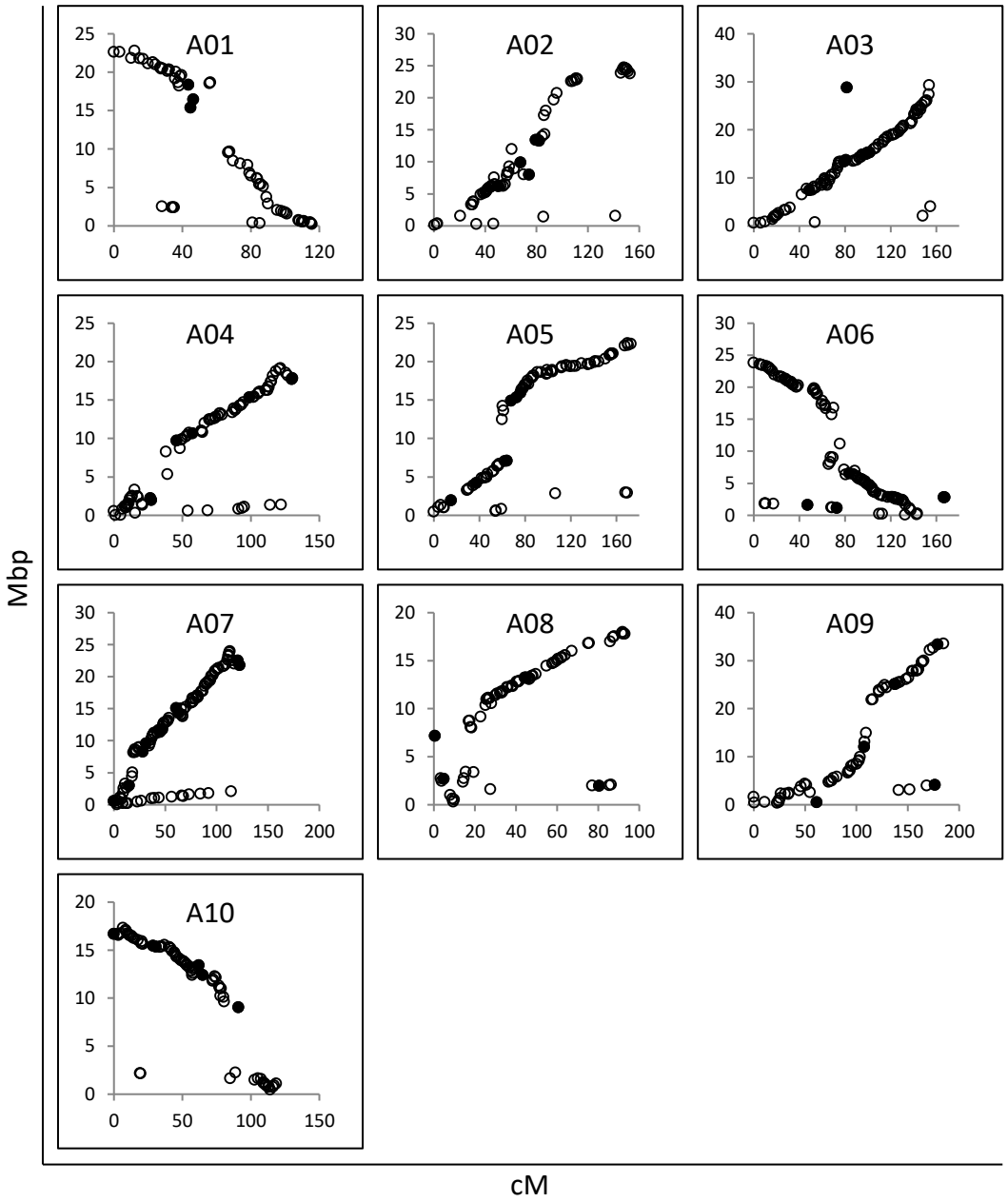
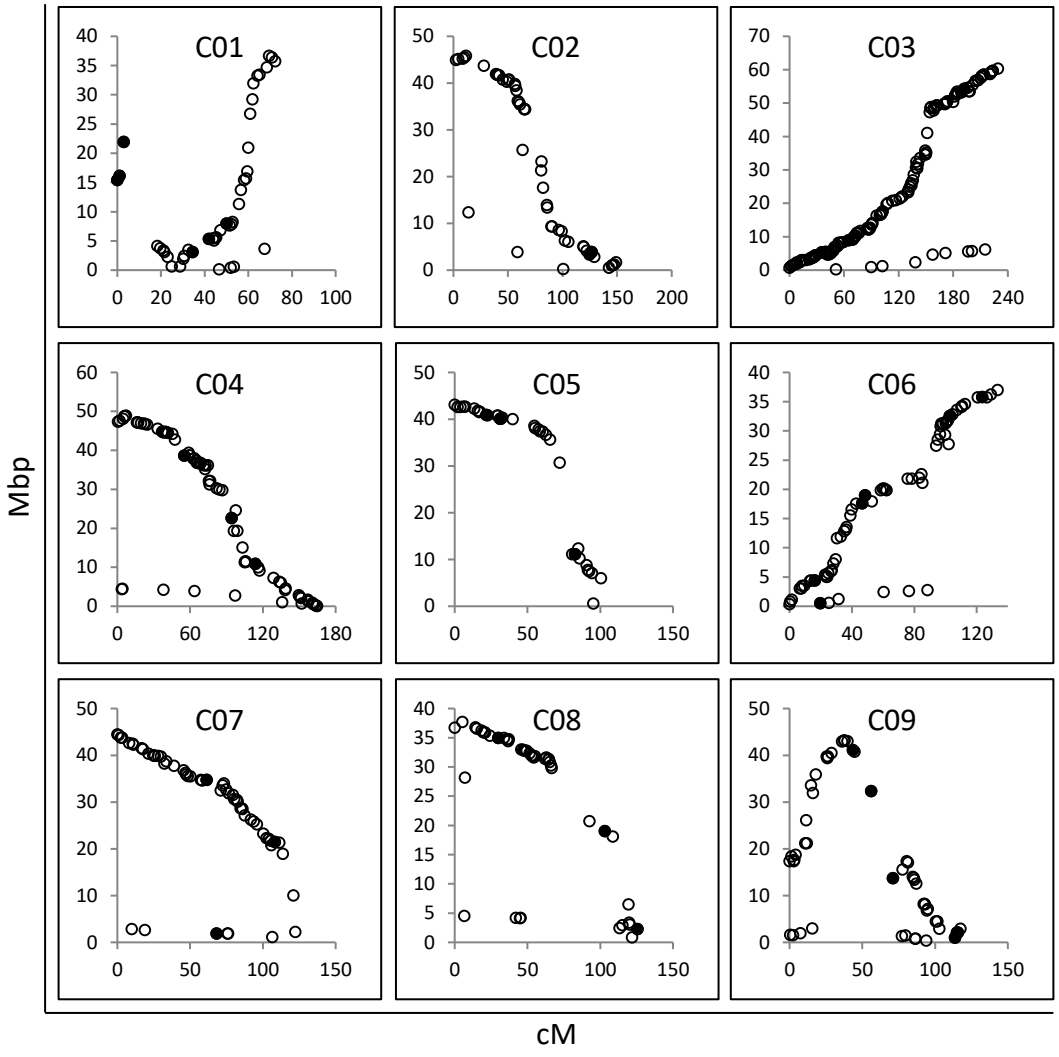
Appendix



Appendix 1: Alignment of SGEDH map with the physical map of *B. napus* Darmor-*bzh* genome assembly (A genome)



Appendix 2: Alignment of SGEDH map with the physical map of *B. napus* Darmor-*bzh* genome assembly (C genome)

Appendix 3: Critical *F*-values calculated by a 1000-permutation test in QTLNetwork software. Critical *F*-values were applied to determine QTL in the SGEDH population

	critical *F*-value	
Trait	EU	China
Oil	13.57	14.02
Oil-reg_corr	13.30	-
Oil-mol_corr	13.33	-
Oil-cond	13.32	-
Oil-reg_corr_NIRS	13.33	13.67
Oil-mol_corr_NIRS	13.57	-
Oil-cond_NIRS	13.29	13.66
Protein	13.21	14.23
Prot.idM	14.00	13.86
GSL	13.48	13.13
GSLidM	13.64	13.64
GC16:0	13.68	-
GC18:1	13.23	-
GC18:2	12.98	-
GC18:3	13.51	-
GC20:1	13.96	-
GC22:1	13.18	-
NIRS22:1	13.20	13.27
TKW	13.65	-
BOF	13.88	14.45
EOF	13.25	13.26
FT	13.56	13.25
PH_EOF	13.76	14.12

Appendix 4: List of candidate genes involved in fatty acid synthesis, modification or TAG synthesis. BLAST search was performed with *A. thaliana* gene sequence against *B. napus* Darmor-*bzh* reference genome (in BRAD); hits on *B. napus* pseudochoromosomes are not presented; Chr. = Chromosome

Gene annotation	Species	Gene ID	Sequence length	Chr.	E-value
ABI3	*A. thaliana*	AT3G24650	3694	3	
B3 domain-containing	*B. napus*	GSBRNA2T00154921001	692	A03	e-165
transcription factor ABI3	*B. napus*	GSBRNA2T00085286001	946	C03	0
BCCP2	*A. thaliana*	AT5G15530	2090	5	
Biotin carboxyl carrier protein 2	*B. napus*	GSBRNA2T00130052001	336	A03	6e-80
	B. napus	GSBRNA2T00135518001	337	A10	7e-52
	B. napus	GSBRNA2T00140460001	330	C03	9e-70
	B. napus	GSBRNA2T00035217001	118	C09	5e-28
ENR1	*A. thaliana*	AT2G05990	2925	2	
Enoyl-ACP reductase (MOD1)	*B. napus*	GSBRNA2T00155011001	145	A03	2e-46
	B. napus	GSBRNA2T00054284001	212	A07	9e-49
	B. napus	GSBRNA2T00002461001	310	C07	6e-50
FAD2	*A. thaliana*	AT3G12120	2766	3	
Fatty acid desaturase 2 (omega-6)/	*B. napus*	GSBRNA2T00000221001	910	A05	0
Oleate desaturase	*B. napus*	GSBRNA2T00111154001	914	C05	0
FAD3	*A. thaliana*	AT2G29980	3463	2	
Fatty acid desaturase 3 (omega-3)/	*B. napus*	GSBRNA2T00142192001	204	A03	5e-91
Linoleate desaturase	*B. napus*	GSBRNA2T00043495001	199	A04	8e-93
	B. napus	GSBRNA2T00048486001	311	A05	3e-80
	B. napus	GSBRNA2T00082771001	204	C03	7e-84
	B. napus	GSBRNA2T00105195001	206	C04	5e-97
FAE1	*A. thaliana*	AT4G34520	1638	4	
Fatty acid elongase 1/	*B. napus*	GSBRNA2T00113693001	447	A03	e-99
3-ketoacyl-CoA synthase (KAS)	*B. napus*	GSBRNA2T00126652001	1525	A08	0
	B. napus	GSBRNA2T00150105001	1525	C03	0
FATA	*A. thaliana*	AT3G25110	2216	3	
Acyl-ACP thioesterase	*B. napus*	GSBRNA2T00154948001	135	A03	2e-40
	B. napus	GSBRNA2T00128191001	277	A07	1e-96
GPAT4	*A. thaliana*	AT1G01610	2650	1	
sn-glycerol-3-phosphate	*B. napus*	GSBRNA2T00150548001	484	A10	e-170
acyltransferase 4 isoform C1	*B. napus*	GSBRNA2T00085343001	483	C05	e-169

Gene annotation	Species	Gene ID	Sequence length	Chr.	E-value
HAD	*A. thaliana*	AT5G10160	1567	5	
3-hydroxyacyl-	*B. napus*	GSBRNA2T00143533001	267	A02	e-102
acyl-carrier-protein dehydratase	*B. napus*	GSBRNA2T00129742001	256	A03	9e-94
	B. napus	GSBRNA2T00068993001	271	C02	e-105
	B. napus	GSBRNA2T00134621001	255	C03	8e-91
KAR	*A. thaliana*	AT1G62610	975	1	
ketoacyl-ACP reductase	*B. napus*	GSBRNA2T00042116001	836	A06	e-171
	B. napus	GSBRNA2T00124954001	641	C03	e-124
	B. napus	GSBRNA2T00151927001	277	C02	4e-42
KASI	*A. thaliana*	AT5G46290	2657	5	
3-ketoacyl-	*B. napus*	GSBRNA2T00079347001	440	A02	e-134
acyl carrier protein synthase I	*B. napus*	GSBRNA2T00054708001	444	A06	e-141
	B. napus	GSBRNA2T00058043001	439	A09	e-112
	B. napus	GSBRNA2T00146768001	447	C02	e-124
	B. napus	GSBRNA2T00128588001	461	C07	e-144
	B. napus	GSBRNA2T00115742001	439	C09	e-109
KASII	*A. thaliana*	AT1G74960	4282	1	
3-ketoacyl-	*B. napus*	GSBRNA2T00083137001	187	A02	1e-64
acyl carrier protein synthase II	*B. napus*	GSBRNA2T00099408001	246	A07	7e-69
	B. napus	GSBRNA2T00143662001	229	C06	2e-84
KASIII	*A. thaliana*	AT1G62640	2876	1	
3-ketoacyl-	*B. napus*	GSBRNA2T00068613001	239	A09	2e-71
acyl carrier protein synthase III	*B. napus*	GSBRNA2T00115818001	236	C09	5e-72
LEC1	*A. thaliana*	AT1G21970	2041	1	
Leafy cotyledon 1	*B. napus*	GSBRNA2T00112245001	311	A07	e-119
	B. napus	GSBRNA2T00102756001	353	A08	5e-25
	B. napus	GSBRNA2T00110047001	311	C07	e-117
	B. napus	GSBRNA2T00066656001	239	C08	3e-14
LPAAT1	*A. thaliana*	AT4G30580	2195	4	
lysophosphatidic acid acyltransferase	*B. napus*	GSBRNA2T00067440001	458	A03	e-130
1/1-acyl-sn-glycerol-3-phosphate	*B. napus*	GSBRNA2T00099722001	458	C07	e-130
acyltransferase 1 (chloroplastic)					
PAH1	*A. thaliana*	AT3G09560	4840	3	
phosphatidic acid		GSBRNA2T00073250001	639	C05	e-140
Phosphohydrolase 1		GSBRNA2T00080692001	639	A05	e-138
		GSBRNA2T00072843001	343	A01	1e-73

Gene annotation	Species	Gene ID	Sequence length	Chr.	E-value
PDAT1	*A. thaliana*	AT5G13640	4480	5	
phospholipid:diacylglycerol	*B. napus*	GSBRNA2T00143366001	525	A02	e-158
acyltransferase 1	*B. napus*	GSBRNA2T00151889001	531	C02	e-171
	B. napus	GSBRNA2T00031534001	508	C09	e-162
PDH (E1 alpha)	*A. thaliana*	At1g01090	1802	1	
Pyruvate Dehydrogenase	*B. napus*	GSBRNA2T00052412001	787	A09	0
(alpha subunit)	*B. napus*	GSBRNA2T00150508001	784	A10	0
	B. napus	GSBRNA2T00085388001	789	C05	0
WRI1	*A. thaliana*	AT3G54320	3944	3	
AP2/EREBP	*B. napus*	GSBRNA2T00098740001	186	A07	3e-49
Transcription Factors	*B. napus*	GSBRNA2T00129553001	194	A09	8e-47
	B. napus	GSBRNA2T00109003001	194	C08	8e-47

Appendix 5: *A. thaliana* protein matches in the genomic region ranging from 53910kbp to 54170kbp on chromosome C03 of the *Brassica napus* Darmor-*bzh* reference genome (Chalhoub *et al.* 2014, http://www.genoscope.cns.fr/brassicanapus/)

A. thaliana Protein match	Gene ID	Symbols/description	Position on *B. napus* genome	Length [bp]
GWSAtT00047376001	AT2G27550.1	ATC, centroradialis-like	(chrC03:53893876..53895582)	1707
GWSAtT00047343001	AT4G21750.1	ATML1, Homeobox-leucine zipper family protein/ lipid-binding START domain-containing protein	(chrC03:53907918..53911949)	4032
GWSAt2T00030191001	AT4G21800.1	QQT2, P-loop containing nucleoside triphosphate hydrolases superfamily protein	(chrC03:53915017..53916431)	1415
GWSAtT00047344001	AT4G04860.1	DER2.2, DERLIN-2.2	(chrC03:53916862..53918334)	1473
GWSAtT00047345001	AT4G21810.1	DER2.1, DERLIN-2.1	(chrC03:53916862..53918334)	1473
GWSAtT00047347001	AT4G21840.1	ATMSRB8\ MSRB8, methionine sulfoxide reductase B8	(chrC03:53938047..53938831)	785
GWSAtT00047348001	AT4G21850.1	ATMSRB9\ MSRB9, methionine sulfoxide reductase B9	(chrC03:53938062..53938831)	770
GWSAtT00047346001	AT4G21830.1	ATMSRB7\ MSRB7, methionine sulfoxide reductase B7	(chrC03:53938047..53938828)	782
GWSAt2T00030193001	AT1G48970.1	NagB/RpiA/CoA transferase-like superfamily protein	(chrC03:53978654..53979201)	548
GWSAt2T00030194001	AT2G44070.1	NagB/RpiA/CoA transferase-like superfamily protein	(chrC03:53979058..53979204)	147
GWSAtT00047349001	AT4G21865.1	unknown protein	(chrC03:53985423..53985930)	508
GWSAtT00047350001	AT4G21870.1	HSP20-like chaperones superfamily protein	(chrC03:53991611..53992102)	492
GWSAtT00047351001	AT4G21895.1	DNA binding	(chrC03:53993386..53994090)	705

continued on next page

Appendix 6: *continued from previous page*

A. thaliana Protein match	Gene ID	Symbols/description	Position on *B. napus* genome	Length [bp]
GWSAt2T00030197001	AT1G16560.1	Per1-like family protein	(chrC03:54019269..54020081)	813
GWSAtT00047352001	AT4G21903.2	MATE efflux family protein	(chrC03:54040692..54057787)	17096
GWSAtT00047353001	AT4G21910.4	MATE efflux family protein	(chrC03:54053483..54057787)	4305
GWSAt2T00030205001	AT3G44480.1	RPP1\ cog1, Disease resistance protein (TIR-NBS-LRR class) family	(chrC03:54090305..54093415)	3111
GWSAt2T00030203001	AT3G44630.3	Disease resistance protein (TIR-NBS-LRR class) family	(chrC03:54090317..54093415)	3099
GWSAt2T00030200001	AT5G38340.1	Disease resistance protein (TIR-NBS-LRR class) family	(chrC03:54090323..54093424)	3102
GWSAt2T00030207001	AT5G38350.1	Disease resistance protein (NBS-LRR class) family	(chrC03:54090323..54093403)	3081
GWSAt2T00030202001	AT3G44400.1	Disease resistance protein (TIR-NBS-LRR class) family	(chrC03:54090332..54093415)	3084
GWSAt2T00030208001	AT3G44670.1	Disease resistance protein (TIR-NBS-LRR class) family	-	-
GWSAt2T00030211001	AT3G25510.1	disease resistance protein (TIR-NBS-LRR class)	-	-
GWSAt2T00030213001	AT3G52900.1	Family of unknown function (DUF662)	(chrC03:54158392..54159677)	1286
GWSAt2T00030217001	AT4G22060.1	F-box family protein with a domain of unknown function (DUF295)	(chrC03:54163083..54164160)	1078
GWSAtT00047356001	AT4G22090.1	Pectin lyase-like superfamily protein	-	-
GWSAtT00047358001	AT4G22080.1	RHS14, root hair specific 14	-	-

Appendix 6: QTL detected for seed oil content (%) in the SGEDH population using MIM (WinQTLCart) in Europe

QTL	Linkage Group	Position [cM]	Confidence Interval [cM][a]	LOD	A[b]	R^{2c}	TR[d]
E_Oil-1	A08	25.1	22.6 - 27.1	40.14	1.04	38.4	83.9
E_Oil-2	A10	16.8	9.7 - 38.9	7.51	-0.36	3.4	
E_Oil-3	C03	193.4	190.4 - 195.4	21.12	0.71	21.1	
E_Oil-4	C03	37.7	34.7 - 41.7	18.03	0.63	16.4	
E_Oil-5	C05	50.2	38.9 - 55.2	4.43	-0.27	4.6	

a LOD-1.5 support interval which has a confidence level close to 95%
b additive effect; positive additive effect indicating that the alleles increasing the trait were derived from SGDH14
c percentage of phenotypic variance explained by each QTL
d percentage of phenotypic variance explained by all QTL

Appendix 7: QTL detected for regression corrected seed oil content (%) in the SGEDH population using MIM (WinQTLCart) in Europe

QTL	Linkage Group	Position [cM]	Confidence Interval [cM][a]	LOD	A[b]	R^{2c}	TR[d]
E_Oil-reg_corr-1	A10	19.8	9.7 - 26.9	4.06	-0.23	6.80	58.5
E_Oil-reg_corr-2	C04	32.3	29.8 - 39.3	6.90	0.29	8.80	
E_Oil-reg_corr-3	C05	6.0	0.0 - 16.4	3.86	0.29	14.10	
E_Oil-reg_corr-4	C05	40.7	34.7 - 49.7	7.34	0.41	23.10	
E_Oil-reg_corr-5	C07	38.9	20.7 - 65.3	4.54	-0.23	5.70	

a LOD-1.5 support interval which has a confidence level close to 95%
b additive effect; positive additive effect indicating that the alleles increasing the trait were derived from SGDH14
c percentage of phenotypic variance explained by each QTL
d percentage of phenotypic variance explained by all QTL

Appendix 8: QTL detected for protein content (%) in the SGEDH population using MIM (WinQTLCart) in Europe

QTL	Linkage Group	Position [cM]	Confidence Interval [cM][a]	LOD	A[b]	R^{2c}	TR[d]
E_Protein-1	A04	66.8	54.4 - 91.5	4.53	-0.14	6.8	62.4
E_Protein-2	A07	23.7	7.7 - 27.7	10.89	0.23	9.9	
E_Protein-3	A08	3.0	0.0 - 6.9	6.00	0.16	7.1	
E_Protein-4	A09	55.7	47.5 - 64.7	8.36	-0.21	7.4	
E_Protein-5	A10	18.8	14.8 - 24.9	9.38	0.21	10.9	
E_Protein-6	C04	51.4	26.8 - 62.6	8.04	-0.20	9.5	
E_Protein-7	C06	105.1	86.2 - 114.6	7.12	-0.18	6.0	
E_Protein-8	C07	63.3	50.2 - 76.3	4.85	0.15	4.7	

a LOD-1.5 support interval which has a confidence level close to 95%

b additive effect; positive additive effect indicating that the alleles increasing the trait were derived from SGDH14

c percentage of phenotypic variance explained by each QTL

d percentage of phenotypic variance explained by all QTL

Appendix 9: QTL detected for GC determined erucic acid content (%) in the SGEDH population using MIM (WinQTLCart) in Europe

QTL	Linkage Group	Position [cM]	Confidence Interval [cM][a]	LOD	A[b]	R^{2c}	TR[d]
E_GC22:1-1	A08	0.0	0.0 - 1.0	31.03	5.23	21.3	97.1
E_GC22:1-2	A08	25.1	25.1 - 27.1	47.37	7.73	33.9	
E_GC22:1-3	C03	201.2	199.2 - 202.2	97.12	10.22	41.5	

a LOD-1.5 support interval which has a confidence level close to 95%

b additive effect; positive additive effect indicating that the alleles increasing the trait were derived from SGDH14

c percentage of phenotypic variance explained by each QTL

d percentage of phenotypic variance explained by all QTL

Appendix 10: QTL detected for oil content (%) in the SGEDH population using MIM (WinQTLCart) in China

QTL	Linkage Group	Position [cM]	Confidence Interval [cM][a]	LOD	A[b]	R^{2c}	TR[d]
C_Oil9-1	A02	138.5	130.8 - 142.5	3.57	-0.29	2	80.5
C_Oil9-2	A06	72.2	51.1 - 78.8	4.37	0.30	4.6	
C_Oil9-3	A07	89.2	84.7 - 99.7	7.35	0.39	3.2	
C_Oil9-4	A08	25.1	21.6 - 27.1	31.6	1.01	33.8	
C_Oil9-5	A10	26.9	14.8 - 39.9	5.49	-0.35	3.8	
C_Oil9-6	C03	194.4	191.4 - 200.2	22.95	0.80	23.4	
C_Oil9-7	C05	40.7	35.7 - 46.7	8.72	0.46	9.6	

a LOD-1.5 support interval which has a confidence level close to 95%

b additive effect; positive additive effect indicating that the alleles increasing the trait were derived from SGDH14

c percentage of phenotypic variance explained by each QTL

d percentage of phenotypic variance explained by all QTL

Appendix 11: QTL detected for protein content (%) in the SGEDH population using MIM (WinQTLCart) in China

QTL	Linkage Group	Position [cM]	Confidence Interval [cM][a]	LOD	A[b]	R^{2c}	TR[d]
C_Protein-1	A05	133.6	90.4 - 161.9	3.29	0.18	6	38.5
C_Protein-2	A09	54.7	35.3 - 65.7	7.2	-0.28	10.9	
C_Protein-3	C03	124.0	116.9 - 143.3	4.44	-0.21	8.3	
C_Protein-4	C04	39.3	28.8 - 45.3	4.11	-0.20	6	
C_Protein-5	C08	113.3	95.7 - 117.3	5.2	-0.22	7.3	

a LOD-1.5 support interval which has a confidence level close to 95%

b additive effect; positive additive effect indicating that the alleles increasing the trait were derived from SGDH14

c percentage of phenotypic variance explained by each QTL

d percentage of phenotypic variance explained by all QTL

Appendix 12: QTL detected for seed oil, regression corrected oil and erucic acid contents (%) in the SGEDH population (QTLNetwork configuration modification: window size 5cM)

QTL	Linkage Group	Position [cM]	Confidence Interval [cM]	A[a]	R^{2b}	V(A)/V(P)[c]	V(I)/V(P)[d]	V(G)/V(P)[e]
E_Oil-1	A08	24.5	20.5-27.1	1.21	50.5			
E_Oil-2	C03	200.2	196.4-204.6	0.61	33.0	67.9	1.0	68.9
E_Oil-3	C07	49.5	38.6-55.2	-0.37	10.0			
E_Oil-reg_corr-1	A10	17.8	12.6-24.9	-0.26	11.2			
E_Oil-reg_corr-2	C04	47.3	42.3-47.4	0.24	4.1	51.8	3.9	55.7
E_Oil-reg_corr-3	C05	39.7	35.7-43.7	0.64	34.6			
E_Oil-reg_corr-4	C07	38.9	38.6-44.9	-0.26	7.2			
E_GC22:1-1	A08	25.1	23.5-30.8	11.82	64.1			
E_GC22:1-2	C03	201.2	199.2-203.2	8.83	46.9	87.2	0.6	87.8
E_GC22:1-3	C04	75.6	70.0-87.3	-1.90	8.0			

a additive effect; positive additive effect indicating that the alleles increasing the trait were derived from SGDH14
b percentage of phenotypic variance explained by each QTL
c variance of additive effects/phenotypic variance – total contribution of additive effect QTL in %
d variance of epistatic effects/phenotypic variance in %
e variance of genetic main effects/phenotypic variance in %

Appendix 13: Marker distribution, size, density and mean distance between non-co-segregation markers of each linkage group in the linkage map of the SGEDH population

Linkage group	No. of markers per linkage group	Size (cM)	Marker density (cM^{-1})	Mean distance between markers (cM)
A01	74	115.6	0.64	1.58
A02	89	152.8	0.58	1.74
A03	116	171.4	0.68	1.49
A04	99	130.3	0.76	1.33
A05	100	172.7	0.58	1.74
A06	124	167.9	0.74	1.37
A07	123	122.4	1.00	1.00
A08	80	92.9	0.86	1.18
A09	78	184.7	0.42	2.40
A10	96	122.3	0.79	1.29
C01	54	75.2	0.72	1.42
C02	72	149.1	0.48	2.10
C03	172	229.2	0.75	1.34
C04	101	164.7	0.61	1.65
C05	44	100.6	0.44	2.34
C06	82	133.5	0.61	1.65
C07	67	122.3	0.55	1.85
C08	56	125.6	0.45	2.28
C09	66	117.5	0.56	1.81
A genome	979.0	1432.9	0.68	1.46
C genome	714.0	1217.7	0.59	1.71
Whole genome	1693.0	2650.6	0.64	1.58

Appendix 14: Linkage map of SGEDH population showing all 1693 individual marker positions; DArT (*brPb*) and AFLP (*E*) markers are indicated in italics, framework map markers are highlighted in grey

Marker	Marker type	LG	Position (cM)	*B. napus* physical position	Chi² 1:1 (*p*-value)	Chi² 1:1 Significance
Bn-A01-p28380414	SNP	A01	0.0	22637901	0.7307	
Bn-scaff_18621_1-p183795	SNP	A01	3.4	22642039	0.1675	
Bn-A01-p28278192	SNP	A01	4.3		0.2695	
Bn-A01-p27907422	SNP	A01	9.1		0.6788	
Bn-A01-p27988223	SNP	A01	10.1	21842399	0.8340	
Bn-A01-p28497900	SNP	A01	12.1	22791037	0.7307	
Bn-A01-p28161207	SNP	A01	13.5		1.0000	
Bn-A01-p27954294	SNP	A01	15.0	21800046	0.9443	
Bn-A01-p27796877	SNP	A01	15.5		0.8332	
Bn-A01-p27722989	SNP	A01	16.9	21730776	0.4902	
Bn-A01-p27125649	SNP	A01	19.8	21118808	0.2673	
Bn-scaff_16691_1-p1350013	SNP	A01	22.7	21281386	0.7307	
Bn-scaff_21884_1-p720411	SNP	A01	24.1	21006073	0.6282	
Bn-Scaffold000164-p232469	SNP	A01	27.0	20622303	0.6788	
Bn-A01-p24697185	SNP	A01	27.5	20465114	0.5809	
Bn-A01-p24921355	SNP	A01	28.0	2519349	0.7815	
Bn-A01-p24707425	SNP	A01	28.5	20475451	0.6282	
Bn-A01-p24575577	SNP	A01	30.4		0.4467	
Bn-A01-p24369455	SNP	A01	31.3	20147030	0.6774	
Bn-A01-p24504495	SNP	A01	31.8	20308678	0.7825	
Bn-A01-p24526982	SNP	A01	32.3	20331886	0.5809	
Bn-A01-p23095567	SNP	A01	33.7	2392685	0.3018	
Bn-A01-p23087707	SNP	A01	34.7	2401294	0.4902	
Bn-A01-p23080525	SNP	A01	35.2	2410168	0.3708	
Bn-A01-p23321507	SNP	A01	35.6	19192316	0.4902	
Bn-A01-p23905191	SNP	A01	36.1	20068435	0.4881	
Bn-A01-p22051502	SNP	A01	37.6	18668714	0.3018	
Bn-A01-p21633202	SNP	A01	38.0	18220392	0.2419	
Bn-A01-p22948925	SNP	A01	38.5	19438818	0.3018	
Bn-A06-p9371495	SNP	A01	39.5	19610782	0.4489	
brPb-662426	*DArT*	*A01*	*43.6*	*18351862*	*0.1736*	
brPb-839314	*DArT*	*A01*	*44.7*	*15377075*	*0.2876*	
brPb-807746	*DArT*	*A01*	*46.4*	*16460419*	*0.5214*	
brPb-808818	*DArT*	*A01*	*50.7*		*0.3914*	
Bn-A01-p21931622	SNP	A01	55.9	18540246	0.2695	
Bn-A01-p22051510	SNP	A01	56.4	18668722	0.3340	
Bn-A01-p10640881	SNP	A01	66.7	9554078	0.8897	
Bn-A01-p10720611	SNP	A01	67.2	9633899	1.0000	
Bn-A01-p10740084	SNP	A01	67.7	9654037	0.8897	
Bn-A01-p10297732	SNP	A01	68.6		0.7307	
Bn-A01-p9996458	SNP	A01	69.1		0.6299	

Marker	Marker type	LG	Position (cM)	*B. napus* physical position	Chi² 1:1 (*p*-value)	Chi² 1:1 Significance
Bn-A01-p9853365	SNP	A01	69.6	8480102	0.5355	
Bn-A01-p9999485	SNP	A01	70.0		0.6299	
Bn-A01-p9476520	SNP	A01	73.8	8110180	0.1133	
Bn-A01-p9369257	SNP	A01	77.2		0.5355	
Bn-A01-p9254925	SNP	A01	78.1	7912126	0.5355	
Bn-A01-p7560785	SNP	A01	79.1	6901073	0.3708	
Bn-A01-p7137897	SNP	A01	80.0	6517900	0.2419	
Bn-A01-p7834871	SNP	A01	81.0	429124	0.3708	
Bn-A01-p6787930	SNP	A01	83.3	6219488	0.3018	
Bn-A01-p6687839	SNP	A01	83.8	6129214	0.3708	
Bn-A01-p5943392	SNP	A01	84.8	5433257	0.5355	
Bn-A01-p5730640	SNP	A01	85.2	352104	0.4489	
Bn-A01-p5944398	SNP	A01	85.7	5434227	0.5355	
Bn-A01-p5635471	SNP	A01	87.1	5145028	0.3018	
Bn-A01-p4084366	SNP	A01	89.0	3747936	0.1909	
Bn-A01-p3183888	SNP	A01	90.0	2894111	0.1133	
Bn-A01-p2538180	SNP	A01	95.3	2035020	0.9449	
Bn-A01-p2453758	SNP	A01	97.7	1939461	0.7295	
Bn-A01-p2340048	SNP	A01	98.2		0.8356	
Bn-A01-p2318972	SNP	A01	99.1	1792893	0.9449	
Bn-A01-p2245780	SNP	A01	100.1	1729866	0.7295	
Bn-A01-p2069488	SNP	A01	101.0	1561728	0.5336	
Bn-A01-p1990755	SNP	A01	101.5		0.6282	
Bn-A01-p1890102	SNP	A01	102.0		0.7295	
Bn-A01-p1149351	SNP	A01	107.8	752932	0.6266	
Bn-A01-p1062520	SNP	A01	108.3	647588	0.7282	
Bn-A01-p941792	SNP	A01	109.8	557682	0.6266	
Bn-A01-p974934	SNP	A01	110.2	591531	0.5316	
Bn-A01-p1012990	SNP	A01	110.7	631908	0.6266	
Bn-A01-p951341	SNP	A01	111.2	566890	0.5316	
Bn-A01-p820317	SNP	A01	114.6	448979	0.1099	
Bn-A01-p713562	SNP	A01	115.1	327077	0.1444	
Bn-A01-p644410	SNP	A01	115.6	235132	0.1866	
Bn-A02-p2643078	SNP	A02	0.0		0.0000	***
Bn-A02-p1232964	SNP	A02	0.5	148050	0.0000	***
Bn-A02-p2785185	SNP	A02	2.4	337265	0.0000	***
Bn-A02-p2799146	SNP	A02	2.8	349083	0.0000	***
E37M62-99E	*AFLP*	*A02*	*18.5*		*0.0000*	***
Bn-A02-p4183553	SNP	A02	20.6	1558970	0.0000	***
Bn-A02-p4259381	SNP	A02	21.6		0.0000	***
Bn-A02-p6039769	SNP	A02	27.8		0.0000	***
Bn-A02-p6099790	SNP	A02	29.2	3292685	0.0000	***
Bn-A02-p6147730	SNP	A02	30.1	3316092	0.0000	***

Marker	Marker type	LG	Position (cM)	*B. napus* physical position	Chi² 1:1 (*p*-value)	Chi² 1:1 Significance
Bn-A02-p6523609	SNP	A02	30.6	3735650	0.0000	***
Bn-A02-p6555729	SNP	A02	31.1	3775620	0.0000	***
brPb-840545	*DArT*	*A02*	*31.6*		*0.0000*	***
Bn-A02-p7350064	SNP	A02	32.1		0.0000	***
Bn-A02-p23491463	SNP	A02	33.0	257220	0.0000	***
Bn-A02-p7371108	SNP	A02	33.5		0.0000	***
Bn-A02-p7848143	SNP	A02	35.9		0.0000	***
Bn-A02-p7882027	SNP	A02	36.4	4867464	0.0000	***
Bn-A02-p8111245	SNP	A02	38.3	5094851	0.0000	***
Bn-A02-p8199891	SNP	A02	38.7	5191279	0.0000	***
Bn-A02-p8215285	SNP	A02	40.2	5196181	0.0000	***
Bn-A02-p8412911	SNP	A02	40.6	5364573	0.0000	***
Bn-A02-p8549373	SNP	A02	41.1	5516531	0.0000	***
Bn-A02-p8702373	SNP	A02	41.6	5669465	0.0000	***
Bn-A02-p8849037	SNP	A02	42.1	5806608	0.0000	***
Bn-A02-p9195997	SNP	A02	43.0	6002614	0.0000	***
Bn-A02-p9229113	SNP	A02	44.0	6032547	0.0000	***
Bn-A02-p9339514	SNP	A02	44.4	6144906	0.0000	***
Bn-A02-p9465668	SNP	A02	44.9	6272697	0.0000	***
Bn-A02-p9846691	SNP	A02	46.3	306194	0.0000	***
Bn-A02-p10700544	SNP	A02	46.8	7568848	0.0000	***
Bn-A02-p9684927	SNP	A02	47.3	6491491	0.0000	***
brPb-661469	*DArT*	*A02*	*49.8*	*6207621*	*0.0000*	***
Bn-A02-p9371363	SNP	A02	50.7	6192352	0.0000	***
Bn-A02-p9480465	SNP	A02	52.2	6286841	0.0000	***
Bn-A02-p9526960	SNP	A02	52.6		0.0000	***
Bn-A02-p9420837	SNP	A02	54.0	6240291	0.0000	***
Bn-A02-p9707309	SNP	A02	55.5	6512051	0.0000	***
Bn-A02-p10587170	SNP	A02	56.0		0.0000	***
Bn-A02-p11086855	SNP	A02	56.9	7845560	0.0000	***
Bn-A02-p11320789	SNP	A02	57.4	8327442	0.0000	***
Bn-A02-p11682974	SNP	A02	58.4	8405396	0.0000	***
Bn-A02-p12552323	SNP	A02	58.8	9278837	0.0000	***
Bn-A02-p15569271	SNP	A02	60.8	11995042	0.0000	***
Bn-A02-p12220436	SNP	A02	62.7	8930059	0.0000	***
brPb-839048	*DArT*	*A02*	*64.2*		*0.0000*	***
E33M49-213D	*AFLP*	*A02*	*64.7*		*0.0001*	***
brPb-670777	*DArT*	*A02*	*66.7*		*0.0000*	***
brPb-660621	*DArT*	*A02*	*67.6*	*9900773*	*0.0000*	***
Bn-A02-p13197154	SNP	A02	69.6		0.0000	***
Bn-A02-p11365167	SNP	A02	70.0	8025075	0.0000	***
brPb-657868	*DArT*	*A02*	*74.3*	*7959492*	*0.0000*	***
E44M62-172D	*AFLP*	*A02*	*75.3*		*0.0000*	***

Marker	Marker type	LG	Position (cM)	*B. napus* physical position	Chi² 1:1 (*p*-value)	Chi² 1:1 Significance
brPb-662889	*DArT*	*A02*	*79.5*	*13408492*	*0.0009*	***
E34M49-091D	*AFLP*	*A02*	*80.6*		*0.0000*	***
brPb-838519	*DArT*	*A02*	*81.1*	*13408492*	*0.0000*	***
E33M47-104D	*AFLP*	*A02*	*81.6*		*0.0000*	***
brPb-840119	*DArT*	*A02*	*82.1*	*13246920*	*0.0000*	***
Bn-scaff_23401_1-p317800	SNP	A02	84.5	13968963	0.0000	***
Bn-A02-p18917772	SNP	A02	85.4	1427623	0.0000	***
Bn-A02-p18101171	SNP	A02	85.9	17261389	0.0000	***
Bn-A02-p17156661	SNP	A02	86.4	14324675	0.0000	***
Bn-A02-p18989840	SNP	A02	87.3	17993227	0.0000	***
Bn-A02-p20363776	SNP	A02	93.0		0.0000	***
Bn-scaff_15712_6-p623654	SNP	A02	93.5	19660126	0.0000	***
Bn-A02-p22433753	SNP	A02	95.9	20736961	0.0000	***
Bn-A02-p24462762	SNP	A02	107.0	22582345	0.0000	***
Bn-scaff_17623_1-p648231	SNP	A02	108.0	22605615	0.0000	***
Bn-scaff_17623_1-p472440	SNP	A02	109.4	22716435	0.0000	***
Bn-A02-p24909771	SNP	A02	110.8	23001097	0.0000	***
Bn-A02-p24731379	SNP	A02	111.3	22820799	0.0000	***
Bn-A02-p24851624	SNP	A02	111.8	22942112	0.0000	***
brPb-657639	*DArT*	*A02*	*119.7*		*0.0000*	***
brPb-808824	*DArT*	*A02*	*128.6*		*0.0000*	***
Bn-A02-p25456985	SNP	A02	141.3	1543353	0.0000	***
Bn-A02-p25827208	SNP	A02	142.7		0.0000	***
Bn-A02-p26013503	SNP	A02	143.2		0.0000	***
Bn-A02-p26193572	SNP	A02	145.6	23874264	0.0000	***
Bn-A02-p27055844	SNP	A02	147.0	24377602	0.0000	***
Bn-A02-p27755843	SNP	A02	147.5		0.0000	***
Bn-A02-p27743910	SNP	A02	148.0	24724585	0.0000	***
Bn-A02-p27288011	SNP	A02	148.5	24605342	0.0000	***
Bn-A02-p27336483	SNP	A02	148.9	24633245	0.0000	***
Bn-A02-p27504018	SNP	A02	149.4		0.0000	***
Bn-A02-p27203061	SNP	A02	149.9	24526336	0.0000	***
Bn-A02-p27052742	SNP	A02	150.4	24374540	0.0000	***
Bn-A02-p26398002	SNP	A02	151.3	24112871	0.0000	***
Bn-A02-p26322916	SNP	A02	151.8		0.0000	***
Bn-A02-p26154951	SNP	A02	152.8	23778913	0.0000	***
Bn-A03-p766322	SNP	A03	0.0	634722	0.0195	*
brPb-661557	*DArT*	*A03*	*2.9*		*0.0375*	*
Bn-A03-p731558	SNP	A03	6.0	596896	0.0195	*
Bn-A03-p1046864	SNP	A03	9.8	879430	0.2164	
Bn-A03-p1899032	SNP	A03	16.0		0.0993	
Bn-A03-p1755651	SNP	A03	16.4	1354935	0.0741	
Bn-A03-p2271873	SNP	A03	17.4	1838499	0.0741	

Marker	Marker type	LG	Position (cM)	*B. napus* physical position	Chi² 1:1 (*p*-value)	Chi² 1:1 Significance
Bn-A03-p2412263	SNP	A03	18.3	1971847	0.0741	
Bn-A03-p2532699	SNP	A03	18.8	2086254	0.0993	
Bn-A03-p2666271	SNP	A03	19.8	2218691	0.0852	
Bn-A03-p2695311	SNP	A03	20.2	2247567	0.0631	
Bn-A03-p3099901	SNP	A03	21.7	2665882	0.0714	
E33M49-206E	*AFLP*	*A03*	*26.2*		*0.2900*	
Bn-A03-p3692048	SNP	A03	26.8	3249527	0.2673	
Bn-A03-p3799804	SNP	A03	28.2	3354699	0.1116	
Bn-A03-p4235658	SNP	A03	31.6	3773246	0.0838	
E32M47-252D	*AFLP*	*A03*	*33.1*		*0.2282*	
Bn-A03-p6849607	SNP	A03	41.2		0.0134	*
Bn-A03-p7218582	SNP	A03	42.1	6493920	0.0449	*
Bn-A03-p7434488	SNP	A03	43.1		0.1308	
Bn-scaff_19248_1-p357191	SNP	A03	46.4	7720293	0.0280	*
brPb-659931	*DArT*	*A03*	*48.8*	*7399131*	*0.0459*	*
Bn-A03-p8152842	SNP	A03	51.1	7448758	0.0280	*
Bn-A03-p8833016	SNP	A03	53.0	8137329	0.0134	*
Bn-A03-p9115020	SNP	A03	53.5	740194	0.0195	*
Bn-A03-p8637914	SNP	A03	54.0	7927482	0.0134	*
Bn-A03-p9567767	SNP	A03	58.7	8832086	0.0004	***
Bn-scaff_23954_1-p141044	SNP	A03	59.6	8480166	0.0010	***
Bn-A03-p10342088	SNP	A03	61.5	9513101	0.0004	***
Bn-A03-p10714724	SNP	A03	62.0	9868260	0.0002	***
Bn-A03-p10010569	SNP	A03	62.5	9204974	0.0004	***
Bn-A03-p9537155	SNP	A03	63.4	8807126	0.0004	***
Bn-A03-p9179896	SNP	A03	64.4	8462796	0.0010	***
Bn-A03-p10293071	SNP	A03	66.3	9464740	0.0004	***
Bn-A03-p11053758	SNP	A03	67.2	10172103	0.0004	***
Bn-A03-p11534030	SNP	A03	68.6	10666214	0.0016	**
Bn-A03-p11751694	SNP	A03	71.0	10886976	0.0001	***
Bn-A03-p12826949	SNP	A03	73.3	11964387	0.0000	***
Bn-A03-p13451851	SNP	A03	73.8	12570127	0.0000	***
Bn-A03-p13863828	SNP	A03	74.3	12967579	0.0000	***
Bn-A03-p14112910	SNP	A03	74.7	13297228	0.0000	***
Bn-scaff_18482_1-p677287	SNP	A03	76.2	13349463	0.0000	***
brPb-662736	*DArT*	*A03*	*79.1*	*13336975*	*0.0000*	***
brPb-809799	*DArT*	*A03*	*80.7*	*13663262*	*0.0001*	***
brPb-662347	*DArT*	*A03*	*81.7*	*28782432*	*0.0000*	***
E33M49-285D	*AFLP*	*A03*	*82.2*		*0.0000*	***
E34M49-274D	*AFLP*	*A03*	*83.7*		*0.0000*	***
Bn-A03-p14306440	SNP	A03	87.0	13490998	0.0000	***
Bn-A03-p14434665	SNP	A03	88.5	13603423	0.0000	***
Bn-A03-p14585799	SNP	A03	90.3	13757171	0.0001	***

Marker	Marker type	LG	Position (cM)	*B. napus* physical position	Chi² 1:1 (*p*-value)	Chi² 1:1 Significance
Bn-A03-p14962288	SNP	A03	90.8		0.0002	***
Bn-A03-p15103575	SNP	A03	92.2	14214995	0.0000	***
Bn-A03-p15141821	SNP	A03	92.7	14250283	0.0001	***
Bn-A03-p15351982	SNP	A03	94.1	14389227	0.0000	***
Bn-A03-p15749083	SNP	A03	95.1	14820911	0.0000	***
Bn-A03-p15768536	SNP	A03	96.0	14841143	0.0000	***
Bn-A03-p15811901	SNP	A03	97.4	14886171	0.0000	***
Bn-A03-p15871284	SNP	A03	97.9	14957225	0.0000	***
Bn-A03-p15954297	SNP	A03	98.4	15032281	0.0000	***
Bn-A03-p15852439	SNP	A03	98.8	14936605	0.0000	***
Bn-A03-p16103891	SNP	A03	99.8	15205946	0.0000	***
Bn-A03-p16158248	SNP	A03	100.2	15252905	0.0000	***
brPb-671327	*DArT*	*A03*	*101.7*	*15254628*	*0.0000*	***
E34M49-436E	*AFLP*	*A03*	*103.2*		*0.0000*	***
Bn-A03-p16811562	SNP	A03	105.2	15967232	0.0000	***
Bn-A03-p17019251	SNP	A03	106.6	16173130	0.0000	***
Bn-A03-p17139931	SNP	A03	107.0	16286197	0.0000	***
Bn-A03-p17985410	SNP	A03	109.4	16992492	0.0000	***
Bn-A03-p18405869	SNP	A03	113.2	17402430	0.0000	***
Bn-A03-p18455932	SNP	A03	113.7		0.0000	***
Bn-A03-p18855689	SNP	A03	114.1	17870241	0.0000	***
Bn-A03-p19065774	SNP	A03	114.6	18006758	0.0000	***
Bn-A03-p19441544	SNP	A03	117.0	18420261	0.0000	***
Bn-A03-p19573902	SNP	A03	117.4	18536283	0.0001	***
Bn-A03-p20000930	SNP	A03	120.7	18872657	0.0000	***
Bn-A03-p20151244	SNP	A03	121.2	19014872	0.0000	***
E33M49-239E	*AFLP*	*A03*	*122.2*		*0.0000*	***
Bn-A03-p20114910	SNP	A03	122.7	18984085	0.0000	***
Bn-A03-p20358050	SNP	A03	124.1	19224899	0.0000	***
Bn-A03-p20786060	SNP	A03	127.0	19597629	0.0000	***
Bn-A03-p21237671	SNP	A03	128.4	20003067	0.0000	***
Bn-A03-p21478889	SNP	A03	129.3	20250542	0.0000	***
Bn-A03-p21820847	SNP	A03	130.7	20604665	0.0000	***
Bn-A03-p22065637	SNP	A03	131.2	20881836	0.0000	***
brPb-661458	*DArT*	*A03*	*133.3*		*0.0003*	***
Bn-A03-p22574983	SNP	A03	137.3	21347046	0.0001	***
Bn-A03-p22590442	SNP	A03	137.7	21368434	0.0001	***
Bn-A03-p22882585	SNP	A03	138.2		0.0001	***
Bn-A03-p23023607	SNP	A03	138.7	21810591	0.0001	***
Bn-A03-p24046452	SNP	A03	140.1		0.0004	***
Bn-A03-p24753667	SNP	A03	140.6	23207312	0.0006	***
Bn-A03-p24971360	SNP	A03	141.5	23385684	0.0002	***
Bn-A03-p25801783	SNP	A03	142.5	24182990	0.0002	***

Marker	Marker type	LG	Position (cM)	*B. napus* physical position	Chi² 1:1 (*p*-value)	Chi² 1:1 Significance
Bn-A03-p25591818	SNP	A03	143.0	23956740	0.0004	***
Bn-A03-p24971161	SNP	A03	143.4	23385485	0.0002	***
Bn-A03-p25393494	SNP	A03	143.9		0.0004	***
Bn-A03-p25851789	SNP	A03	144.4	24222908	0.0002	***
Bn-A03-p26311193	SNP	A03	145.8	24704852	0.0004	***
Bn-A03-p25868537	SNP	A03	146.3	24234827	0.0006	***
Bn-A03-p26132481	SNP	A03	146.7		0.0004	***
Bn-A03-p26740723	SNP	A03	147.7	25162708	0.0010	***
Bn-A03-p27066000	SNP	A03	148.2	2079613	0.0006	***
Bn-A03-p27398352	SNP	A03	149.1		0.0016	**
Bn-A03-p27445819	SNP	A03	149.6	25729522	0.0010	***
Bn-A03-p27720045	SNP	A03	151.0	25945974	0.0006	***
Bn-A03-p27906760	SNP	A03	151.5	26134324	0.0010	***
Bn-A03-p28977058	SNP	A03	153.3	27387270	0.0010	***
Bn-A03-p31327366	SNP	A03	153.8	29263757	0.0016	**
Bn-A01-p22363809	SNP	A03	154.3		0.0048	**
Bn-scaff_16923_1-p34465	SNP	A03	154.8	4033619	0.0025	**
E32M49-083D	*AFLP*	*A03*	*157.4*		*0.0031*	**
E34M49-254D	*AFLP*	*A03*	*158.8*		*0.0258*	*
E34M49-412D	*AFLP*	*A03*	*162.7*		*0.0231*	*
E32M49-284D	*AFLP*	*A03*	*166.1*		*0.0184*	*
E39M62-138E	*AFLP*	*A03*	*168.2*		*0.0408*	*
E32M49-213E	*AFLP*	*A03*	*171.4*		*0.0034*	**
Bn-A04-p669182	SNP	A04	0.0	545915	0.0741	
Bn-A04-p1032336	SNP	A04	0.5		0.0993	
Bn-A04-p1043111	SNP	A04	0.9	36679	0.0741	
Bn-A04-p1048288	SNP	A04	4.7	30423	0.1308	
Bn-A04-p1062119	SNP	A04	6.1	808616	0.2718	
Bn-A04-p1417481	SNP	A04	8.0	1172772	0.1696	
Bn-A04-p1231047	SNP	A04	8.5	980412	0.1308	
Bn-A04-p1330321	SNP	A04	9.0	1094802	0.1696	
Bn-A04-p1623151	SNP	A04	9.4	1349461	0.2164	
Bn-A04-p1745832	SNP	A04	9.9	1471426	0.2718	
Bn-A04-p1815404	SNP	A04	10.9	1540065	0.1696	
Bn-A04-p2048587	SNP	A04	11.3	1932984	0.1308	
Bn-A04-p2498254	SNP	A04	11.8	2211909	0.0993	
Bn-A04-p2669971	SNP	A04	12.7	2374354	0.0545	
Bn-A04-p2841305	SNP	A04	13.2	2529785	0.0741	
Bn-A04-p3631563	SNP	A04	15.1	3299941	0.0394	*
Bn-A04-p3283282	SNP	A04	15.6	289605	0.0280	*
Bn-A04-p2780974	SNP	A04	17.0	2480917	0.0741	
Bn-A04-p2657695	SNP	A04	17.5	2361541	0.0545	
Bn-A04-p2104072	SNP	A04	18.9		0.1308	

Marker	Marker type	LG	Position (cM)	*B. napus* physical position	Chi² 1:1 (*p*-value)	Chi² 1:1 Significance
Bn-A04-p1774031	SNP	A04	20.3	1510202	0.2718	
Bn-A04-p1589006	SNP	A04	20.8	1341378	0.2164	
brPb-657687	*DArT*	*A04*	*24.1*		*0.0993*	
brPb-658988	*DArT*	*A04*	*26.6*	*2211621*	*0.1531*	
brPb-839945	*DArT*	*A04*	*27.2*	*1986738*	*0.2531*	
E45M49-436E	*AFLP*	*A04*	*36.6*		*0.3685*	
Bn-A04-p6968899	SNP	A04	38.1	8255363	0.2164	
Bn-A04-p5183306	SNP	A04	39.0	5349887	0.1308	
Bn-A04-p7616574	SNP	A04	40.9		0.3363	
brPb-842031	*DArT*	*A04*	*43.9*		*0.2626*	
brPb-660920	*DArT*	*A04*	*45.9*	*9698384*	*0.2718*	
Bn-A04-p8299391	SNP	A04	47.8		0.4098	
Bn-A04-p7373248	SNP	A04	48.2	8697322	0.3363	
Bn-A04-p8473768	SNP	A04	49.2	9809154	0.3363	
Bn-A04-p8587019	SNP	A04	49.7	9899845	0.2718	
Bn-A04-p8575908	SNP	A04	51.5		0.5827	
Bn-A04-p9119079	SNP	A04	52.5	10211790	0.7835	
Bn-A04-p9309213	SNP	A04	53.4	10427628	1.0000	
Bn-A04-p9484747	SNP	A04	53.9	586309	0.8907	
Bn-A04-p9623505	SNP	A04	54.8	10751182	0.8907	
brPb-841683	*DArT*	*A04*	*57.4*	*10637420*	*0.4401*	
Bn-A04-p9878192	SNP	A04	63.8	11017412	0.7835	
Bn-A04-p9808875	SNP	A04	64.3	10937173	0.6803	
Bn-A04-p9658559	SNP	A04	64.7	10803206	0.7835	
Bn-A04-p10841936	SNP	A04	66.2	12001712	0.6803	
Bn-A04-p11243983	SNP	A04	68.5	629314	1.0000	
Bn-A04-p11420466	SNP	A04	69.5	12471774	0.7835	
Bn-scaff_24979_1-p134759	SNP	A04	69.9	12405205	0.6803	
Bn-A04-p11449464	SNP	A04	70.9	12506260	0.4922	
Bn-A04-p11503303	SNP	A04	72.3	12559435	0.2718	
Bn-A04-p11595428	SNP	A04	73.7	12652738	0.4922	
Bn-A04-p11795779	SNP	A04	74.2	12796786	0.4098	
Bn-A04-p11923310	SNP	A04	74.7		0.4922	
Bn-A04-p11818064	SNP	A04	75.2		0.4098	
Bn-A04-p11957518	SNP	A04	76.1	12923125	0.4098	
Bn-A04-p12259499	SNP	A04	77.1	13248139	0.5827	
Bn-A04-p12283561	SNP	A04	77.5	13272510	0.4922	
Bn-A04-p12087644	SNP	A04	78.9	13065298	0.4098	
E32M47-129E	*AFLP*	*A04*	*81.4*		*0.2649*	
E39M62-369D	*AFLP*	*A04*	*83.5*		*0.7740*	
Bn-A04-p12670129	SNP	A04	86.6	13394741	0.5827	
Bn-A04-p13140266	SNP	A04	87.5	13904971	0.6788	
Bn-A04-p12959668	SNP	A04	88.5	13648348	0.5827	

Marker	Marker type	LG	Position (cM)	*B. napus* physical position	Chi² 1:1 (*p*-value)	Chi² 1:1 Significance
Bn-A04-p13122589	SNP	A04	89.4	13880694	0.5827	
Bn-A04-p13293545	SNP	A04	90.8	834704	0.8907	
Bn-A04-p13370433	SNP	A04	91.3		0.7835	
Bn-A04-p13583325	SNP	A04	91.8	14254994	0.8907	
Bn-A04-p13645293	SNP	A04	92.2	14288651	0.7835	
Bn-A04-p13738279	SNP	A04	93.2	14381023	0.5827	
Bn-A04-p13866861	SNP	A04	93.6	956098	0.4922	
Bn-A04-p14156506	SNP	A04	94.6	14687848	0.4922	
Bn-A04-p14332759	SNP	A04	95.1	1083056	0.5827	
brPb-662662	*DArT*	*A04*	*98.9*	*15331393*	*0.3708*	
Bn-A04-p14923168	SNP	A04	101.8	15369184	0.7835	
Bn-A04-p14986920	SNP	A04	102.3	15435680	0.6803	
Bn-A04-p15399118	SNP	A04	105.1	15791837	0.8907	
Bn-A04-p15530059	SNP	A04	105.6	15952175	0.8902	
Bn-A04-p15631388	SNP	A04	106.5	16069820	1.0000	
Bn-A04-p15939956	SNP	A04	108.9		0.8907	
Bn-A04-p16090875	SNP	A04	110.8	16283217	0.6803	
Bn-A04-p16104213	SNP	A04	112.2	16299441	0.4098	
Bn-A04-p16584084	SNP	A04	112.7	16747317	0.4922	
Bn-A04-p16514488	SNP	A04	113.1	16678079	0.4098	
Bn-A04-p16751677	SNP	A04	113.6		0.4922	
Bn-A04-p17170974	SNP	A04	114.1	1335605	0.4098	
Bn-scaff_20270_1-p138318	SNP	A04	114.6	17363975	0.3363	
Bn-A04-p17312041	SNP	A04	115.0	17436928	0.2718	
Bn-A04-p18065896	SNP	A04	116.0	18147068	0.4098	
Bn-A04-p18595858	SNP	A04	118.4	18723702	0.1308	
Bn-A01-p25429100	SNP	A04	120.7	19025100	0.0993	
Bn-scaff_27676_1-p33891	SNP	A04	121.7	19140278	0.1696	
Bn-A01-p25175675	SNP	A04	122.1	1379378	0.0993	
Bn-A04-p18530009	SNP	A04	124.5		0.1308	
Bn-A04-p18457550	SNP	A04	125.5	18546486	0.2164	
Bn-A04-p18390795	SNP	A04	126.4		0.3363	
Bn-A04-p18112711	SNP	A04	126.9	18198401	0.4098	
E45M49-111E	*AFLP*	*A04*	*128.8*		*0.1888*	
brPb-660337	*DArT*	*A04*	*129.8*	*17697052*	*0.4098*	
brPb-809834	*DArT*	*A04*	*130.3*	*17835073*	*0.5256*	
Bn-Scaffold000403-p12455	SNP	A05	0.0	461334	0.0000	***
Bn-A05-p917769	SNP	A05	3.8	1050679	0.0000	***
Bn-A05-p1237296	SNP	A05	6.1	1353802	0.0000	***
Bn-A05-p895522	SNP	A05	8.5	1028820	0.0000	***
Bn-A05-p895276	SNP	A05	9.0	1028587	0.0000	***
brPb-660860	*DArT*	*A05*	*10.9*		*0.0000*	***
brPb-842067	*DArT*	*A05*	*15.2*	*1912098*	*0.0000*	***

Marker	Marker type	LG	Position (cM)	*B. napus* physical position	Chi² 1:1 (*p*-value)	Chi² 1:1 Significance
Bn-A05-p3246311	SNP	A05	29.1	3275313	0.0000	***
Bn-A05-p3275289	SNP	A05	29.6	3298294	0.0000	***
Bn-A05-p3548736	SNP	A05	30.5	3465096	0.0000	***
Bn-A05-p3649985	SNP	A05	31.0		0.0000	***
Bn-A05-p4333409	SNP	A05	33.4		0.0000	***
Bn-A05-p3988958	SNP	A05	34.3	3854713	0.0000	***
E33M47-155E	*AFLP*	*A05*	*36.2*		*0.0000*	***
brPb-660062	*DArT*	*A05*	*37.3*	*4177128*	*0.0000*	***
Bn-A05-p4525705	SNP	A05	39.4		0.0000	***
Bn-scaff_23907_1-p261237	SNP	A05	41.7	4834543	0.0000	***
Bn-A05-p5038148	SNP	A05	43.6	4859009	0.0000	***
Bn-A05-p5100352	SNP	A05	44.6	4920049	0.0000	***
Bn-A05-p5133417	SNP	A05	46.0	4948094	0.0000	***
Bn-A05-p5251613	SNP	A05	46.5	5036043	0.0000	***
Bn-A05-p5627413	SNP	A05	46.9	5375973	0.0000	***
Bn-A05-p5926631	SNP	A05	51.2	5664379	0.0000	***
Bn-A05-p6055523	SNP	A05	52.6	5805343	0.0000	***
Bn-A05-p6154871	SNP	A05	54.0	545467	0.0000	***
Bn-A05-p6494313	SNP	A05	54.5	610568	0.0000	***
Bn-A05-p6984362	SNP	A05	55.4	6411896	0.0000	***
Bn-A05-p7031053	SNP	A05	56.4	6456989	0.0000	***
Bn-A05-p7246705	SNP	A05	57.4	6671048	0.0000	***
Bn-A05-p7741244	SNP	A05	58.8		0.0000	***
Bn-A05-p7778186	SNP	A05	59.2	833212	0.0000	***
Bn-A05-p12457173	SNP	A05	59.7	12459849	0.0000	***
Bn-A05-p17262653	SNP	A05	60.2	14220462	0.0000	***
Bn-A05-p13907424	SNP	A05	61.2	13641790	0.0000	***
Bn-A05-p7552614	SNP	A05	62.1	7060706	0.0000	***
brPb-662191	*DArT*	*A05*	*64.0*	*7093245*	*0.0000*	***
E39M62-254D	*AFLP*	*A05*	*65.1*		*0.0000*	***
brPb-662228	*DArT*	*A05*	*67.8*	*14893104*	*0.0000*	***
E35M47-199E	*AFLP*	*A05*	*69.3*		*0.0000*	***
E32M47-132D	*AFLP*	*A05*	*71.4*		*0.0000*	***
brPb-839815	*DArT*	*A05*	*72.4*	*15322345*	*0.0000*	***
brPb-670950	*DArT*	*A05*	*73.4*		*0.0000*	***
brPb-661887	*DArT*	*A05*	*75.9*	*15945685*	*0.0000*	***
Bn-A05-p18101769	SNP	A05	76.9	16381082	0.0000	***
Bn-A05-p18137410	SNP	A05	77.8	16423714	0.0000	***
Bn-A05-p18391581	SNP	A05	78.3	16659608	0.0000	***
Bn-A05-p18543933	SNP	A05	79.2		0.0000	***
Bn-A05-p18730286	SNP	A05	79.7	16983395	0.0000	***
Bn-A05-p18820057	SNP	A05	81.1	17078598	0.0000	***
Bn-A05-p19267100	SNP	A05	82.1	17501969	0.0000	***

Marker	Marker type	LG	Position (cM)	*B. napus* physical position	Chi² 1:1 (*p*-value)	Chi² 1:1 Significance
Bn-A05-p18807426	SNP	A05	83.0	17065968	0.0000	***
Bn-A05-p19622826	SNP	A05	85.0	17822505	0.0000	***
Bn-A05-p19728614	SNP	A05	86.4	17930801	0.0000	***
Bn-A05-p19919111	SNP	A05	86.9	18142185	0.0000	***
Bn-A05-p19973413	SNP	A05	87.4	18188757	0.0000	***
Bn-A05-p20422081	SNP	A05	90.7	18633417	0.0000	***
Bn-A05-p20020286	SNP	A05	92.1		0.0000	***
Bn-A05-p20425648	SNP	A05	93.5	18636346	0.0000	***
Bn-A05-p20747913	SNP	A05	95.9		0.0000	***
Bn-A05-p20178007	SNP	A05	98.7	18381573	0.0000	***
Bn-A05-p20712388	SNP	A05	99.2	18915544	0.0000	***
Bn-Scaffold002092-p583	SNP	A05	99.7	18521878	0.0000	***
E44M62-280D	*AFLP*	*A05*	*102.2*		*0.0000*	***
Bn-A05-p20563653	SNP	A05	102.7	18763414	0.0000	***
Bn-A05-p20697473	SNP	A05	103.2		0.0000	***
Bn-A05-p20714302	SNP	A05	103.7	18917433	0.0000	***
Bn-A05-p20485998	SNP	A05	104.1	18685808	0.0000	***
Bn-A05-p20825280	SNP	A05	106.5	2840496	0.0000	***
Bn-A05-p20972649	SNP	A05	110.8		0.0000	***
Bn-A05-p21057936	SNP	A05	111.7	19215707	0.0000	***
Bn-scaff_21369_1-p239290	SNP	A05	112.2	19371525	0.0000	***
brPb-660131	*DArT*	*A05*	*113.6*		*0.0000*	***
E33M47-150E	*AFLP*	*A05*	*114.6*		*0.0000*	***
Bn-A05-p21375513	SNP	A05	116.2	19544199	0.0000	***
Bn-scaff_21369_1-p108745	SNP	A05	116.6	19436624	0.0000	***
Bn-A05-p21221296	SNP	A05	119.5	19386916	0.0004	***
Bn-A05-p21240761	SNP	A05	122.3	19399758	0.0000	***
Bn-A05-p21267598	SNP	A05	124.2	19427934	0.0002	***
Bn-A05-p21375514	SNP	A05	126.6		0.0000	***
Bn-A05-p21579642	SNP	A05	129.5	19765824	0.0000	***
Bn-A05-p21480994	SNP	A05	134.7	19658445	0.0010	***
Bn-A05-p21484113	SNP	A05	135.2	19661558	0.0001	***
Bn-A05-p21538700	SNP	A05	137.1	19721966	0.0002	***
Bn-scaff_17441_1-p388434	SNP	A05	140.9	19936494	0.0073	**
Bn-A05-p21889289	SNP	A05	141.4	20036310	0.0109	*
Bn-A05-p21853739	SNP	A05	144.3	19995465	0.0006	***
Bn-A05-p22182628	SNP	A05	147.2		0.0145	*
Bn-A05-p22282814	SNP	A05	150.1	20349428	0.0019	**
Bn-A05-p22780670	SNP	A05	154.0	20854886	0.1099	
Bn-A05-p22797664	SNP	A05	155.0	20872117	0.3018	
Bn-A05-p22911906	SNP	A05	155.5	21027776	0.2374	
Bn-A05-p22943215	SNP	A05	155.9	21054060	0.0993	
Bn-A05-p22948751	SNP	A05	156.9	21059386	0.1655	

Marker	Marker type	LG	Position (cM)	*B. napus* physical position	Chi² 1:1 (*p*-value)	Chi² 1:1 Significance
E35M62-252E	*AFLP*	*A05*	*163.9*		*0.4445*	
Bn-A05-p23512396	SNP	A05	167.4	22090281	0.6266	
Bn-A02-p26921384	SNP	A05	167.9	2953115	0.5316	
Bn-A08-p16956285	SNP	A05	169.3	2911175	0.8348	
Bn-A02-p26854880	SNP	A05	169.8	22381796	0.7282	
Bn-A02-p26640222	SNP	A05	170.3	22133099	0.8897	
Bn-A02-p26710300	SNP	A05	172.7	22302346	0.9451	
Bn-A06-p25471463	SNP	A06	0.0	23837637	0.6803	
Bn-A06-p24695860	SNP	A06	5.2	23595271	0.4922	
Bn-A06-p24599972	SNP	A06	6.2		0.3363	
Bn-A06-p24557108	SNP	A06	6.6	23504740	0.4098	
Bn-A06-p24496051	SNP	A06	7.6	23440008	0.2718	
Bn-A06-p24340830	SNP	A06	9.5	1859635	0.4922	
Bn-scaff_15754_1-p496098	SNP	A06	10.0	1850507	0.3708	
Bn-A06-p24327317	SNP	A06	10.4	1845400	0.3018	
Bn-A06-p24297216	SNP	A06	10.9	23296226	0.2718	
Bn-A06-p24142937	SNP	A06	12.3	23133507	0.5355	
Bn-A06-p24045825	SNP	A06	12.8	23045817	0.4922	
Bn-A06-p24076443	SNP	A06	13.3		0.4098	
Bn-A06-p23983648	SNP	A06	13.7	22967491	0.4922	
Bn-A06-p23968879	SNP	A06	14.2		0.4922	
Bn-A06-p23795599	SNP	A06	14.7	22776542	0.6299	
Bn-A06-p23542209	SNP	A06	16.1	22518900	0.7307	
E32M47-241D	*AFLP*	*A06*	*16.6*		*0.6666*	
Bn-A06-p23341715	SNP	A06	17.1	1782506	0.6803	
Bn-A06-p22922325	SNP	A06	18.1	21972047	0.4922	
Bn-A06-p22647955	SNP	A06	21.9	21689171	0.0741	
Bn-A06-p22605217	SNP	A06	23.7	21643954	0.1308	
Bn-A06-p22555168	SNP	A06	24.2	21597560	0.0993	
Bn-A06-p22505664	SNP	A06	24.7	21528638	0.0741	
Bn-A06-p22271048	SNP	A06	27.0	21304697	0.0280	*
Bn-A06-p22268128	SNP	A06	28.0	21301737	0.0280	*
Bn-A06-p22074841	SNP	A06	28.5	21111173	0.0394	*
Bn-A06-p21926982	SNP	A06	29.9	20949452	0.0993	
Bn-A06-p21917612	SNP	A06	30.3	20939334	0.0741	
Bn-A06-p21884761	SNP	A06	30.8		0.0993	
Bn-A06-p21848747	SNP	A06	31.3	20865884	0.0741	
Bn-A06-p21825957	SNP	A06	32.7	20842565	0.0545	
Bn-scaff_18520_1-p486046	SNP	A06	33.2	20531364	0.0741	
Bn-A06-p21224575	SNP	A06	34.1	20468466	0.1308	
Bn-A06-p21318925	SNP	A06	34.6	20363781	0.1696	
Bn-A06-p18717323	SNP	A06	37.0	20070622	0.4922	
Bn-A06-p18706102	SNP	A06	37.9	20059090	0.6803	

Marker	Marker type	LG	Position (cM)	*B. napus* physical position	Chi² 1:1 (*p*-value)	Chi² 1:1 Significance
Bn-A06-p21415201	SNP	A06	38.8	20298214	0.4922	
E39M62-193E	*AFLP*	*A06*	*42.2*		*0.4860*	
brPb-661510	*DArT*	*A06*	*47.1*	*1604474*	*0.8907*	
Bn-A06-p18127830	SNP	A06	51.8	19529301	0.6803	
Bn-A06-p18455125	SNP	A06	52.8	19824222	0.6803	
Bn-A06-p18364834	SNP	A06	53.3	19735683	0.5827	
Bn-A06-p18131525	SNP	A06	53.7	19533061	0.6803	
Bn-A06-p17734402	SNP	A06	54.7	19172543	0.4922	
Bn-A06-p17566259	SNP	A06	55.1	18956568	0.5827	
Bn-A06-p17549603	SNP	A06	55.6	18945217	0.4922	
Bn-A06-p17208027	SNP	A06	56.1		0.4098	
Bn-A06-p15893014	SNP	A06	59.4	17351418	0.8907	
Bn-A06-p16362555	SNP	A06	59.9	17866136	0.7835	
Bn-A06-p15791244	SNP	A06	62.2	17298191	0.6803	
Bn-A06-p15632434	SNP	A06	62.7	17132332	0.7835	
Bn-A06-p15210596	SNP	A06	63.2	16683468	0.6803	
Bn-A06-p8434088	SNP	A06	65.5	7982753	0.4098	
Bn-A06-p8809314	SNP	A06	66.9	8303243	0.6803	
Bn-A06-p9562200	SNP	A06	67.4	9087418	0.5827	
Bn-A06-p13726921	SNP	A06	67.9	1199271	0.4922	
Bn-A06-p14121814	SNP	A06	68.4	15738257	0.5827	
Bn-A06-p13728321	SNP	A06	68.8	1200459	0.4922	
Bn-A06-p9517147	SNP	A06	69.3	9014008	0.5827	
Bn-A06-p15285770	SNP	A06	69.8	16759065	0.6803	
E35M62-143D	*AFLP*	*A06*	*71.2*		*0.7269*	
brPb-658956	*DArT*	*A06*	*72.8*	*1143152*	*0.3363*	
Bn-A06-p12356525	SNP	A06	75.3	11152462	0.4922	
Bn-A06-p7589883	SNP	A06	79.1	7107268	0.2164	
Bn-A06-p6920925	SNP	A06	80.5	6375006	0.4098	
brPb-808961	*DArT*	*A06*	*83.8*	*6528626*	*0.4922*	
Bn-A06-p6955437	SNP	A06	87.2	6410107	0.4098	
Bn-A06-p7437586	SNP	A06	88.6	6943260	0.2164	
Bn-A06-p6744737	SNP	A06	90.0	6131668	0.4098	
Bn-A06-p6636202	SNP	A06	90.5	6031166	0.4922	
Bn-A06-p6458266	SNP	A06	90.9	5897495	0.5827	
Bn-A06-p6341389	SNP	A06	91.4	5792303	0.4922	
Bn-A06-p6204652	SNP	A06	92.8	5639597	0.7307	
Bn-A06-p6198128	SNP	A06	93.8	5627992	1.0000	
Bn-A06-p6182593	SNP	A06	94.7	5603682	0.7835	
Bn-A06-p6091453	SNP	A06	95.2	5536979	0.8907	
Bn-A06-p5930501	SNP	A06	96.1	5364528	0.8907	
Bn-A06-p5878990	SNP	A06	97.6	5300722	0.7835	
Bn-A06-p5669960	SNP	A06	98.0	5119657	0.8907	

Marker	Marker type	LG	Position (cM)	*B. napus* physical position	Chi² 1:1 (*p*-value)	Chi² 1:1 Significance
Bn-A06-p5593050	SNP	A06	98.5	5044622	1.0000	
Bn-A06-p4901091	SNP	A06	99.9	4796382	0.8907	
Bn-A06-p4881785	SNP	A06	101.3	4755815	0.5827	
Bn-A06-p4597746	SNP	A06	102.8	4414849	0.8907	
Bn-A06-p4436168	SNP	A06	103.7	4266437	0.8907	
Bn-A06-p4179318	SNP	A06	104.2	3979080	0.7835	
Bn-A06-p3953661	SNP	A06	104.6	3743186	0.6803	
Bn-A06-p3867353	SNP	A06	105.6	3634902	0.4922	
Bn-A06-p3805018	SNP	A06	107.0	3565565	0.7835	
Bn-A06-p3587032	SNP	A06	107.5		0.6803	
Bn-A06-p3491241	SNP	A06	109.8	232344	1.0000	
Bn-A06-p3414128	SNP	A06	110.3	3231109	0.8907	
Bn-A06-p3361377	SNP	A06	111.2	3179145	0.6803	
Bn-A06-p3257163	SNP	A06	112.7	201651	0.4098	
Bn-A06-p3167411	SNP	A06	113.1	3042973	0.3363	
Bn-A06-p3093741	SNP	A06	115.5		0.2718	
Bn-A06-p2949562	SNP	A06	117.4	2847938	0.2718	
Bn-A06-p2940743	SNP	A06	120.7	2840356	0.0394	*
Bn-A06-p2938890	SNP	A06	121.6	2838485	0.0741	
Bn-A06-p2883231	SNP	A06	122.1	2781094	0.0993	
Bn-A06-p2870413	SNP	A06	123.0	2768252	0.1696	
Bn-A06-p2851834	SNP	A06	123.5	2753135	0.2164	
Bn-A06-p2617079	SNP	A06	124.5	2574090	0.1308	
Bn-A06-p2599150	SNP	A06	124.9	2555169	0.1696	
Bn-A06-p2756792	SNP	A06	125.4	2679399	0.1308	
Bn-A06-p2586825	SNP	A06	126.3	2547287	0.0741	
Bn-A06-p2437284	SNP	A06	127.3	2448753	0.0394	*
Bn-A06-p2300342	SNP	A06	129.2	2313720	0.0394	*
Bn-A06-p2412998	SNP	A06	129.6	2425890	0.0280	*
Bn-A06-p2341154	SNP	A06	130.1	2356210	0.0394	*
Bn-A06-p2292578	SNP	A06	130.6	2299465	0.0280	*
Bn-A06-p2274318	SNP	A06	131.1	2280545	0.0394	*
Bn-A06-p1777127	SNP	A06	132.5	91400	0.0134	*
Bn-A06-p1636449	SNP	A06	133.0	1690832	0.0195	*
Bn-A06-p1615726	SNP	A06	133.4		0.0280	*
Bn-A06-p1345642	SNP	A06	134.8		0.0741	
Bn-A06-p1071392	SNP	A06	136.3	1142318	0.1696	
Bn-A06-p1016417	SNP	A06	136.7	1005504	0.2164	
Bn-A06-p781674	SNP	A06	137.7	787768	0.2164	
Bn-A06-p163388	SNP	A06	142.9	132977	0.0993	
Bn-A06-p307436	SNP	A06	143.4	266475	0.0741	
brPb-658647	*DArT*	*A06*	*148.6*		*0.0304*	*
brPb-809008	*DArT*	*A06*	*166.4*	*2792764*	*0.0477*	*

Marker	Marker type	LG	Position (cM)	*B. napus* physical position	Chi² 1:1 (*p*-value)	Chi² 1:1 Significance
brPb-670236	*DArT*	*A06*	*167.4*	*2792764*	*0.0084*	**
E32M49-205D	*AFLP*	*A06*	*167.9*		*0.0101*	*
brPb-842247	*DArT*	*A07*	*0.0*	*561912*	*0.8907*	
Bn-Scaffold000996-p195	SNP	A07	2.4	586344	0.5827	
Bn-A07-p885219	SNP	A07	2.8	45191	0.4922	
Bn-A07-p178437	SNP	A07	3.3	279606	0.5827	
Bn-A07-p959523	SNP	A07	5.7	1110210	0.4922	
Bn-A07-p1135440	SNP	A07	7.1	963684	0.5827	
Bn-A07-p1210412	SNP	A07	7.6	874286	0.4922	
Bn-A07-p1437386	SNP	A07	8.5	201778	0.3363	
Bn-A07-p1800556	SNP	A07	9.0	1897346	0.2718	
Bn-A10-p11607526	SNP	A07	9.4	2569273	0.2164	
Bn-A10-p12331294	SNP	A07	9.9		0.1696	
Bn-A10-p12574115	SNP	A07	10.9	3247984	0.0993	
Bn-A10-p11918400	SNP	A07	11.8	232813	0.1696	
Bn-scaff_17084_1-p341281	SNP	A07	12.3	2643808	0.2419	
Bn-A07-p1443503	SNP	A07	13.2	208437	0.3363	
brPb-670852	*DArT*	*A07*	*14.7*	*2984918*	*0.1845*	
Bn-A10-p12491298	SNP	A07	15.7		0.0993	
Bn-A07-p5291077	SNP	A07	17.1		0.0394	*
Bn-A10-p3266570	SNP	A07	17.5	4388073	0.0545	
Bn-A08-p9536971	SNP	A07	18.0	5034412	0.0394	*
Bn-A07-p6244683	SNP	A07	19.0	8173788	0.0195	*
Bn-A07-p6297941	SNP	A07	19.9	8218562	0.0091	**
Bn-A07-p6735264	SNP	A07	20.4	8665683	0.0060	**
Bn-A07-p6283126	SNP	A07	20.8	8203354	0.0091	**
Bn-A07-p6523973	SNP	A07	21.8	8460249	0.0091	**
Bn-A07-p6460270	SNP	A07	22.7	464575	0.0091	**
Bn-A07-p6878338	SNP	A07	23.2		0.0025	**
Bn-A07-p6981435	SNP	A07	23.7	8884037	0.0016	**
Bn-scaff_16541_1-p263947	SNP	A07	24.6	8975277	0.0004	***
Bn-A07-p7200963	SNP	A07	25.6		0.0016	**
Bn-A07-p6976405	SNP	A07	26.5	605755	0.0039	**
brPb-660097	*DArT*	*A07*	*28.0*	*8247016*	*0.0038*	**
E34M49-146E	*AFLP*	*A07*	*29.4*		*0.0022*	**
brPb-663295	*DArT*	*A07*	*31.4*	*9571178*	*0.0001*	***
Bn-A07-p7584364	SNP	A07	34.3	9190911	0.0010	***
Bn-A07-p8128951	SNP	A07	35.2	9573861	0.0004	***
Bn-A07-p8696690	SNP	A07	36.2	10121431	0.0001	***
Bn-A07-p8852716	SNP	A07	36.7	975622	0.0001	***
Bn-A07-p9079474	SNP	A07	37.1	10541888	0.0001	***
Bn-A07-p9402974	SNP	A07	37.6	10780972	0.0002	***
Bn-A07-p9565657	SNP	A07	38.1	10901610	0.0001	***

Marker	Marker type	LG	Position (cM)	*B. napus* physical position	Chi² 1:1 (*p*-value)	Chi² 1:1 Significance
Bn-A07-p9921856	SNP	A07	39.0	11205379	0.0004	***
Bn-A07-p9632473	SNP	A07	39.5	1042490	0.0002	***
Bn-A07-p10209154	SNP	A07	42.0	11374691	0.0000	***
Bn-A07-p9923514	SNP	A07	42.4	11206947	0.0001	***
Bn-A07-p10455192	SNP	A07	43.4	11647529	0.0000	***
Bn-A07-p10004245	SNP	A07	43.8	1066095	0.0000	***
brPb-661786	*DArT*	*A07*	*44.8*	*11332122*	*0.0000*	***
brPb-661422	*DArT*	*A07*	*46.3*	*11773160*	*0.0002*	***
Bn-A07-p10554944	SNP	A07	47.3	11734336	0.0001	***
Bn-A07-p11044717	SNP	A07	47.7	12267076	0.0000	***
Bn-Scaffold000317-p23246	SNP	A07	48.2	12629937	0.0001	***
Bn-A02-p126006	SNP	A07	48.7	12758954	0.0001	***
Bn-A02-p212959	SNP	A07	49.2	12833976	0.0002	***
Bn-A02-p300727	SNP	A07	51.2	12926340	0.0000	***
Bn-A02-p459981	SNP	A07	51.6	13077219	0.0000	***
Bn-A02-p500418	SNP	A07	52.1	13125319	0.0000	***
Bn-A02-p551481	SNP	A07	52.6	13173662	0.0000	***
Bn-A02-p918384	SNP	A07	53.5	13543711	0.0000	***
Bn-A02-p972896	SNP	A07	54.0		0.0000	***
Bn-A07-p11572985	SNP	A07	56.4	1213131	0.0001	***
E32M49-188D	*AFLP*	*A07*	*57.8*		*0.0001*	***
E32M49-193E	*AFLP*	*A07*	*58.3*		*0.0000*	***
E35M62-318D	*AFLP*	*A07*	*60.3*		*0.0002*	***
brPb-671124	*DArT*	*A07*	*60.8*	*15074931*	*0.0001*	***
brPb-659002	*DArT*	*A07*	*63.2*	*14326004*	*0.0004*	***
Bn-A07-p11979910	SNP	A07	65.1	14097304	0.0001	***
Bn-A07-p12921727	SNP	A07	65.6	15026946	0.0002	***
Bn-A07-p12546889	SNP	A07	66.1	1400519	0.0001	***
Bn-A07-p11870301	SNP	A07	66.5	14028105	0.0002	***
Bn-A07-p11629852	SNP	A07	67.0	13778172	0.0001	***
Bn-A07-p11943948	SNP	A07	67.5	1306060	0.0002	***
Bn-A07-p12734895	SNP	A07	67.9	14831219	0.0001	***
Bn-A07-p13167844	SNP	A07	69.8	15251749	0.0001	***
Bn-A07-p13787534	SNP	A07	73.2	1591548	0.0006	***
Bn-A07-p13963849	SNP	A07	74.6	15890459	0.0001	***
Bn-A07-p14046607	SNP	A07	76.0	15965574	0.0006	***
Bn-A07-p14759961	SNP	A07	76.5	16644393	0.0004	***
Bn-A07-p14225642	SNP	A07	77.0	16093010	0.0006	***
Bn-A07-p14645683	SNP	A07	77.4	16540846	0.0004	***
Bn-A07-p14780968	SNP	A07	78.4		0.0004	***
Bn-scaff_15746_1-p1299	SNP	A07	79.3	16524094	0.0004	***
Bn-A07-p14922137	SNP	A07	80.7	16794612	0.0006	***
Bn-A07-p15162837	SNP	A07	81.2	17075670	0.0010	***

Marker	Marker type	LG	Position (cM)	*B. napus* physical position	Chi² 1:1 (*p*-value)	Chi² 1:1 Significance
Bn-A07-p14895551	SNP	A07	81.7	16764376	0.0006	***
Bn-scaff_16064_1-p816197	SNP	A07	82.1	16990844	0.0010	***
Bn-A07-p15441391	SNP	A07	84.0	1677737	0.0060	**
Bn-A07-p15771342	SNP	A07	85.0	17677086	0.0134	*
Bn-A07-p15801291	SNP	A07	85.9		0.0280	*
Bn-A07-p15829285	SNP	A07	86.4	17734570	0.0195	*
Bn-A07-p16167393	SNP	A07	87.8		0.0060	**
Bn-A07-p16557893	SNP	A07	88.3	18536350	0.0091	**
Bn-A07-p16931589	SNP	A07	89.2	18852096	0.0195	*
Bn-A07-p17017277	SNP	A07	90.6	18931037	0.0545	
Bn-A07-p17186393	SNP	A07	91.1	19103647	0.0741	
Bn-A07-p17434468	SNP	A07	92.1	1807582	0.1308	
Bn-A07-p17477080	SNP	A07	92.5	19366860	0.0993	
Bn-A07-p17409328	SNP	A07	93.0	19306687	0.1308	
Bn-A07-p17587315	SNP	A07	93.9	19464629	0.1308	
Bn-A07-p18201301	SNP	A07	94.9	20094052	0.2164	
Bn-A07-p18308378	SNP	A07	96.3	20209920	0.4098	
Bn-A07-p18957162	SNP	A07	98.2	20769036	0.7835	
Bn-A07-p19118066	SNP	A07	98.7	20923897	0.6803	
Bn-A07-p19352007	SNP	A07	100.5	21142560	0.8907	
Bn-scaff_25466_1-p192846	SNP	A07	101.0	21223186	0.7835	
Bn-scaff_19937_1-p20028	SNP	A07	102.0	21340844	0.7835	
Bn-A07-p19795126	SNP	A07	105.7	21608240	0.4098	
Bn-A07-p19865996	SNP	A07	107.2	21681393	0.3363	
Bn-A07-p19912379	SNP	A07	107.6		0.4098	
Bn-A07-p20611621	SNP	A07	108.6		0.5827	
Bn-A07-p20230189	SNP	A07	109.1	21986831	0.4922	
Bn-A07-p21078249	SNP	A07	110.5	22624570	0.5827	
Bn-A07-p21678440	SNP	A07	111.4	23223777	0.5827	
Bn-A07-p21994050	SNP	A07	111.9	23385746	0.6803	
Bn-A07-p22382675	SNP	A07	112.4	23779520	0.8907	
Bn-A07-p22542288	SNP	A07	112.8	23956661	1.0000	
Bn-A07-p21871055	SNP	A07	114.2	2058120	0.6803	
Bn-A07-p20986876	SNP	A07	115.7	22540038	0.5827	
Bn-A07-p20894218	SNP	A07	116.1		0.4922	
Bn-A07-p20248654	SNP	A07	117.1	21998627	0.4922	
brPb-660246	*DArT*	*A07*	*119.0*		*0.4031*	
brPb-657676	*DArT*	*A07*	*120.5*	*22509387*	*0.2419*	
brPb-663103	*DArT*	*A07*	*122.4*	*21795193*	*0.2971*	
E44M62-106E	*AFLP*	*A08*	*0.0*		*0.0024*	***
brPb-663594	*DArT*	*A08*	*0.5*	*7145468*	*0.0031*	***
Bn-A08-p7260514	SNP	A08	2.4		0.0060	**
Bn-A08-p3284482	SNP	A08	3.3	2761770	0.0025	**

Marker	Marker type	LG	Position (cM)	*B. napus* physical position	Chi² 1:1 (*p*-value)	Chi² 1:1 Significance
Bn-A08-p2995827	SNP	A08	3.8	2476740	0.0016	**
brPb-663528	*DArT*	*A08*	*4.9*	*2700177*	*0.0004*	***
E33M47-82E	*AFLP*	*A08*	*5.9*		*0.0094*	**
Bn-A08-p1123899	SNP	A08	8.0	1013089	0.0134	*
Bn-A08-p694721	SNP	A08	8.9	633910	0.0060	**
Bn-A08-p372048	SNP	A08	9.4	313830	0.0091	**
Bn-A08-p570304	SNP	A08	9.9	518503	0.0134	*
Bn-A08-p2922263	SNP	A08	14.1	2377343	0.0016	**
Bn-A08-p3270600	SNP	A08	14.6	2747756	0.0025	**
Bn-A08-p4000353	SNP	A08	15.6	3400709	0.0060	**
Bn-A08-p6066021	SNP	A08	16.0		0.0091	**
Bn-A08-p10795996	SNP	A08	17.0	8702053	0.0091	**
Bn-A08-p10844808	SNP	A08	17.4	8746377	0.0060	**
Bn-A08-p10067927	SNP	A08	17.9	8069051	0.0091	**
Bn-A08-p10026421	SNP	A08	18.4	8026173	0.0060	**
Bn-A08-p6101880	SNP	A08	18.9		0.0091	**
Bn-A08-p3964840	SNP	A08	19.3	3386722	0.0060	**
Bn-A08-p11308315	SNP	A08	22.8	9168980	0.0280	*
Bn-A08-p12699181	SNP	A08	25.1	10373996	0.0394	*
Bn-A08-p13214532	SNP	A08	25.6	10959819	0.0280	*
Bn-A08-p13284369	SNP	A08	26.1	11051768	0.0195	*
Bn-A08-p13363830	SNP	A08	26.5	11110719	0.0134	*
Bn-A08-p13325281	SNP	A08	27.0	11067320	0.0195	*
Bn-A08-p13200580	SNP	A08	27.5	1606733	0.0280	*
Bn-A08-p12638473	SNP	A08	28.0	10557498	0.0394	*
Bn-A08-p13616733	SNP	A08	29.8	11367526	0.0195	*
Bn-A08-p13830207	SNP	A08	30.8	11563063	0.0394	*
Bn-scaff_18559_1-p248231	SNP	A08	32.2	11635179	0.0134	*
Bn-A08-p14045962	SNP	A08	32.7	11749118	0.0195	*
Bn-A08-p13932634	SNP	A08	33.1	11662900	0.0134	*
Bn-A08-p14117791	SNP	A08	33.6	11815421	0.0195	*
Bn-A08-p14597858	SNP	A08	35.0		0.0545	
Bn-A08-p14538807	SNP	A08	35.5	12242058	0.0394	*
Bn-A08-p14493385	SNP	A08	36.0	12192650	0.0280	*
Bn-A08-p14813540	SNP	A08	37.9	12376779	0.0993	
Bn-A08-p14759185	SNP	A08	38.3	12320936	0.0741	
Bn-A08-p15053710	SNP	A08	39.8		0.1696	
Bn-A08-p15231480	SNP	A08	40.2	12789541	0.1308	
Bn-A08-p15243115	SNP	A08	40.7	12801818	0.0993	
Bn-A08-p15239295	SNP	A08	41.2	12797960	0.1308	
Bn-A08-p15322011	SNP	A08	41.6	12889609	0.0993	
brPb-659418	*DArT*	*A08*	*42.7*		*0.0016*	**
brPb-658031	*DArT*	*A08*	*44.3*	*13214891*	*0.0005*	***

Marker	Marker type	LG	Position (cM)	*B. napus* physical position	Chi² 1:1 (*p*-value)	Chi² 1:1 Significance
brPb-809516	*DArT*	*A08*	*44.9*	*13214891*	*0.1425*	
Bn-A08-p15710460	SNP	A08	45.8	13216541	0.2164	
Bn-A08-p15489686	SNP	A08	46.3	13061411	0.1696	
Bn-A08-p15614498	SNP	A08	46.8	13145012	0.2164	
Bn-A08-p15945454	SNP	A08	47.7	13407152	0.3363	
Bn-A08-p15991441	SNP	A08	48.2	13450684	0.2718	
Bn-A08-p16132462	SNP	A08	49.6	13593811	0.3363	
Bn-A08-p16514789	SNP	A08	52.0		0.5827	
Bn-A08-p16990347	SNP	A08	54.8	14465850	0.2718	
Bn-A08-p17291598	SNP	A08	57.6	14720820	0.7835	
Bn-A08-p17327135	SNP	A08	58.1	14773111	0.8907	
Bn-A08-p17393046	SNP	A08	59.1	14852028	0.8907	
Bn-A08-p17449410	SNP	A08	59.5		1.0000	
Bn-A08-p17586676	SNP	A08	60.0	15039202	0.8907	
Bn-A08-p17735673	SNP	A08	60.5	15175604	1.0000	
Bn-A08-p17816612	SNP	A08	61.9	15264416	0.8907	
Bn-A08-p17932590	SNP	A08	62.4	15372403	1.0000	
Bn-A08-p18112619	SNP	A08	63.8	15559843	0.6803	
Bn-A08-p18605816	SNP	A08	67.1	16019728	0.4098	
Bn-A08-p18720560	SNP	A08	68.1		0.5827	
Bn-A08-p19428204	SNP	A08	75.2	16803479	0.3363	
Bn-A08-p19454590	SNP	A08	75.7	16825035	0.4098	
Bn-A08-p19553756	SNP	A08	77.1	2001776	0.4922	
brPb-661602	*DArT*	*A08*	*80.4*	*1962473*	*0.7835*	
Bn-A08-p19573947	SNP	A08	85.3	2026256	0.4922	
Bn-A08-p19676751	SNP	A08	85.8	17007110	0.5827	
Bn-A08-p20134420	SNP	A08	86.3	2075051	0.4922	
Bn-A08-p20216535	SNP	A08	87.2	17422359	0.6803	
Bn-A05-p8120997	SNP	A08	87.7	17544012	0.5827	
Bn-A08-p20373400	SNP	A08	91.5	17834189	0.2718	
Bn-A08-p20490539	SNP	A08	92.0	17971842	0.2164	
Bn-A08-p20306567	SNP	A08	92.4	17778164	0.2718	
Bn-A08-p20305036	SNP	A08	92.9	17775526	0.3363	
Bn-A09-p616919	SNP	A09	0.0	1637190	0.1142	
Bn-A09-p297029	SNP	A09	0.5	394410	0.0091	**
Bn-scaff_15783_1-p273092	SNP	A09	10.4	565946	0.0000	***
Bn-scaff_22749_1-p202280	SNP	A09	22.7	389015	0.0000	***
Bn-scaff_17526_1-p2206429	SNP	A09	24.1	837094	0.0000	***
Bn-scaff_15783_1-p350376	SNP	A09	24.6	524248	0.0000	***
Bn-A09-p2208929	SNP	A09	25.7	1382721	0.0000	***
Bn-A09-p1603989	SNP	A09	26.2	2323846	0.0000	***
Bn-A09-p1634652	SNP	A09	30.5	2292134	0.0000	***
Bn-scaff_17526_1-p221576	SNP	A09	33.8	2208477	0.0000	***

Marker	Marker type	LG	Position (cM)	*B. napus* physical position	Chi² 1:1 (*p*-value)	Chi² 1:1 Significance
Bn-scaff_19783_1-p71479	SNP	A09	34.4	2448273	0.0000	***
Bn-A09-p3047090	SNP	A09	44.0	2966543	0.0000	***
Bn-A09-p4300103	SNP	A09	45.9	3765540	0.0000	***
Bn-A09-p4447029	SNP	A09	49.7	4358752	0.0000	***
Bn-A09-p3908310	SNP	A09	51.2	4095068	0.0000	***
Bn-A01-p9004629	SNP	A09	54.9	2580895	0.0000	***
brPb-661324	*DArT*	*A09*	*61.2*	*504924*	*0.0000*	***
E32M47-231D	*AFLP*	*A09*	*69.5*		*0.0000*	***
Bn-A09-p4831706	SNP	A09	72.9	4740301	0.0000	***
Bn-A09-p5255753	SNP	A09	74.4	4931273	0.0000	***
Bn-A09-p5455608	SNP	A09	75.8	5015175	0.0000	***
Bn-A09-p7329993	SNP	A09	77.7	5542420	0.0000	***
Bn-A09-p7047022	SNP	A09	81.0	5815858	0.0000	***
E35M62-368E	*AFLP*	*A09*	*84.6*		*0.0014*	**
Bn-A09-p6010593	SNP	A09	91.7	6668908	0.0002	***
Bn-A09-p7454442	SNP	A09	92.1	6949288	0.0004	***
Bn-A09-p7560188	SNP	A09	93.5	7077469	0.0016	**
Bn-A09-p8591730	SNP	A09	95.0	8020106	0.0025	**
Bn-A09-p9098267	SNP	A09	96.9	8279745	0.0134	*
Bn-A09-p9379347	SNP	A09	100.2	8460797	0.1308	
Bn-A09-p10064024	SNP	A09	102.0	9050884	0.0394	*
Bn-A09-p10172534	SNP	A09	102.5	9249533	0.0280	*
Bn-A09-p13827633	SNP	A09	103.5	9946476	0.0545	
Bn-A09-p13251498	SNP	A09	104.4		0.0280	*
brPb-663436	*DArT*	*A09*	*107.5*	*12020749*	*0.0993*	
Bn-A09-p17342092	SNP	A09	108.0	13123322	0.1483	
Bn-A09-p11727553	SNP	A09	109.4	14981748	0.0993	
Bn-A09-p23515910	SNP	A09	114.6	21894307	0.3363	
Bn-A09-p23552530	SNP	A09	116.0	21931881	0.1696	
Bn-A05-p15797839	SNP	A09	119.8		0.0545	
Bn-A09-p24854950	SNP	A09	120.3		0.0741	
Bn-A09-p24921401	SNP	A09	120.7		0.0545	
Bn-A09-p24942194	SNP	A09	121.2		0.0394	*
Bn-A09-p25738255	SNP	A09	121.7	23823204	0.0545	
Bn-A09-p25372544	SNP	A09	122.2	23516972	0.0394	*
Bn-A09-p25493173	SNP	A09	122.6		0.0545	
Bn-A09-p26244223	SNP	A09	125.5	24365261	0.0060	**
Bn-A09-p26864664	SNP	A09	127.4	24929899	0.0010	***
Bn-A09-p26301343	SNP	A09	129.2	24386373	0.0060	**
E44M62-99D	*AFLP*	*A09*	*130.7*		*0.0251*	*
E33M47-385E	*AFLP*	*A09*	*136.7*		*0.0004*	***
brPb-808599	*DArT*	*A09*	*137.2*	*25119502*	*0.0001*	***
Bn-A09-p27103683	SNP	A09	138.2	25104229	0.0000	***

Marker	Marker type	LG	Position (cM)	*B. napus* physical position	Chi² 1:1 (*p*-value)	Chi² 1:1 Significance
Bn-A09-p27351028	SNP	A09	140.5	25392191	0.0006	***
Bn-A09-p27436536	SNP	A09	141.0	2994306	0.0004	***
Bn-A09-p27628825	SNP	A09	142.4	25547079	0.0016	**
Bn-A09-p28212233	SNP	A09	148.1	26138085	0.0001	***
Bn-A09-p28266331	SNP	A09	148.6		0.0001	***
Bn-A09-p28518619	SNP	A09	150.5	26412035	0.0010	***
Bn-A09-p29134207	SNP	A09	151.9	3164770	0.0006	***
Bn-A09-p30002747	SNP	A09	154.2	27828143	0.0010	***
Bn-A09-p30010889	SNP	A09	155.2	27836342	0.0025	**
Bn-A09-p30108430	SNP	A09	158.5	27923796	0.0001	***
Bn-A09-p30119978	SNP	A09	159.4	27937900	0.0002	***
Bn-A09-p30280052	SNP	A09	159.9	28059010	0.0004	***
Bn-A09-p25899239	SNP	A09	160.4	28210312	0.0006	***
Bn-A09-p5040112	SNP	A09	163.7	29637725	0.0025	**
Bn-A09-p5157169	SNP	A09	164.2	29733948	0.0039	**
Bn-A09-p31884693	SNP	A09	164.6		0.0025	**
Bn-A09-p31905309	SNP	A09	165.1		0.0016	**
Bn-A09-p32104974	SNP	A09	165.6	29995410	0.0025	**
Bn-A09-p33875531	SNP	A09	168.4	3977457	0.0060	**
Bn-A09-p34995048	SNP	A09	171.2	32205427	0.0280	*
Bn-A09-p35508720	SNP	A09	174.5	32655355	0.0091	**
brPb-840102	*DArT*	*A09*	*176.5*	*4089451*	*0.0098*	**
brPb-661730	*DArT*	*A09*	*179.0*	*33364165*	*0.0016*	**
Bn-A09-p36402581	SNP	A09	182.3		0.0025	**
Bn-A09-p36551108	SNP	A09	184.7	33561792	0.0039	**
brPb-809539	*DArT*	*A10*	*0.0*	*16674875*	*0.0280*	*
Bn-scaff_21636_1-p137494	SNP	A10	2.8	16670308	0.0025	**
Bn-A10-p16737974	SNP	A10	3.3	16535774	0.0019	**
Bn-A10-p16924111	SNP	A10	4.7	16729401	0.0091	**
Bn-A10-p17500897	SNP	A10	6.6	17299341	0.0060	**
Bn-A10-p17470876	SNP	A10	8.1		0.0039	**
Bn-A10-p17262954	SNP	A10	8.5	17087915	0.0048	**
Bn-A10-p17148313	SNP	A10	9.0	16960203	0.0031	**
Bn-A10-p16918661	SNP	A10	10.0	16723858	0.0073	**
Bn-A10-p16824950	SNP	A10	10.9	16627400	0.0031	**
Bn-A10-p16718558	SNP	A10	11.4	16510586	0.0016	**
Bn-A10-p16703268	SNP	A10	12.8	16493815	0.0025	**
Bn-A10-p16522658	SNP	A10	13.7	16316296	0.0016	**
Bn-A10-p15254551	SNP	A10	14.7	16234650	0.0012	**
Bn-A10-p15265878	SNP	A10	15.6		0.0004	***
Bn-A10-p15442975	SNP	A10	17.5	16049634	0.0025	**
Bn-A10-p15591443	SNP	A10	18.9	2182756	0.0006	***
Bn-A10-p15606935	SNP	A10	19.4	2137016	0.0004	***

Marker	Marker type	LG	Position (cM)	*B. napus* physical position	Chi² 1:1 (*p*-value)	Chi² 1:1 Significance
Bn-A10-p15863143	SNP	A10	19.9	15695789	0.0006	***
Bn-A10-p15651383	SNP	A10	20.4	15915998	0.0004	***
Bn-A10-p15894309	SNP	A10	20.8	15670009	0.0006	***
Bn-A10-p15948040	SNP	A10	21.3	15618261	0.0010	***
E45M49-297E	*AFLP*	*A10*	*24.8*		*0.0057*	**
brPb-670588	*DArT*	*A10*	*28.6*	*15444463*	*0.0007*	***
brPb-809027	*DArT*	*A10*	*30.5*	*15343574*	*0.0002*	***
Bn-A10-p16285282	SNP	A10	33.5	15306586	0.0000	***
Bn-A10-p16215165	SNP	A10	34.0	15363433	0.0000	***
Bn-A10-p16190708	SNP	A10	34.9	15381939	0.0000	***
Bn-A10-p16021831	SNP	A10	36.8	15545593	0.0001	***
Bn-scaff_17750_1-p1692483	SNP	A10	40.1	15301191	0.0000	***
Bn-A10-p16317798	SNP	A10	40.6	15273770	0.0000	***
Bn-A10-p16424917	SNP	A10	41.5	15164117	0.0000	***
Bn-A10-p14962307	SNP	A10	42.5	14897418	0.0000	***
Bn-A10-p14809502	SNP	A10	43.9	14762080	0.0000	***
Bn-A10-p14728923	SNP	A10	44.3	14684577	0.0000	***
Bn-A10-p14472446	SNP	A10	45.3	14409444	0.0000	***
Bn-A10-p14354612	SNP	A10	45.8	14295891	0.0000	***
Bn-A10-p14103279	SNP	A10	47.6	14101620	0.0000	***
Bn-A10-p13913564	SNP	A10	48.6		0.0000	***
Bn-A10-p13875041	SNP	A10	49.1	13907075	0.0000	***
Bn-A10-p13815263	SNP	A10	50.5	13851859	0.0000	***
Bn-A10-p13798602	SNP	A10	50.9	13834339	0.0000	***
Bn-A10-p13677412	SNP	A10	51.4	13718053	0.0000	***
Bn-A10-p13638489	SNP	A10	51.9	13678934	0.0000	***
Bn-A10-p13455391	SNP	A10	52.8	13502402	0.0000	***
Bn-A10-p13375342	SNP	A10	53.3	13415791	0.0000	***
Bn-A10-p13393956	SNP	A10	53.8	13436702	0.0000	***
Bn-A10-p13142215	SNP	A10	55.2	13181101	0.0000	***
Bn-A08-p4902282	SNP	A10	56.6	12841613	0.0000	***
Bn-A10-p11042627	SNP	A10	57.1	12401828	0.0000	***
Bn-A10-p11311425	SNP	A10	57.6	12610338	0.0000	***
Bn-A10-p12996989	SNP	A10	58.0	13029666	0.0000	***
Bn-A10-p13139635	SNP	A10	59.0	13178585	0.0000	***
Bn-A10-p13318061	SNP	A10	59.4		0.0000	***
E44M62-288D	*AFLP*	*A10*	*61.0*		*0.0000*	***
brPb-663056	*DArT*	*A10*	*62.0*	*13419646*	*0.0000*	***
brPb-663284	*DArT*	*A10*	*64.8*	*12406226*	*0.0000*	***
brPb-661264	*DArT*	*A10*	*68.7*		*0.0000*	***
E33M47-178E	*AFLP*	*A10*	*69.1*		*0.0000*	***
Bn-A10-p10395990	SNP	A10	72.0	11786976	0.0000	***
Bn-A10-p10562649	SNP	A10	72.5	11938280	0.0000	***

Marker	Marker type	LG	Position (cM)	*B. napus* physical position	Chi² 1:1 (*p*-value)	Chi² 1:1 Significance
Bn-A10-p10866946	SNP	A10	73.5	12249791	0.0000	***
Bn-A10-p10777142	SNP	A10	74.4	12136830	0.0000	***
Bn-A10-p9846287	SNP	A10	76.8	11292790	0.0000	***
Bn-A10-p9615362	SNP	A10	77.2	11034012	0.0000	***
Bn-A10-p8834914	SNP	A10	77.7	10246833	0.0000	***
Bn-A10-p9558311	SNP	A10	78.2	11004371	0.0000	***
Bn-A10-p9887525	SNP	A10	78.7		0.0000	***
Bn-A10-p8723171	SNP	A10	80.1	10123624	0.0000	***
Bn-A10-p7988223	SNP	A10	80.5	9647659	0.0000	***
Bn-A10-p2065917	SNP	A10	84.8	1648732	0.0000	***
Bn-A10-p634822	SNP	A10	88.1		0.0000	***
Bn-A10-p1354965	SNP	A10	88.6	2246432	0.0000	***
Bn-A10-p7454604	SNP	A10	89.1		0.0000	***
brPb-658735	*DArT*	*A10*	*91.0*	*9034334*	*0.0000*	***
brPb-660669	*DArT*	*A10*	*92.5*		*0.0000*	***
E33M47-227D	*AFLP*	*A10*	*100.6*		*0.0000*	***
Bn-A10-p2374648	SNP	A10	102.7	1488746	0.0000	***
Bn-A10-p2171166	SNP	A10	103.1		0.0000	***
Bn-A10-p2096482	SNP	A10	105.1	1624221	0.0000	***
Bn-A10-p2260170	SNP	A10	107.4	1577525	0.0000	***
Bn-A10-p2687844	SNP	A10	108.9	1222375	0.0000	***
Bn-A10-p2804340	SNP	A10	109.4	1140201	0.0000	***
Bn-A10-p2819947	SNP	A10	109.8	1123105	0.0000	***
Bn-A10-p3809773	SNP	A10	110.8	1010599	0.0000	***
Bn-A10-p3903845	SNP	A10	111.3	918947	0.0000	***
Bn-A10-p4104015	SNP	A10	112.7	760137	0.0001	***
Bn-A10-p4076884	SNP	A10	113.2	784818	0.0002	***
Bn-A10-p4212056	SNP	A10	113.7		0.0000	***
Bn-A10-p4356450	SNP	A10	114.1	474901	0.0000	***
Bn-A10-p4311291	SNP	A10	115.1		0.0002	***
Bn-A10-p4118433	SNP	A10	116.0	747781	0.0002	***
Bn-A10-p3905100	SNP	A10	117.0	917471	0.0000	***
Bn-A10-p2833336	SNP	A10	118.4	1106811	0.0000	***
brPb-659419	*DArT*	*A10*	*120.8*		*0.0000*	***
brPb-658597	*DArT*	*A10*	*122.3*		*0.0000*	***
brPb-657840	*DArT*	*C01*	*0.0*	*15392292*	*0.0000*	***
brPb-661486	*DArT*	*C01*	*1.0*	*16137582*	*0.0000*	***
brPb-663378	*DArT*	*C01*	*2.9*	*21932884*	*0.0000*	***
E32M47-123D	*AFLP*	*C01*	*3.4*		*0.0000*	***
E39M62-103D	*AFLP*	*C01*	*7.8*		*0.0000*	***
E39M62-111E	*AFLP*	*C01*	*8.3*		*0.0000*	***
E32M47-175D	*AFLP*	*C01*	*10.9*		*0.0000*	***
Bn-C13838053-p225	SNP	C01	18.3	4135768	0.0000	***

Marker	Marker type	LG	Position (cM)	*B. napus* physical position	Chi² 1:1 (*p*-value)	Chi² 1:1 Significance
Bn-scaff_15838_5-p929569	SNP	C01	19.7	3759560	0.0000	***
Bn-scaff_15838_5-p478688	SNP	C01	21.1	3326131	0.0000	***
Bn-scaff_15838_5-p227630	SNP	C01	21.6	3078855	0.0000	***
Bn-scaff_15838_1-p1917196	SNP	C01	23.0	2273565	0.0000	***
Bn-scaff_19244_1-p693772	SNP	C01	25.0	628240	0.0000	***
Bn-scaff_20809_1-p163800	SNP	C01	26.9		0.0000	***
Bn-scaff_19244_1-p699318	SNP	C01	28.8	633739	0.0000	***
Bn-scaff_15838_1-p1572850	SNP	C01	30.2	1942644	0.0000	***
Bn-scaff_15838_1-p2039607	SNP	C01	30.7	2411780	0.0000	***
Bn-scaff_15838_5-p625786	SNP	C01	32.6	3450388	0.0000	***
brPb-660268	*DArT*	*C01*	*34.5*	*3056412*	*0.0000*	***
brPb-670827	*DArT*	*C01*	*40.5*		*0.0000*	***
brPb-838658	*DArT*	*C01*	*42.0*	*5324890*	*0.0000*	***
Bn-scaff_19193_1-p927967	SNP	C01	43.8	5409479	0.0000	***
Bn-scaff_19193_1-p1250152	SNP	C01	44.3	5057305	0.0000	***
Bn-scaff_19193_1-p832198	SNP	C01	44.8	5495867	0.0000	***
Bn-scaff_19193_1-p704672	SNP	C01	45.3	5610497	0.0000	***
Bn-scaff_17731_1-p165802	SNP	C01	46.7	116130	0.0000	***
Bn-scaff_17731_1-p569481	SNP	C01	47.2	6798070	0.0000	***
brPb-808002	*DArT*	*C01*	*50.0*	*7974068*	*0.0000*	***
Bn-scaff_17827_1-p817715	SNP	C01	51.4	7628573	0.0000	***
Bn-scaff_20210_1-p267408	SNP	C01	51.9	396159	0.0000	***
Bn-scaff_17827_1-p963588	SNP	C01	52.4	7866709	0.0000	***
Bn-scaff_20210_1-p171615	SNP	C01	52.9	8211890	0.0000	***
Bn-scaff_16443_1-p1306	SNP	C01	53.3	563170	0.0000	***
Bn-scaff_17369_1-p1041740	SNP	C01	55.7	11298284	0.0000	***
Bn-scaff_27129_1-p103216	SNP	C01	56.6	13683268	0.0000	***
Bn-A01-p11249993	SNP	C01	58.1	15394740	0.0000	***
Bn-scaff_20126_1-p457	SNP	C01	58.5		0.0000	***
Bn-A01-p11471489	SNP	C01	59.0	15689357	0.0000	***
Bn-scaff_20250_1-p164979	SNP	C01	59.5	16850781	0.0000	***
Bn-scaff_25373_1-p399	SNP	C01	59.9	20911383	0.0000	***
Bn-scaff_19564_1-p17934	SNP	C01	60.9	26746237	0.0000	***
Bn-scaff_20452_1-p146457	SNP	C01	61.4		0.0000	***
Bn-scaff_16055_1-p785275	SNP	C01	61.8	29173129	0.0000	***
Bn-A01-p21277841	SNP	C01	62.3	31948919	0.0000	***
Bn-scaff_16055_1-p409784	SNP	C01	62.8		0.0000	***
Bn-scaff_15844_1-p6065	SNP	C01	64.2	33233101	0.0000	***
Bn-scaff_15844_1-p184165	SNP	C01	65.2	33411621	0.0000	***
Bn-A01-p23120368	SNP	C01	66.1		0.0000	***
Bn-scaff_24466_1-p76815	SNP	C01	67.5	3614103	0.0000	***
Bn-scaff_17515_1-p220339	SNP	C01	68.5	34651059	0.0000	***
Bn-A01-p24915384	SNP	C01	69.4	36655396	0.0000	***

Marker	Marker type	LG	Position (cM)	*B. napus* physical position	Chi² 1:1 (*p*-value)	Chi² 1:1 Significance
Bn-scaff_15936_1-p226371	SNP	C01	70.9	36365924	0.0000	***
Bn-scaff_22115_1-p155376	SNP	C01	72.3	35743933	0.0000	***
Bn-A01-p26518304	SNP	C01	75.2		0.0000	***
brPb-670980	*DArT*	*C02*	*0.0*		*0.1308*	
Bn-scaff_26086_1-p11779	SNP	C02	2.4	44930141	0.0533	
Bn-scaff_16139_1-p1393867	SNP	C02	4.3	45156052	0.0852	
Bn-scaff_16139_1-p1337221	SNP	C02	8.6	45207812	0.4489	
Bn-scaff_16139_1-p1027358	SNP	C02	10.0	45482093	0.6803	
Bn-scaff_16139_1-p721705	SNP	C02	11.4	45788397	0.8907	
Bn-scaff_16139_1-p435514	SNP	C02	13.8	12309438	0.4076	
Bn-scaff_16139_1-p260488	SNP	C02	14.8		0.5809	
Bn-scaff_17721_1-p49987	SNP	C02	27.5		0.0190	*
Bn-scaff_17721_1-p638632	SNP	C02	28.0	43688864	0.0272	*
Bn-scaff_15918_1-p161116	SNP	C02	36.6		0.0394	*
Bn-scaff_15712_12-p21418	SNP	C02	39.0	41940070	0.1290	
Bn-scaff_17109_1-p593012	SNP	C02	40.0	41769868	0.0728	
Bn-scaff_17109_1-p626573	SNP	C02	40.9	41712928	0.0384	*
Bn-scaff_17109_1-p683105	SNP	C02	41.9	41639026	0.0160	*
Bn-scaff_17109_4-p114755	SNP	C02	45.2	40738569	0.0130	*
Bn-scaff_17831_1-p219290	SNP	C02	47.1		0.0130	*
Bn-scaff_17088_1-p4312	SNP	C02	48.1		0.0130	*
Bn-scaff_17831_1-p215355	SNP	C02	49.1	40284877	0.0091	**
Bn-scaff_17109_4-p159217	SNP	C02	50.0	40699432	0.0058	**
Bn-scaff_17109_4-p101748	SNP	C02	51.0	40753783	0.0130	*
Bn-scaff_15712_9-p660357	SNP	C02	55.7		0.0272	*
Bn-scaff_22144_1-p193415	SNP	C02	56.2	39783146	0.0384	*
Bn-scaff_15712_9-p618165	SNP	C02	56.7	39345051	0.0328	*
Bn-scaff_15712_2-p321760	SNP	C02	57.6	38436073	0.0272	*
Bn-scaff_18360_1-p873769	SNP	C02	58.6	3849294	0.0545	
Bn-scaff_18360_1-p360342	SNP	C02	59.1	36165181	0.0394	*
Bn-scaff_18360_1-p31167	SNP	C02	60.0	35859321	0.0160	*
Bn-scaff_17289_1-p655703	SNP	C02	61.0	35396803	0.0195	*
Bn-scaff_21131_1-p49530	SNP	C02	61.9		0.0195	*
Bn-scaff_22451_2-p166198	SNP	C02	63.3	25649403	0.0134	*
Bn-scaff_16449_1-p248131	SNP	C02	64.8	34353013	0.0195	*
Bn-scaff_16449_1-p181175	SNP	C02	65.2	34425302	0.0231	*
Bn-scaff_16449_1-p244840	SNP	C02	65.7	34356264	0.0195	*
brPb-841932	*DArT*	*C02*	*71.0*		*0.2718*	
E35M62-185D	*AFLP*	*C02*	*75.9*		*0.2098*	
Bn-scaff_20735_1-p44052	SNP	C02	79.8		0.0160	*
Bn-scaff_16903_1-p398198	SNP	C02	80.3	21324474	0.0280	*
Bn-scaff_16300_1-p614082	SNP	C02	80.8	23190745	0.0195	*
Bn-scaff_17077_1-p199562	SNP	C02	82.2	17610189	0.0280	*

Marker	Marker type	LG	Position (cM)	*B. napus* physical position	Chi² 1:1 (*p*-value)	Chi² 1:1 Significance
Bn-scaff_20979_1-p153226	SNP	C02	84.1		0.0459	*
Bn-A02-p10775283	SNP	C02	85.5	13931225	0.0394	*
Bn-scaff_27039_1-p73433	SNP	C02	86.0	13363912	0.0280	*
Bn-scaff_21224_1-p99003	SNP	C02	89.8	9296310	0.0134	*
Bn-scaff_20461_1-p314199	SNP	C02	90.3	9353770	0.0195	*
Bn-scaff_15712_5-p649917	SNP	C02	95.5		0.1696	
Bn-scaff_15712_5-p941560	SNP	C02	96.4	8561569	0.2718	
Bn-scaff_15712_13-p33062	SNP	C02	99.3	8297230	0.1696	
Bn-scaff_19467_1-p26963	SNP	C02	100.7	199921	0.0728	
Bn-scaff_16269_1-p296261	SNP	C02	102.1	6303237	0.1133	
Bn-scaff_16269_1-p533948	SNP	C02	105.4	6059666	0.0091	**
Bn-scaff_17522_1-p224957	SNP	C02	106.4		0.0231	*
Bn-scaff_17522_1-p746125	SNP	C02	108.3		0.0195	*
E33M49-186D	*AFLP*	*C02*	*111.3*		*0.0136*	*
E33M49-185E	*AFLP*	*C02*	*112.3*		*0.0223*	*
brPb-839257	*DArT*	*C02*	*115.5*		*0.0027*	**
Bn-scaff_17522_1-p1607150	SNP	C02	118.5		0.0016	**
Bn-scaff_22527_1-p155460	SNP	C02	119.0	5070252	0.0025	**
Bn-scaff_22527_1-p276311	SNP	C02	119.5	4942588	0.0039	**
Bn-scaff_15714_1-p210487	SNP	C02	121.9	4145857	0.0010	***
Bn-scaff_15714_1-p785730	SNP	C02	124.2		0.0006	***
Bn-scaff_15714_1-p1026389	SNP	C02	124.7	3274964	0.0012	**
Bn-scaff_15714_1-p727914	SNP	C02	125.2	3599776	0.0006	***
Bn-scaff_15714_1-p489734	SNP	C02	126.1	3840813	0.0006	***
brPb-838939	*DArT*	*C02*	*126.6*	*3859213*	*0.0066*	**
Bn-scaff_15714_1-p1210756	SNP	C02	128.2		0.0025	**
Bn-A02-p3493190	SNP	C02	129.1	2790204	0.0019	**
Bn-scaff_15714_1-p3001398	SNP	C02	142.4	518239	0.0993	
Bn-scaff_22970_1-p335296	SNP	C02	145.3	966861	0.3708	
Bn-scaff_22970_1-p242460	SNP	C02	145.7	1032250	0.1909	
Bn-scaff_22970_1-p32371	SNP	C02	147.2	1188795	0.0741	
Bn-A02-p1705187	SNP	C02	149.1	1667077	0.1483	
Bn-A03-p623390	SNP	C03	0.0	688444	0.3363	
Bn-scaff_16614_1-p1250990	SNP	C03	0.9		0.5355	
Bn-scaff_16614_1-p889881	SNP	C03	1.9	1188743	0.4098	
Bn-scaff_16614_1-p846820	SNP	C03	2.4	1242591	0.3708	
Bn-scaff_16614_1-p571357	SNP	C03	4.3	1538331	0.1483	
Bn-scaff_16614_1-p424532	SNP	C03	5.2	1665521	0.2419	
Bn-scaff_16614_1-p233213	SNP	C03	6.2	1872289	0.1483	
Bn-scaff_16614_1-p226165	SNP	C03	7.6	1879242	0.0631	
Bn-scaff_15844_1-p159209	SNP	C03	8.1	2282310	0.0852	
Bn-scaff_27198_1-p300707	SNP	C03	11.4	2356068	0.4489	
Bn-scaff_18936_1-p97644	SNP	C03	12.3	2740202	0.4902	

Marker	Marker type	LG	Position (cM)	*B. napus* physical position	Chi² 1:1 (*p*-value)	Chi² 1:1 Significance
Bn-scaff_18936_1-p277517	SNP	C03	12.8	2886860	0.4076	
Bn-scaff_18936_1-p384730	SNP	C03	13.8	2978046	0.4076	
E35M47-107E	*AFLP*	*C03*	*16.2*		*0.2649*	
Bn-scaff_18936_1-p439378	SNP	C03	17.8	3021607	0.2695	
Bn-A03-p2737190	SNP	C03	21.6	3151758	0.2695	
Bn-scaff_18936_1-p890286	SNP	C03	23.0	3419605	0.0993	
Bn-scaff_18936_1-p981056	SNP	C03	23.5	3513253	0.0741	
Bn-scaff_15877_1-p105023	SNP	C03	24.0	3725020	0.1866	
Bn-scaff_15877_1-p122377	SNP	C03	25.4	3741371	0.2374	
Bn-scaff_15877_1-p143213	SNP	C03	25.9	3766805	0.2971	
Bn-scaff_15877_1-p273957	SNP	C03	26.9	3921452	0.3685	
Bn-scaff_15877_1-p454328	SNP	C03	27.4	4099146	0.3662	
Bn-scaff_15877_1-p718090	SNP	C03	28.3	4415333	0.2164	
Bn-scaff_15877_1-p744560	SNP	C03	29.3	4441977	0.1308	
brPb-670046	*DArT*	*C03*	*32.9*		*0.6162*	
brPb-659783	*DArT*	*C03*	*33.4*		*0.7762*	
brPb-670934	*DArT*	*C03*	*35.1*	*5310826*	*0.8852*	
E44M62-73E	*AFLP*	*C03*	*35.6*		*0.8864*	
Bn-scaff_22728_1-p1363526	SNP	C03	38.7	5304614	0.1655	
Bn-scaff_22728_1-p1267084	SNP	C03	39.7	5435942	0.2718	
Bn-scaff_22728_1-p1393497	SNP	C03	40.2	5264572	0.4881	
Bn-scaff_21778_1-p184325	SNP	C03	40.7	4984948	0.2718	
Bn-scaff_15877_1-p908058	SNP	C03	41.6	4638640	0.1696	
Bn-scaff_21778_1-p287475	SNP	C03	42.6	5080193	0.2718	
Bn-scaff_15877_1-p985222	SNP	C03	43.1	4717091	0.2164	
Bn-scaff_15877_1-p1022276	SNP	C03	43.5	4747486	0.2718	
Bn-scaff_21778_1-p211639	SNP	C03	45.4	5007377	0.5827	
Bn-scaff_22728_1-p1080976	SNP	C03	46.4	5611091	0.2718	
Bn-scaff_22728_1-p938985	SNP	C03	48.3	5736222	0.0993	
Bn-scaff_18322_1-p2560823	SNP	C03	48.8	6665525	0.1308	
Bn-scaff_18322_1-p2149073	SNP	C03	49.7	6947278	0.1696	
Bn-scaff_18322_1-p2385043	SNP	C03	50.2	6837185	0.2164	
Bn-scaff_18322_1-p2378816	SNP	C03	50.7	6843416	0.1290	
Bn-scaff_18322_1-p2266068	SNP	C03	51.2	240911	0.0741	
Bn-scaff_18322_1-p2028484	SNP	C03	52.6	7083075	0.1696	
Bn-scaff_18322_1-p1794782	SNP	C03	53.0	7327999	0.2164	
Bn-scaff_18322_1-p881842	SNP	C03	54.0	8149532	0.1463	
Bn-scaff_18322_1-p826786	SNP	C03	55.9	8204548	0.0838	
Bn-scaff_18322_1-p803101	SNP	C03	57.4	8234764	0.0545	
Bn-scaff_18322_1-p677584	SNP	C03	58.3		0.0545	
Bn-scaff_18322_1-p679268	SNP	C03	59.3	8383959	0.0231	*
Bn-scaff_18322_1-p361468	SNP	C03	60.7		0.0394	*
brPb-838972	*DArT*	*C03*	*64.9*	*8968623*	*0.0280*	*

Marker	Marker type	LG	Position (cM)	*B. napus* physical position	Chi² 1:1 (*p*-value)	Chi² 1:1 Significance
Bn-scaff_21312_1-p1376602	SNP	C03	68.8	9075214	0.0280	*
Bn-scaff_21312_1-p1306149	SNP	C03	69.7	9149335	0.0545	
Bn-scaff_21312_1-p694502	SNP	C03	70.2	9753350	0.0394	*
Bn-scaff_21312_1-p546514	SNP	C03	70.7	9862898	0.0545	
Bn-scaff_21312_1-p169902	SNP	C03	71.6	10156097	0.0993	
Bn-scaff_21312_1-p313911	SNP	C03	72.1	10005760	0.0741	
Bn-scaff_21312_1-p90644	SNP	C03	72.6	10233936	0.0993	
Bn-scaff_23954_1-p971859	SNP	C03	73.5	10948613	0.0545	
Bn-scaff_23954_1-p635109	SNP	C03	74.5	11205263	0.0993	
Bn-scaff_23954_1-p865303	SNP	C03	75.4	11057063	0.0545	
Bn-scaff_23954_1-p768646	SNP	C03	76.3	11135002	0.0993	
Bn-scaff_23954_1-p72433	SNP	C03	77.8	11751541	0.2164	
brPb-659157	*DArT*	*C03*	*80.2*		*0.2396*	
brPb-663720	*DArT*	*C03*	*81.6*		*0.3363*	
E33M49-268D	*AFLP*	*C03*	*82.6*		*0.2948*	
Bn-scaff_16002_1-p1800806	SNP	C03	85.5	12576009	0.2164	
Bn-scaff_16002_1-p2144304	SNP	C03	86.0	12251622	0.2718	
Bn-scaff_16002_1-p2298646	SNP	C03	86.9	12092818	0.4098	
Bn-scaff_16002_1-p2010458	SNP	C03	87.9	12400523	0.2718	
Bn-scaff_16002_1-p1676442	SNP	C03	88.8	12709584	0.2718	
Bn-scaff_16002_1-p1154894	SNP	C03	89.7	920502	0.4098	
Bn-scaff_16002_1-p734704	SNP	C03	90.2	13588111	0.3363	
Bn-scaff_16002_1-p482742	SNP	C03	90.7	13828655	0.2718	
Bn-scaff_16002_1-p176561	SNP	C03	91.2	14102902	0.2164	
Bn-scaff_16352_1-p1097366	SNP	C03	95.9	16291033	0.8907	
Bn-scaff_16352_1-p1287850	SNP	C03	99.2	16502913	0.7835	
Bn-scaff_15782_1-p172469	SNP	C03	99.7	16639919	0.8364	
Bn-scaff_15782_1-p135470	SNP	C03	100.6	16670883	0.6299	
Bn-scaff_20646_1-p1254998	SNP	C03	101.1	16841780	0.6803	
Bn-scaff_20646_1-p744773	SNP	C03	101.6	17322217	0.5827	
Bn-scaff_20646_1-p290576	SNP	C03	102.0	1140696	0.4922	
Bn-scaff_20646_1-p498394	SNP	C03	102.5	17526837	0.4098	
Bn-scaff_20646_1-p182686	SNP	C03	103.0		0.4922	
Bn-scaff_19523_1-p261625	SNP	C03	103.5		0.4098	
Bn-scaff_18482_1-p699135	SNP	C03	106.3		0.5827	
Bn-scaff_18482_1-p680661	SNP	C03	106.8	19686048	0.4922	
Bn-scaff_18482_1-p268565	SNP	C03	108.2	20084717	0.2718	
Bn-scaff_22067_1-p139587	SNP	C03	112.9	20725718	0.4098	
Bn-scaff_17521_1-p1385225	SNP	C03	116.7	20865512	1.0000	
Bn-scaff_17521_1-p842968	SNP	C03	120.9	21372098	0.3363	
Bn-scaff_17521_1-p353123	SNP	C03	123.3	21872048	0.1696	
Bn-scaff_17521_1-p193675	SNP	C03	124.3	22023262	0.2718	
Bn-scaff_17298_1-p13882	SNP	C03	124.7		0.2164	

Marker	Marker type	LG	Position (cM)	*B. napus* physical position	Chi² 1:1 (*p*-value)	Chi² 1:1 Significance
Bn-scaff_17298_1-p1181494	SNP	C03	130.0	23299456	0.1696	
Bn-scaff_17298_1-p1218613	SNP	C03	130.4	23339513	0.1308	
Bn-scaff_17298_1-p2096293	SNP	C03	130.9	24097476	0.0993	
Bn-scaff_23350_1-p765525	SNP	C03	132.3	24937585	0.1308	
Bn-scaff_16310_1-p45097	SNP	C03	133.8	25952943	0.0993	
Bn-scaff_20103_1-p133338	SNP	C03	134.2	25352453	0.0741	
Bn-scaff_16310_1-p46451	SNP	C03	134.7		0.0993	
Bn-scaff_20079_1-p21999	SNP	C03	135.6	26814745	0.0545	
Bn-scaff_16092_1-p845686	SNP	C03	136.6	28543961	0.0993	
Bn-scaff_18917_1-p433571	SNP	C03	137.5		0.1696	
Bn-scaff_16130_2-p707487	SNP	C03	138.5	2346818	0.0993	
Bn-scaff_15695_1-p564807	SNP	C03	138.9	30660549	0.0741	
Bn-scaff_17869_1-p661079	SNP	C03	139.4	32376712	0.0545	
Bn-scaff_15695_1-p776379	SNP	C03	140.4	30383742	0.0993	
Bn-scaff_18855_1-p405082	SNP	C03	141.3	31763661	0.0545	
Bn-scaff_17044_1-p531625	SNP	C03	143.7	33528101	0.0394	*
Bn-scaff_18356_1-p292961	SNP	C03	148.9	34607781	0.2718	
Bn-scaff_25323_1-p6946	SNP	C03	149.3	35725920	0.3363	
Bn-scaff_18356_1-p272201	SNP	C03	149.8	34629778	0.2718	
Bn-scaff_19310_1-p432772	SNP	C03	150.3	35229125	0.3363	
Bn-scaff_21330_1-p291816	SNP	C03	151.2	41016905	0.3363	
Bn-A08-p17190098	SNP	C03	154.1	47241633	0.4922	
Bn-scaff_16372_1-p94487	SNP	C03	155.0	48587680	0.6803	
Bn-scaff_16665_1-p20050	SNP	C03	155.5	48779387	0.5827	
Bn-scaff_17440_1-p659691	SNP	C03	156.4		0.5827	
Bn-scaff_19740_1-p203934	SNP	C03	157.4	4617696	0.5827	
Bn-A08-p16966023	SNP	C03	158.3	47771339	0.5827	
Bn-scaff_17440_1-p25854	SNP	C03	158.8	48460619	0.6803	
Bn-C13868303-p376	SNP	C03	159.3	48754940	0.5827	
Bn-scaff_17440_1-p20595	SNP	C03	159.7		0.6803	
Bn-scaff_24631_1-p338156	SNP	C03	161.1	49195016	0.4098	
Bn-scaff_24631_1-p415987	SNP	C03	161.6	49299796	0.4922	
Bn-scaff_24631_1-p422231	SNP	C03	162.6	49305933	0.6803	
Bn-scaff_16394_2-p1431681	SNP	C03	170.2	49774450	0.6803	
Bn-scaff_24631_1-p738703	SNP	C03	170.6	49659645	0.5827	
Bn-scaff_16394_2-p1373926	SNP	C03	171.6	5068962	0.7835	
Bn-scaff_16394_2-p1264969	SNP	C03	172.0	49972081	0.6803	
Bn-scaff_16394_2-p1075976	SNP	C03	172.5	50176349	0.7835	
Bn-scaff_16394_2-p1023437	SNP	C03	173.0		0.8907	
Bn-scaffold19029-p392	SNP	C03	173.5	50495268	1.0000	
brPb-809548	*DArT*	*C03*	*176.8*		*0.6788*	
Bn-scaff_16394_2-p883473	SNP	C03	179.8	50360672	0.8907	
Bn-scaff_16394_2-p992866	SNP	C03	180.2		1.0000	

Marker	Marker type	LG	Position (cM)	*B. napus* physical position	Chi² 1:1 (*p*-value)	Chi² 1:1 Significance
Bn-scaff_16182_1-p319123	SNP	C03	181.7	51969264	0.6803	
Bn-scaff_26846_1-p30953	SNP	C03	182.6	52628184	0.8907	
Bn-scaff_26846_1-p38699	SNP	C03	183.6	52632098	0.8907	
Bn-scaff_17042_1-p180246	SNP	C03	184.0	53306239	1.0000	
Bn-scaff_17042_1-p296096	SNP	C03	184.5	53438349	0.8907	
Bn-scaff_18669_1-p41786	SNP	C03	185.0	52863596	1.0000	
E32M47-199D	*AFLP*	*C03*	*188.4*		*0.5754*	
brPb-660636	*DArT*	*C03*	*188.9*	*53172809*	*0.8364*	
brPb-839425	*DArT*	*C03*	*192.3*	*54303404*	*0.2971*	
Bn-scaff_16394_2-p207187	SNP	C03	195.8	54518639	0.4098	
Bn-scaff_17042_1-p418599	SNP	C03	196.3	5575682	0.4922	
Bn-scaff_17042_1-p319410	SNP	C03	197.2	53459665	0.6803	
Bn-scaff_17042_1-p361073	SNP	C03	198.1	53504427	0.4922	
Bn-scaff_15794_1-p310593	SNP	C03	199.6		0.2718	
Bn-scaff_15794_1-p157188	SNP	C03	200.5	5677670	0.4098	
Bn-scaff_22466_1-p1371888	SNP	C03	201.4	55417714	0.2142	
E35M62-223E	*AFLP*	*C03*	*203.6*		*0.1939*	
Bn-scaff_17592_1-p386456	SNP	C03	204.6	56649414	0.4098	
Bn-scaff_17119_1-p148890	SNP	C03	207.0	56814530	0.6803	
Bn-scaff_17821_1-p138457	SNP	C03	207.4	56881371	0.7835	
Bn-scaff_17119_1-p349622	SNP	C03	207.9		0.6803	
Bn-scaff_23761_1-p249628	SNP	C03	210.3	57481852	0.7835	
Bn-scaff_18559_1-p161529	SNP	C03	211.2	58097259	0.5827	
Bn-scaff_18559_1-p157785	SNP	C03	212.1	58101018	0.4098	
Bn-scaff_19047_1-p114228	SNP	C03	213.6	58602934	0.4922	
Bn-scaff_19047_1-p12619	SNP	C03	215.0	6115326	0.2718	
Bn-scaff_16148_1-p159973	SNP	C03	220.2	58686028	0.3363	
Bn-scaff_23098_1-p232984	SNP	C03	220.7	58743735	0.4098	
Bn-scaff_16755_1-p1426914	SNP	C03	221.6	59368600	0.5827	
Bn-scaff_16755_1-p1208174	SNP	C03	223.0	59584485	0.4922	
Bn-scaff_16755_1-p1184581	SNP	C03	223.5	59606751	0.4098	
Bn-scaff_16755_1-p223829	SNP	C03	229.2	60330444	0.0545	
Bn-scaff_18903_1-p350780	SNP	C04	0.0		1.0000	
Bn-scaff_18903_1-p127936	SNP	C04	0.5	47289390	0.9451	
Bn-scaff_18903_1-p371596	SNP	C04	1.9	47531616	0.7835	
Bn-scaff_18903_1-p568442	SNP	C04	3.3		0.5355	
Bn-scaff_18903_1-p735820	SNP	C04	3.8	4385147	0.4922	
Bn-scaff_18903_1-p757633	SNP	C04	4.3	4413426	0.5827	
Bn-scaff_18903_1-p848057	SNP	C04	4.7	48065045	0.6803	
Bn-scaff_20817_1-p15523	SNP	C04	5.7	48734471	0.4922	
Bn-scaff_20817_1-p17196	SNP	C04	6.1	48732787	0.4098	
Bn-A04-p18918997	SNP	C04	7.1	48844133	0.5827	
Bn-scaff_26787_1-p53113	SNP	C04	16.1	47140908	1.0000	

Marker	Marker type	LG	Position (cM)	*B. napus* physical position	Chi² 1:1 (*p*-value)	Chi² 1:1 Significance
Bn-scaff_26787_1-p54705	SNP	C04	16.6	47142500	0.8364	
Bn-scaff_20270_1-p1006352	SNP	C04	17.1	47079845	0.9451	
Bn-A04-p17766173	SNP	C04	19.5	46950993	0.7835	
Bn-scaff_19523_1-p545907	SNP	C04	21.8	46810706	0.3363	
Bn-scaff_20270_1-p632286	SNP	C04	22.8	46752815	0.2164	
Bn-scaff_20270_1-p456204	SNP	C04	24.7	46573131	0.0852	
Bn-scaff_16888_1-p1301583	SNP	C04	32.3		0.0394	*
Bn-scaff_16888_1-p1168071	SNP	C04	32.7		0.0545	
Bn-scaff_16888_1-p1347380	SNP	C04	33.2	45497676	0.0394	*
Bn-scaff_20804_1-p9294	SNP	C04	37.0	44770385	0.0091	**
Bn-scaff_20804_1-p84419	SNP	C04	37.5	44693180	0.0134	*
Bn-scaff_20804_1-p111983	SNP	C04	37.9	4159111	0.0195	*
Bn-scaff_15585_1-p1089867	SNP	C04	38.4	44500183	0.0280	*
Bn-scaff_20804_1-p85446	SNP	C04	39.4	44692157	0.0134	*
Bn-scaff_20804_1-p43582	SNP	C04	39.8	44735690	0.0091	**
Bn-C13873857-p268	SNP	C04	41.2	44543739	0.0280	*
Bn-scaff_15585_1-p1069334	SNP	C04	41.7	44478300	0.0195	*
Bn-scaff_15585_1-p265441	SNP	C04	45.5	44202300	0.1308	
Bn-scaff_16804_2-p261726	SNP	C04	47.4	42745422	0.0394	*
brPb-670011	*DArT*	*C04*	*55.1*	*38602046*	*0.3685*	
E45M49-378E	*AFLP*	*C04*	*55.6*		*0.2396*	
E32M47-122E	*AFLP*	*C04*	*56.1*		*0.4445*	
Bn-scaff_21956_1-p232134	SNP	C04	59.0	39386078	0.0993	
Bn-scaff_16700_1-p126138	SNP	C04	59.5	38672627	0.0741	
Bn-scaff_16700_1-p25306	SNP	C04	60.4		0.1308	
Bn-scaff_15852_1-p103172	SNP	C04	62.8	37802520	0.4098	
Bn-scaff_15852_1-p257905	SNP	C04	63.2	37963134	0.3363	
Bn-scaff_15852_1-p593067	SNP	C04	63.7	3848161	0.2718	
Bn-C13715031-p207	SNP	C04	64.6		0.4098	
Bn-scaff_15798_1-p699844	SNP	C04	65.1	37397803	0.3363	
Bn-scaff_15798_1-p202558	SNP	C04	65.6	36817160	0.2718	
Bn-scaff_15798_1-p104278	SNP	C04	66.1	36720494	0.3363	
Bn-scaff_15798_1-p11929	SNP	C04	67.5	36638464	0.5827	
Bn-scaff_15798_1-p97409	SNP	C04	69.0	36717240	0.3363	
Bn-scaff_19043_1-p440830	SNP	C04	71.3	36165731	0.7835	
Bn-scaff_19208_1-p527194	SNP	C04	72.3	35156307	1.0000	
Bn-scaff_16694_1-p236039	SNP	C04	72.8	36069917	0.8907	
Bn-scaff_16694_1-p296501	SNP	C04	74.6	36135111	0.6803	
Bn-scaff_17525_1-p117065	SNP	C04	75.1		0.5827	
Bn-scaff_16394_1-p842382	SNP	C04	75.6	32137244	0.6803	
Bn-scaff_18062_1-p114555	SNP	C04	76.1	31237430	0.5827	
Bn-scaff_16394_1-p847636	SNP	C04	76.5	32142501	0.6803	
E44M62-208E	*AFLP*	*C04*	*77.0*		*0.8897*	

Marker	Marker type	LG	Position (cM)	*B. napus* physical position	Chi² 1:1 (*p*-value)	Chi² 1:1 Significance
Bn-scaff_27173_1-p1097	SNP	C04	81.9	30270712	0.5827	
Bn-scaff_15779_1-p149362	SNP	C04	82.3		0.4922	
Bn-scaff_18776_1-p508763	SNP	C04	84.2	29899107	0.8907	
Bn-scaff_18776_1-p263330	SNP	C04	86.6	29710854	0.5827	
Bn-scaff_27914_1-p9919	SNP	C04	87.5		0.7835	
brPb-658096	*DArT*	*C04*	*89.4*		*0.9451*	
brPb-671298	*DArT*	*C04*	*94.2*	*22567802*	*0.9449*	
E35M62-273E	*AFLP*	*C04*	*94.7*		*0.7256*	
Bn-scaff_16192_1-p12662	SNP	C04	96.2	19291400	0.7835	
Bn-scaff_16309_1-p144811	SNP	C04	97.1	2686938	1.0000	
Bn-A04-p1472739	SNP	C04	97.6	24597648	0.8907	
Bn-scaff_16309_1-p215682	SNP	C04	98.5		0.8907	
Bn-scaff_16192_1-p12984	SNP	C04	99.0	19291078	0.7835	
Bn-scaff_19253_1-p56432	SNP	C04	103.2	15027977	0.3363	
Bn-scaff_27513_1-p93870	SNP	C04	103.7		0.2718	
Bn-scaff_19821_1-p399685	SNP	C04	105.1	11297665	0.3363	
Bn-scaff_15607_1-p7680	SNP	C04	105.6	11473214	0.2718	
Bn-C13765949-p42	SNP	C04	106.1	11340589	0.3363	
Bn-scaff_19253_1-p152044	SNP	C04	108.0		0.3363	
E33M47-160E	*AFLP*	*C04*	*110.6*		*0.7175*	
brPb-660503	*DArT*	*C04*	*113.9*	*10855937*	*0.3639*	
Bn-scaff_23699_1-p169605	SNP	C04	115.8		0.2718	
Bn-scaff_16095_1-p30079	SNP	C04	116.3	9983172	0.2164	
Bn-scaff_17869_1-p115797	SNP	C04	117.2	9134841	0.2164	
Bn-scaff_23907_1-p3780	SNP	C04	128.7	7268917	0.2164	
Bn-scaff_21369_1-p190637	SNP	C04	132.6		0.8907	
Bn-scaff_15908_1-p1114490	SNP	C04	133.6	6260753	0.8907	
Bn-scaff_15908_1-p885163	SNP	C04	135.0	6031843	0.7835	
Bn-scaff_16534_1-p2435936	SNP	C04	136.0	995885	0.5827	
Bn-scaff_16534_1-p1510638	SNP	C04	138.3	4153883	0.6803	
Bn-scaff_16534_1-p1901223	SNP	C04	138.8	4551279	0.5827	
brPb-660050	*DArT*	*C04*	*141.4*		*0.8282*	
E32M49-110D	*AFLP*	*C04*	*143.7*		*0.2555*	
Bn-scaff_16534_1-p551394	SNP	C04	148.2		0.5827	
Bn-scaff_16534_1-p22373	SNP	C04	149.6	2791744	0.4922	
Bn-scaff_16214_1-p263853	SNP	C04	150.6	2412721	0.3363	
Bn-scaff_28382_1-p131432	SNP	C04	151.5	2093733	0.2164	
Bn-scaff_16356_1-p78632	SNP	C04	152.0	630111	0.2718	
Bn-scaff_27469_1-p101056	SNP	C04	154.8		0.2718	
Bn-scaff_25686_1-p9156	SNP	C04	157.2	1637404	0.3363	
Bn-scaff_16027_1-p386406	SNP	C04	157.7	1269444	0.4098	
Bn-scaff_24859_1-p44428	SNP	C04	161.0	818510	0.2164	
Bn-A05-p458357	SNP	C04	161.4	467061	0.1696	

Marker	Marker type	LG	Position (cM)	*B. napus* physical position	Chi² 1:1 (*p*-value)	Chi² 1:1 Significance
Bn-scaff_15832_1-p52737	SNP	C04	162.4	378647	0.0993	
Bn-scaff_19673_1-p11357	SNP	C04	163.8	175337	0.0394	*
Bn-scaff_23534_1-p36688	SNP	C04	164.3	79235	0.0280	*
Bn-scaff_22933_1-p70880	SNP	C04	164.7	39554	0.0195	*
Bn-scaff_20809_1-p64287	SNP	C05	0.0	43092413	0.2164	
Bn-scaff_21634_1-p20207	SNP	C05	1.9	42609111	0.2164	
Bn-scaff_15676_1-p341508	SNP	C05	2.9		0.7230	
Bn-scaff_21634_1-p19901	SNP	C05	3.9	42608798	0.7230	
Bn-scaff_21634_1-p61428	SNP	C05	5.9	42644580	0.5827	
Bn-scaff_21634_1-p62623	SNP	C05	7.3	42645558	0.3363	
Bn-scaff_20376_1-p336606	SNP	C05	13.4	42240817	0.6197	
Bn-scaff_20270_1-p1434329	SNP	C05	14.9		0.5235	
Bn-scaff_20270_1-p1323200	SNP	C05	15.9	41750615	0.7230	
Bn-scaff_16526_1-p42180	SNP	C05	16.9	41465761	0.9435	
Bn-scaff_20270_1-p1070285	SNP	C05	17.4	41549696	0.8316	
E33M49-454E	*AFLP*	*C05*	*21.2*		*0.3961*	
brPb-838927	*DArT*	*C05*	*21.7*	*40851858*	*0.3317*	
Bn-scaff_20219_1-p152816	SNP	C05	22.7	40792867	0.9435	
Bn-scaff_20219_1-p128114	SNP	C05	24.2		0.7230	
Bn-scaff_20219_1-p52642	SNP	C05	29.2	40731490	0.1290	
Bn-scaff_20219_1-p50816	SNP	C05	29.7	40729821	0.5235	
brPb-840901	*DArT*	*C05*	*31.2*	*40064093*	*0.8356*	
Bn-scaff_17441_1-p958418	SNP	C05	31.7	40077590	0.8907	
Bn-scaff_17441_3-p28950	SNP	C05	32.7	40322684	0.6803	
Bn-scaff_29028_1-p9948	SNP	C05	38.8		0.1696	
Bn-A05-p22111286	SNP	C05	39.9	39985396	0.0993	
Bn-scaff_17441_1-p269557	SNP	C05	43.7		0.2419	
Bn-scaff_21369_1-p406725	SNP	C05	54.7	38540195	0.6803	
Bn-scaff_21369_1-p871257	SNP	C05	55.2	38057288	0.7835	
Bn-A09-p4436808	SNP	C05	56.6		0.4922	
Bn-scaff_23408_1-p96806	SNP	C05	57.6	37805451	0.3363	
Bn-scaff_18826_1-p17445	SNP	C05	58.5	37349567	0.3363	
Bn-scaff_18826_1-p97796	SNP	C05	60.4	37280327	0.6803	
Bn-A05-p19943528	SNP	C05	61.8		1.0000	
Bn-scaff_18826_1-p633463	SNP	C05	62.8	36604519	1.0000	
Bn-scaff_16770_1-p317634	SNP	C05	65.6	35621473	0.7835	
Bn-scaff_15695_2-p413042	SNP	C05	72.3	30679776	0.4098	
Bn-scaff_15969_1-p200804	SNP	C05	74.6		0.4922	
Bn-scaff_20125_1-p151394	SNP	C05	80.8	11066535	0.7835	
brPb-808840	*DArT*	*C05*	*82.8*	*11071733*	*0.6299*	
Bn-scaff_21338_1-p782134	SNP	C05	84.9	12304517	0.7835	
Bn-C13793433-p23	SNP	C05	85.8	10190075	1.0000	
Bn-scaff_22183_1-p645188	SNP	C05	90.6	8734997	0.4098	

Marker	Marker type	LG	Position (cM)	*B. napus* physical position	Chi² 1:1 (*p*-value)	Chi² 1:1 Significance
Bn-scaff_16045_1-p147607	SNP	C05	91.5	7747845	0.4489	
Bn-scaff_18181_1-p195543	SNP	C05	92.5	7443681	0.3363	
Bn-scaff_18181_1-p622258	SNP	C05	94.4	7039846	0.1308	
Bn-scaff_18181_1-p814209	SNP	C05	95.3	570629	0.1133	
Bn-scaff_15712_10-p1182534	SNP	C05	100.6	5938847	0.5355	
Bn-scaff_16670_1-p167090	SNP	C06	0.0	309233	0.1308	
Bn-scaff_18362_1-p90481	SNP	C06	0.5	779381	0.1696	
Bn-scaff_18549_1-p451733	SNP	C06	1.4	1072771	0.1696	
Bn-scaff_16984_1-p340898	SNP	C06	5.2		0.2718	
Bn-scaff_16984_1-p204543	SNP	C06	6.6	3026037	0.4922	
Bn-scaff_16984_1-p200546	SNP	C06	7.6	3030033	0.3363	
Bn-scaff_16485_1-p557881	SNP	C06	8.0	3468110	0.4098	
Bn-scaff_16485_1-p587862	SNP	C06	9.4	3496623	0.4922	
Bn-scaff_16485_1-p668116	SNP	C06	11.8		0.1696	
Bn-scaff_18344_1-p119313	SNP	C06	12.7		0.2718	
Bn-scaff_18344_1-p291478	SNP	C06	13.2	4304541	0.2164	
Bn-scaff_18344_1-p341615	SNP	C06	13.7	4365263	0.2718	
brPb-660333	*DArT*	*C06*	*16.1*	*4369658*	*0.2649*	
brPb-840224	*DArT*	*C06*	*19.7*	*494749*	*0.7825*	
Bn-scaff_20773_1-p340868	SNP	C06	22.9	5384872	0.7835	
Bn-scaff_27421_1-p16736	SNP	C06	23.3	5077391	0.6803	
Bn-scaff_27421_1-p89824	SNP	C06	23.8	5002061	0.5827	
Bn-scaff_27421_1-p32784	SNP	C06	24.3	5065268	0.6803	
Bn-scaff_20773_1-p123341	SNP	C06	24.7	5669221	0.7835	
Bn-scaff_16647_1-p239957	SNP	C06	25.2	559889	0.6803	
Bn-scaff_16647_1-p162647	SNP	C06	26.6	5963420	0.4098	
Bn-scaff_16647_1-p1229	SNP	C06	27.1	6153639	0.4922	
Bn-scaff_23199_1-p39784	SNP	C06	28.1	7267152	0.3363	
Bn-scaff_17917_1-p67000	SNP	C06	29.5	7975448	0.2718	
Bn-scaff_18002_1-p240843	SNP	C06	30.4	11595721	0.4098	
Bn-scaff_24402_1-p8022	SNP	C06	31.4	1194966	0.5827	
Bn-scaff_17088_3-p61230	SNP	C06	32.8	11905621	0.8907	
Bn-scaff_18439_1-p706567	SNP	C06	35.1	12826352	0.7835	
Bn-scaff_18439_1-p953290	SNP	C06	36.1	13080622	1.0000	
Bn-scaff_16510_1-p714006	SNP	C06	36.5	13547313	0.8907	
Bn-scaff_16903_1-p230137	SNP	C06	38.0		0.7835	
Bn-scaff_15818_1-p237481	SNP	C06	38.9	15527980	1.0000	
Bn-scaff_17461_1-p1185821	SNP	C06	39.4		0.8907	
Bn-scaff_15818_2-p128759	SNP	C06	39.8	16553661	0.7835	
Bn-scaff_15818_2-p1169445	SNP	C06	42.7	17546819	0.2718	
brPb-840227	*DArT*	*C06*	*46.5*	*17587065*	*0.3685*	
brPb-670643	*DArT*	*C06*	*48.4*	*18977707*	*0.5827*	
Bn-scaff_18206_3-p99826	SNP	C06	52.2		0.5827	

Marker	Marker type	LG	Position (cM)	*B. napus* physical position	Chi² 1:1 (*p*-value)	Chi² 1:1 Significance
Bn-scaff_15818_2-p1554832	SNP	C06	52.7	17917307	0.4922	
Bn-scaff_15763_1-p233999	SNP	C06	58.4	19830472	0.0631	
Bn-scaff_15763_1-p514953	SNP	C06	59.8	20087711	0.0545	
Bn-scaff_15763_1-p1401447	SNP	C06	60.3	2420591	0.0394	*
Bn-scaff_15763_1-p553985	SNP	C06	60.8	20129667	0.0545	
Bn-scaff_15763_1-p342625	SNP	C06	61.7	19910325	0.0993	
Bn-scaff_15763_1-p233149	SNP	C06	62.2	19829412	0.0741	
Bn-scaff_15763_1-p342497	SNP	C06	62.7		0.0993	
E32M47-156D	*AFLP*	*C06*	*66.1*		*0.1866*	
E34M49-073D	*AFLP*	*C06*	*69.0*		*0.0272*	*
E44M62-183D	*AFLP*	*C06*	*70.5*		*0.0511*	
E32M49-391D	*AFLP*	*C06*	*72.0*		*0.3148*	
Bn-scaff_15818_1-p2371026	SNP	C06	75.7	21776563	0.0545	
Bn-scaff_15818_1-p2022143	SNP	C06	76.6	2593048	0.0280	*
Bn-scaff_15818_1-p2355639	SNP	C06	77.5		0.0545	
Bn-scaff_15818_1-p2404767	SNP	C06	78.5	21807925	0.0280	*
Bn-scaff_15818_1-p2532249	SNP	C06	83.2	21940179	0.1696	
Bn-scaff_15818_1-p3172268	SNP	C06	84.6	22566552	0.3363	
Bn-scaff_15746_1-p101764	SNP	C06	85.1	21073990	0.2718	
Bn-scaff_16064_1-p233751	SNP	C06	88.4	2717990	0.1308	
Bn-A07-p16406072	SNP	C06	94.1	27462139	0.6803	
Bn-scaff_16116_1-p605377	SNP	C06	95.0	28471832	0.6803	
Bn-scaff_16547_1-p59615	SNP	C06	96.5	29440247	0.4098	
Bn-scaff_23957_1-p250533	SNP	C06	96.9	30781603	0.3363	
Bn-scaff_23957_1-p346986	SNP	C06	97.4	31207117	0.2718	
Bn-scaff_23957_1-p417072	SNP	C06	98.3	31276700	0.1696	
Bn-scaff_23957_1-p665858	SNP	C06	99.8	29320337	0.1308	
Bn-scaff_23957_1-p432810	SNP	C06	100.2	31297823	0.0993	
Bn-scaff_16874_1-p172867	SNP	C06	100.7	31535621	0.1308	
Bn-scaff_16874_1-p292060	SNP	C06	101.2	31673815	0.0993	
Bn-scaff_16874_1-p411591	SNP	C06	101.6	31817471	0.0741	
Bn-scaff_20741_1-p40404	SNP	C06	102.1	27738141	0.0545	
Bn-scaff_16397_1-p360292	SNP	C06	102.6	32556251	0.0394	*
brPb-660426	*DArT*	*C06*	*103.1*	*32559920*	*0.0418*	*
Bn-scaff_16397_1-p101309	SNP	C06	104.6	32808365	0.0993	
Bn-scaff_20294_1-p378164	SNP	C06	107.4	33582719	0.4098	
Bn-scaff_17799_1-p2456101	SNP	C06	110.2	34051804	0.5827	
Bn-scaff_17799_1-p2278557	SNP	C06	110.7	34227921	0.4922	
Bn-scaff_17799_1-p1999577	SNP	C06	112.6	34569614	0.3363	
Bn-scaff_17799_1-p853567	SNP	C06	120.7	35737936	0.4098	
brPb-838794	*DArT*	*C06*	*123.5*	*35778345*	*0.4098*	
Bn-scaff_17799_1-p859347	SNP	C06	126.4	35732971	0.4098	
Bn-scaff_17799_1-p458187	SNP	C06	129.2	36178610	0.2718	

Marker	Marker type	LG	Position (cM)	*B. napus* physical position	Chi² 1:1 (*p*-value)	Chi² 1:1 Significance
Bn-scaff_24104_1-p65405	SNP	C06	133.5	36993917	0.0993	
Bn-scaff_16110_1-p469361	SNP	C07	0.0	44433003	0.0394	*
Bn-scaff_16110_1-p575479	SNP	C07	0.9	44315051	0.0741	
Bn-scaff_16110_1-p1181825	SNP	C07	2.4	43764295	0.0993	
Bn-scaff_16110_1-p1204999	SNP	C07	3.3	43743748	0.1696	
Bn-scaff_16110_1-p2275269	SNP	C07	8.0	42597493	0.4098	
Bn-A03-p28265602	SNP	C07	10.0	2804600	0.5355	
Bn-scaff_16110_1-p2432728	SNP	C07	10.5	42465135	0.6803	
Bn-scaff_16110_1-p2655836	SNP	C07	10.9	42223309	0.5827	
Bn-scaff_16110_1-p2489509	SNP	C07	11.4		0.4489	
Bn-scaff_16110_1-p3437348	SNP	C07	16.7	41488455	0.5827	
Bn-scaff_16110_1-p3660759	SNP	C07	17.6	41278008	0.4098	
Bn-scaff_16069_1-p4814525	SNP	C07	18.5		0.4098	
Bn-scaff_16069_1-p4819823	SNP	C07	19.0	2618033	0.4922	
Bn-scaff_16069_1-p4228211	SNP	C07	20.0		0.2718	
Bn-scaff_16069_1-p3882959	SNP	C07	21.4	40293214	0.4922	
Bn-scaff_16069_1-p3596469	SNP	C07	24.7	40010148	0.7835	
Bn-scaff_16069_1-p3470600	SNP	C07	25.2	39887479	0.6803	
Bn-scaff_16069_1-p3417121	SNP	C07	25.6	39838720	0.5827	
Bn-scaff_16069_1-p3392606	SNP	C07	28.0	39809008	0.8907	
Bn-scaff_16069_1-p2867813	SNP	C07	29.9	39690395	0.4922	
Bn-scaff_16069_1-p1789002	SNP	C07	32.2	38196399	1.0000	
Bn-scaff_16069_1-p2323317	SNP	C07	33.7	38695286	0.6803	
Bn-scaff_16069_1-p1268741	SNP	C07	38.9	37716443	0.4098	
Bn-scaff_16069_1-p556940	SNP	C07	43.6		0.0993	
Bn-scaff_16069_1-p439239	SNP	C07	45.5	36781133	0.2718	
Bn-scaff_15705_3-p436841	SNP	C07	46.9	36219257	0.2164	
Bn-scaff_15705_3-p129321	SNP	C07	47.4	35900328	0.2718	
Bn-scaff_15705_1-p2590289	SNP	C07	48.3	35531515	0.2718	
Bn-scaff_15705_1-p2504173	SNP	C07	50.2	35449116	0.5827	
Bn-scaff_15705_1-p1403790	SNP	C07	57.3	34707905	0.6803	
Bn-scaff_15705_1-p1214538	SNP	C07	58.3	34553319	0.8907	
brPb-841894	*DArT*	*C07*	*61.3*	*34694123*	*0.7230*	
E35M47-165D	*AFLP*	*C07*	*65.1*		*0.4355*	
E34M49-118D	*AFLP*	*C07*	*66.6*		*0.3270*	
brPb-670555	*DArT*	*C07*	*68.1*	*1817519*	*0.3708*	
Bn-scaff_18520_1-p612557	SNP	C07	70.9	32469059	0.2718	
Bn-scaff_15705_1-p108561	SNP	C07	72.4	33515009	0.4922	
Bn-scaff_15705_1-p577327	SNP	C07	73.3	33974318	0.6803	
Bn-scaff_18520_1-p817618	SNP	C07	74.7	32644625	0.4098	
Bn-scaff_18520_1-p414565	SNP	C07	75.7	1818299	0.2718	
Bn-scaff_18520_1-p404968	SNP	C07	76.1	1827532	0.2164	
Bn-scaff_21711_1-p12893	SNP	C07	76.6	31806671	0.2718	

Marker	Marker type	LG	Position (cM)	*B. napus* physical position	Chi² 1:1 (*p*-value)	Chi² 1:1 Significance
Bn-scaff_17972_1-p146234	SNP	C07	79.4	31461693	0.4098	
Bn-scaff_16514_1-p133007	SNP	C07	79.9		0.3363	
Bn-scaff_17740_1-p555272	SNP	C07	80.4	30601151	0.2718	
Bn-scaff_17740_1-p834125	SNP	C07	81.8	30371629	0.1308	
Bn-scaff_17740_1-p844883	SNP	C07	82.3	30360966	0.0741	
Bn-scaff_16130_1-p2433175	SNP	C07	82.8	30054699	0.0545	
Bn-scaff_16130_1-p916846	SNP	C07	84.7	28627933	0.0134	*
Bn-scaff_16130_1-p910136	SNP	C07	85.6	28605819	0.0280	*
Bn-scaff_16130_1-p800124	SNP	C07	86.1	28502186	0.0195	*
Bn-scaff_15754_1-p651840	SNP	C07	87.5	27102046	0.0060	**
Bn-scaff_19724_1-p166186	SNP	C07	91.7	26237295	0.0091	**
Bn-scaff_19724_1-p557617	SNP	C07	93.6	25823754	0.0039	**
Bn-scaff_21268_1-p102415	SNP	C07	96.0	25181707	0.0280	*
Bn-scaff_18202_1-p379677	SNP	C07	100.2	23217609	0.0039	**
Bn-scaff_18202_1-p1568783	SNP	C07	102.6	22250104	0.0280	*
Bn-scaff_18202_1-p1683343	SNP	C07	104.0	22118953	0.0091	**
Bn-scaff_18501_1-p995878	SNP	C07	105.4	21670927	0.0134	*
Bn-scaff_18501_1-p426392	SNP	C07	105.9	20741594	0.0091	**
Bn-scaff_18501_1-p850558	SNP	C07	106.4	1105056	0.0134	*
brPb-662975	*DArT*	*C07*	*108.3*	*21440197*	*0.0218*	*
Bn-scaff_18501_1-p264708	SNP	C07	111.3	21322269	0.0091	**
Bn-scaff_19106_1-p176043	SNP	C07	113.6	18902919	0.0545	
E39M62-177E	*AFLP*	*C07*	*114.1*		*0.0375*	*
Bn-scaff_17461_1-p1193733	SNP	C07	120.9	9960996	0.0195	*
Bn-scaff_15762_1-p717223	SNP	C07	122.3	2201365	0.0060	**
Bn-scaff_20947_1-p137826	SNP	C08	0.0	36705377	0.3018	
Bn-scaff_21269_1-p309569	SNP	C08	1.9		0.1909	
Bn-scaff_20901_1-p2019548	SNP	C08	5.2	37694422	0.4922	
Bn-scaff_16389_1-p688011	SNP	C08	6.6	4498088	0.6299	
Bn-scaff_16389_1-p907642	SNP	C08	7.1	28127520	0.6803	
Bn-scaff_20947_1-p93326	SNP	C08	14.3	36745442	0.3018	
Bn-scaff_16445_1-p307890	SNP	C08	15.2	36515197	0.2718	
Bn-scaff_16445_1-p591427	SNP	C08	18.6	36217699	0.3363	
Bn-scaff_16445_1-p895858	SNP	C08	19.5	35905726	0.4922	
Bn-scaff_16445_1-p933254	SNP	C08	20.5	35852130	0.4922	
Bn-scaff_16445_1-p925530	SNP	C08	20.9	35858936	0.6299	
Bn-scaff_16445_1-p1443287	SNP	C08	24.3	35331835	0.1483	
Bn-scaff_16445_1-p1639969	SNP	C08	25.7		0.1483	
brPb-670302	*DArT*	*C08*	*30.0*	*34952631*	*0.3018*	
Bn-A09-p33660289	SNP	C08	33.8	34965592	0.1133	
Bn-scaff_16445_1-p2125574	SNP	C08	36.2	34737240	0.2419	
Bn-scaff_16445_1-p2523413	SNP	C08	36.7	34371263	0.2718	
Bn-scaff_16445_1-p2119017	SNP	C08	37.1	34743849	0.2419	

Marker	Marker type	LG	Position (cM)	*B. napus* physical position	Chi² 1:1 (*p*-value)	Chi² 1:1 Significance
Bn-scaff_16197_1-p135491	SNP	C08	42.0	4172989	0.4922	
Bn-scaff_16197_1-p689617	SNP	C08	44.8	4127932	0.8907	
Bn-scaff_16197_1-p724640	SNP	C08	45.3	4167496	0.7835	
Bn-scaff_16197_1-p1137119	SNP	C08	45.8	32958180	0.6803	
Bn-scaff_16197_1-p1166542	SNP	C08	47.2	32929279	1.0000	
Bn-scaff_16197_1-p1348777	SNP	C08	47.7	32767525	0.8907	
Bn-scaff_16197_1-p1367016	SNP	C08	48.6	32753070	0.6803	
Bn-scaff_16197_1-p1382140	SNP	C08	49.5	32737137	0.4922	
Bn-scaff_16197_1-p1413767	SNP	C08	50.0		0.4098	
Bn-scaff_16197_1-p1830408	SNP	C08	51.4	32311654	0.2164	
Bn-scaff_16197_1-p2310244	SNP	C08	52.9	31904079	0.0993	
Bn-scaff_16197_1-p2405266	SNP	C08	53.8	31790087	0.1696	
Bn-scaff_16197_1-p2605576	SNP	C08	54.3	31605425	0.1308	
Bn-scaff_16197_1-p2367309	SNP	C08	55.2	31828785	0.1308	
E39M62-95D	*AFLP*	*C08*	*56.7*		*0.0687*	
E37M62-271E	*AFLP*	*C08*	*60.4*		*0.0064*	**
Bn-scaff_16197_1-p2872732	SNP	C08	62.0	31384792	0.0280	*
Bn-scaff_16197_1-p2626407	SNP	C08	62.9	31578417	0.0545	
Bn-scaff_16197_1-p3061092	SNP	C08	64.3	31236965	0.0195	*
Bn-scaff_16197_1-p2958698	SNP	C08	64.8	31316166	0.0280	*
Bn-scaff_16361_1-p3355395	SNP	C08	65.3	30830072	0.0195	*
Bn-scaff_16361_1-p2750042	SNP	C08	66.2	30245443	0.0195	*
Bn-scaff_16361_1-p2263072	SNP	C08	66.7	29800595	0.0134	*
Bn-scaff_16361_1-p2750581	SNP	C08	67.1		0.0195	*
brPb-660785	*DArT*	*C08*	*68.6*		*0.0167*	*
Bn-scaff_16231_1-p1176976	SNP	C08	92.6	20727271	0.0394	*
brPb-663376	*DArT*	*C08*	*103.1*	*19013385*	*0.1216*	
Bn-scaff_19242_1-p390109	SNP	C08	108.6	18065063	0.0741	
Bn-scaff_22350_1-p80848	SNP	C08	111.4		0.0741	
Bn-scaff_16545_1-p172608	SNP	C08	113.3	2405829	0.0741	
Bn-scaff_18275_1-p1067723	SNP	C08	115.2	2888744	0.1308	
Bn-scaff_16273_1-p360866	SNP	C08	119.0		0.2164	
Bn-scaff_18310_1-p332839	SNP	C08	119.5	6435227	0.1696	
Bn-scaff_17807_1-p364572	SNP	C08	119.9	3357643	0.1696	
Bn-scaff_27765_1-p53066	SNP	C08	120.4	3024527	0.2164	
Bn-scaff_17367_1-p687815	SNP	C08	120.9		0.2718	
Bn-scaff_18607_1-p283263	SNP	C08	121.8	801987	0.2718	
brPb-660345	*DArT*	*C08*	*125.6*	*2261923*	*0.2718*	
Bn-scaff_15650_1-p714363	SNP	C09	0.0	17312803	0.5827	
Bn-scaff_15650_1-p368565	SNP	C09	0.5	1580696	0.6803	
Bn-scaff_17109_1-p373778	SNP	C09	1.4	18368561	0.8907	
Bn-scaff_15650_1-p45061	SNP	C09	1.9		0.7835	
Bn-scaff_15650_1-p321251	SNP	C09	2.4	1493349	0.6803	

Marker	Marker type	LG	Position (cM)	*B. napus* physical position	Chi² 1:1 (*p*-value)	Chi² 1:1 Significance
Bn-scaff_15650_1-p683404	SNP	C09	2.8	17345389	0.5827	
Bn-scaff_22835_1-p309344	SNP	C09	3.3	17583602	0.6803	
Bn-scaff_17109_1-p59759	SNP	C09	4.2	18705902	0.8907	
Bn-scaff_18424_1-p878895	SNP	C09	7.6	1891589	0.4098	
Bn-scaff_17339_1-p262780	SNP	C09	10.9	21144841	0.3363	
Bn-scaff_18326_1-p1144589	SNP	C09	11.3	26086220	0.2718	
Bn-scaff_17339_1-p292992	SNP	C09	11.8	21120874	0.3363	
Bn-scaff_17910_1-p126780	SNP	C09	14.6	33576423	0.1308	
Bn-scaff_17367_1-p9887	SNP	C09	15.1		0.0993	
Bn-scaff_20619_1-p306931	SNP	C09	15.6	2972845	0.1308	
Bn-scaff_16246_2-p75351	SNP	C09	16.1	31883285	0.0993	
Bn-scaff_19483_1-p121870	SNP	C09	16.5		0.1308	
Bn-scaff_15838_2-p186362	SNP	C09	17.0		0.1696	
Bn-scaff_21276_1-p345081	SNP	C09	17.9	35841715	0.0993	
Bn-scaff_17799_1-p2937790	SNP	C09	25.5	39679168	0.0134	*
Bn-scaff_15881_1-p118945	SNP	C09	26.0	39330784	0.0091	**
Bn-scaff_17554_1-p19888	SNP	C09	26.5	39720917	0.0134	*
Bn-scaff_19899_1-p651590	SNP	C09	28.8	40474372	0.0195	*
Bn-scaff_17028_1-p450579	SNP	C09	36.0	42936669	0.0993	
Bn-scaff_21841_1-p144151	SNP	C09	37.4	43109282	0.0741	
Bn-scaff_21841_1-p174651	SNP	C09	37.9	43128718	0.1463	
Bn-A10-p12810049	SNP	C09	39.8	42966699	0.0728	
Bn-scaff_15576_1-p225938	SNP	C09	43.1		0.0394	*
brPb-808246	*DArT*	*C09*	*43.6*	*41099048*	*0.0216*	*
Bn-scaff_19899_1-p356624	SNP	C09	44.7	40719229	0.0091	**
brPb-839212	*DArT*	*C09*	*56.0*	*32331167*	*0.0741*	
E33M47-146D	*AFLP*	*C09*	*66.1*		*0.6215*	
brPb-660506	*DArT*	*C09*	*71.1*	*13660845*	*0.6803*	
E35M47-80D	*AFLP*	*C09*	*72.6*		*0.4378*	
E32M47-285E	*AFLP*	*C09*	*74.7*		*0.4261*	
E44M62-167E	*AFLP*	*C09*	*75.3*		*0.2673*	
E35M62-167D	*AFLP*	*C09*	*75.8*		*0.3293*	
Bn-scaff_20202_1-p179338	SNP	C09	77.2	1271394	0.5827	
Bn-scaff_17888_1-p437754	SNP	C09	77.7	15529668	0.4922	
Bn-A09-p15852129	SNP	C09	79.6	1452810	0.8907	
Bn-scaff_15650_1-p750450	SNP	C09	80.5	17284661	0.8907	
Bn-scaff_15650_1-p924603	SNP	C09	81.5	17078535	0.8907	
Bn-scaff_18100_1-p344652	SNP	C09	84.8	13975500	0.5827	
Bn-scaff_18100_1-p96955	SNP	C09	85.3	13737502	0.6803	
Bn-scaff_17174_1-p114224	SNP	C09	85.7	13322851	0.7835	
Bn-scaff_17174_1-p258626	SNP	C09	86.2	732717	0.8907	
Bn-scaff_17174_1-p272081	SNP	C09	86.7	727617	0.7835	
Bn-scaff_18839_1-p1092979	SNP	C09	87.2	12521285	0.6803	

Marker	Marker type	LG	Position (cM)	*B. napus* physical position	Chi² 1:1 (*p*-value)	Chi² 1:1 Significance
E33M47-132D	*AFLP*	*C09*	*91.1*		*0.1065*	
Bn-scaff_17487_1-p1890044	SNP	C09	92.1	8175527	0.2164	
Bn-scaff_17487_1-p1909011	SNP	C09	92.6	8186841	0.1696	
Bn-scaff_17487_1-p1782018	SNP	C09	93.0	8067659	0.2164	
Bn-scaff_17487_1-p1258245	SNP	C09	94.0	336971	0.1308	
Bn-scaff_17487_1-p527387	SNP	C09	94.4	6790708	0.1696	
Bn-scaff_17487_1-p781216	SNP	C09	94.9	7096622	0.1308	
Bn-scaff_17190_1-p1119408	SNP	C09	100.6	4482056	0.3363	
Bn-scaff_17190_1-p1116406	SNP	C09	101.5	4479050	0.4922	
Bn-scaff_19783_1-p384442	SNP	C09	103.0	2854636	0.6299	
Bn-A09-p2419287	SNP	C09	103.4		0.5355	
Bn-scaff_16486_1-p88236	SNP	C09	107.8		0.1133	
E45M49-173E	*AFLP*	*C09*	*112.2*		*0.6299*	
E35M62-233E	*AFLP*	*C09*	*113.2*		*0.8886*	
brPb-671034	*DArT*	*C09*	*113.7*	*985076*	*0.6299*	
brPb-660449	*DArT*	*C09*	*115.1*	*2058650*	*0.5336*	
brPb-663692	*DArT*	*C09*	*115.6*	*2041777*	*0.6788*	
Bn-A09-p2730673	SNP	C09	117.5	2894200	0.5827	