FIELD EVALUATION AND MOLECULAR STUDY OF A DOUBLED HAPLOID POPULATION OF *Brassica napus* SEGREGATING FOR LINOLENIC ACID CONTENT

BY

ALLISON CATHERINE BROWN HOEPPNER

A Thesis Submitted to the Faculty of Graduate Studies
in Partial Fulfilment of the Requirements for the Degree of

MASTER OF SCIENCE

Department of Plant Science
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THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

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ABSTRACT

Brown Hoeppner, Allison Catherine. M.Sc., The University of Manitoba, May, 2002. Field evaluation and molecular study of a doubled haploid population of *Brassica napus* segregating for linolenic acid content. Major professor: Dr. Rachael Scarth

Linolenic acid (C18:3) is one of several long chain fatty acids present in the oil of rapeseed (*Brassica napus* and *Brassica rapa*). The high level of linolenic acid (8-12%) found in conventional *B. napus* cultivars leads to oxidative rancidity, giving the oil an off-flavour and reducing its shelf life. Although low linolenic acid *B. napus* genotypes exist, breeding for low linolenic acid content is complicated by polygenic inheritance, maternal factors and environmental effects. The first objective of this research project was to evaluate several agronomic and quality traits of a doubled haploid (DH) population of *B. napus* (Reston x LL09) segregating for linolenic acid content in replicated field studies. Low linolenic acid content was correlated with later flowering and later maturity. This suggests that incorporating the low linolenic acid trait into early flowering and maturing cultivars could be more challenging. No correlations were found between linolenic acid content and oil content, protein content or plant height. The second objective of this research project was to convert a Random Amplified Polymorphic DNA (RAPD) marker associated with linolenic acid content into an Allele Specific Amplicon (ASA). The ASA350 locus accounted for 27% of the variation in linolenic acid content and mapped 5.2cM from *fad3*A, one of the genes involved in the synthesis of linolenic acid. Therefore, ASA350 could be used in making selections for low linolenic acid genotypes. The phenotypic and genotypic

distributions obtained from this DH population support the previous reports that two genes control linolenic acid content in *B. napus*.

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1. INTRODUCTION

The oil derived from rapeseed (*Brassica napus* and *Brassica rapa*) is composed primarily of long chain fatty acids including palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2), linolenic (C18:3), eicosenoic (C22:0) and erucic (C22:1) acids. The levels and ratios of the various fatty acids are important factors in determining oil quality and therefore, modifying the fatty acid profile has been a breeding objective over the past few decades. One of the most important advances in oil quality breeding was the reduction of erucic acid, an anti-nutritional component of rapeseed oil, from approximately 50% to 0% (Steffanson and Downey, 1995). Rapeseed oil with reduced levels of erucic acid in the oil was termed 'canola'. Breeders since have focused on altering levels of other fatty acids, including linolenic acid.

Conventional canola quality *B. napus* cultivars contain 8-12% linolenic acid. The high level of linolenic acid is a source of oxidative rancidity and thus gives the oil a distasteful flavour and reduces its shelf life. Furthermore, canola oil must be partially hydrogenated to reduce the linolenic acid content if used for industrial frying. Hydrogenation is an expensive process and leads to the formation of *trans* fatty acids, which pose some nutritional concerns (Scarth et al., 1992). Breeding for canola cultivars with reduced levels of linolenic acid is an important breeding goal.

The range of linolenic acid in *Brassica* germplasm was originally very narrow (6-12%) so chemical mutagenesis was used to broaden the range (2-20%) (Robbelen and Nitch, 1975.) The mutant line M11 was the source of variation used by the University of Manitoba to develop Stellar, the world's first canola quality cultivar with oil low in linolenic acid (Scarth et al., 1988). However, the development of low linolenic acid cultivars has been quite slow despite the availability of low linolenic acid genotypes. Breeding for low linolenic acid is complicated by polygenic inheritance of the trait. Conventional breeding as well as molecular studies have suggested that at least two genes are involved in the genetic control of linolenic acid content (Chen and Beversdorf, 1990; Jourdren et al., 1996a). Maternal and environmental factors may also have an influence on the accumulation of linolenic acid (Thomas and Kondra, 1973; Pleines and Freidt, 1989; Rajcan, 1996). Corrleations have been reported between linolenic acid content and some agronomic and quality traits in field studies (Rajcan et al., 1997). These associations could make breeding for low linolenic acid levels in improved genetic backgrounds more difficult (Rajcan et al., 1997).

Over the past decade, many research groups have developed molecular markers for linolenic acid content in *B. napus*. Breeding for low linolenic acid content using molecular markers is potentially very efficient, as selections could be made based on genotype. Various types of molecular markers associated with linolenic acid content have been developed, including Restriction Fragment Length Polymorphisms (RFLP) and Random Amplified Polymorphic DNA (RAPD)

(Hu et al., 1995; Jourdren et al., 1996a; Thormann et al., 1996; Somers et al., 1998, Hu et al., 1999, Rajcan et al., 1999). Most recently, Barret et al. (1999) and Somers and Rakow (PTC/CA00/01140) identified mutations in *fad3*, one of the genes believed to be responsible for the synthesis of linolenic acid content. Specific markers associated with the mutant alleles were identified.

The understanding of the inheritance of linolenic acid and the effects of reduced linolenic acid is incomplete. The first objective of this research project was to evaluate the association of agronomic and quality traits in a DH population segregating for linolenic acid content in replicated field studies. The second objective was to develop an allele specific amplicon (ASA) DNA-based marker that could be used as a selection tool in breeding for low linolenic acid in *B. napus*.

2. LITERATURE REVIEW

2.1 History of Rapeseed

Origin

The Cruciferae family consists of many important crop species including rapeseed/ oilseed rape *(Brassica napus and Brassica rapa*). It appears that *Brassica* species may have been among the earliest domesticated plants, and the production of rapeseed itself can be traced back to 2000 BC in India (Downey & Robbelen, 1989). Evolution data indicates that *B. rapa* was the first of the current rapeseed species to evolve, likely in the Mediterranean region with a secondary centre of origin in Asia. Its wide distribution, however, has made it difficult to identify the primary centre of origin. *B. napus* is believed to have evolved at least several hundred years later in the Mediterranean region as a result of an interspecific cross between *B. rapa* and *B. oleracea* (Downey and Robbelen, 1989). The relationship among the common *Brassica* species is illustrated in Figure 2.1.

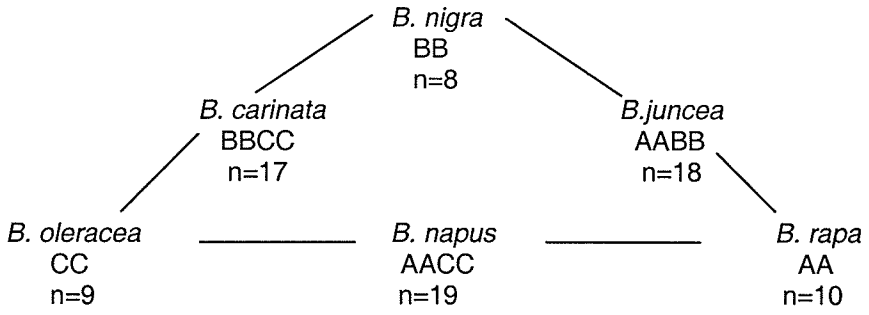

Figure 2.1. U's triangle illustrating the relationship among the *Brassica* species (U, 1935). (A, B, C = genome; n = chromosome number)

Development of canola

Rapeseed was first introduced to Canada from Poland in 1936 by a Polish farmer in Saskatchewan who had received seeds of *B. rapa* from his homeland. *B. napus* was introduced a few years later from Argentina. Originally, the interest in rapeseed production was based on the fact that its oil contained high levels of the long chain fatty acid erucic acid and could be used as a marine engine lubricant. After World War II, the demand for rapeseed oil as a lubricant dropped (Stefansson and Downey, 1995) but the economic need for crop diversification on the Canadian prairies and the desire for a domestic source of edible vegetable oil increased the interest in rapeseed production (Bell, 1982).

Prior to 1960 all known rapeseed varieties contained two anti-nutritional components – erucic acid (in the seed oil) and glucosinolates (in the seed meal). Lab studies found that rapeseed oil high in erucic acid was poorly digested and reduced growth in experimental animals (Sauer and Kramer, 1983). Subsequently, breeding was undertaken to reduce the amount of this fatty acid. In 1959, a line of rapeseed containing low levels of erucic acid, Liho, was identified and breeding for rapeseed cultivars with lower erucic acid began. The first low erucic acid *B. napus* cultivar 'Oro' was released in 1968 followed by the *B. rapa* cultivar 'Span' in 1971 (Eskin et al., 1996). During the 1960's, glucosinolates, the anti-nutritional components of rapeseed meal, were found to contribute to liver disease in poultry, with adverse effects on growth and weight gain in animals (Sauer and Kramer, 1983). The low glucosinolate trait was

identified in the late 1960's and breeding integrated this trait into agronomically adapted low-erucic lines. The first low erucic acid, low glucosinolate *B. napus* cultivar 'Tower' was released in 1974, followed by the first double low *B. rapa* cultivar 'Candle' in 1978 (Bell, 1982).

The Western Canadian Oilseed Crushers Association registered the term 'canola' in 1978, referring to rapeseed cultivars containing less than 5% erucic acid in the oil and less than 3mg/g of aliphatic glucosinolates in the meal (Eskin et al., 1996). The canola trademark was transferred to the Canola Council of Canada and, according to the current variety registration criteria, canola now refers to oilseed rape having less than 1% erucic acid in the seed and less than 18 mmols of glucosinolates per gram of whole seed (Canola Council of Canada, 2002).

Distribution and economic importance

Canola is well adapted to both temperate and warm-temperate zones and is produced in areas of North America, Europe, Asia and Australia (Eskin et al., 1996). Canola production in Canada is primarily in southern Manitoba, central Saskatchewan, central Alberta and the Peace River region of Alberta. Approximately 9.6 millions acres of canola were grown in Canada in 2001, with 4.7 million, 2.9 million and 1.9 million acres grown in Alberta, Saskatchewan and Manitoba respectively. This represented approximately 5.1 million tonnes of seed produced (Canola Council of Canada, 2002).

From the 1960s until the mid 1980s, rapeseed/ canola ranked fifth in world production of oilseeds, behind soybean, cottonseed, groundnut and sunflower (Kimber and McGregor, 1995; Weiss, 2000). Since then, canola production has increased dramatically and on a global scale, canola now ranks second after soybean in oilseed production (Weiss, 2000).

Canola is the second most important commercial and export crop in Canada after wheat. Since the mid 1980's, Canada has been the largest average annual exporter of canola seed (Kimber and McGregor, 1995). Between 1991 and 2001, Canada's export of canola seed increased from 1.9 million tonnes to 4.8 million tonnes. In the same decade, Canadian export of canola oil increased from 203 000 tonnes to 715 000 tonnes (Canola Council of Canada, 2002).

2.2 Breeding for Modified Oil Quality

Fatty acid biosynthesis in rapeseed

Fatty acid synthesis is carried out in the proplastids of developing seeds and in the chloroplasts of leaf tissue (Stumpf, 1989). The first step involves the carboxylation of acetyl CoA by acetyl-CoA carboxylase (ACC) to form malonyl-CoA. Malonyl-CoA is the substrate for the elongation reactions that extend the fatty acyl chain. The fatty acid synthetase (FAS) complex sequentially adds two carbon units derived from malonyl-CoA onto the carboxyl end of the growing fatty acid chain.

In rapeseed, the predominant end product of fatty acid synthetase (seven cycles) is palmitoyl (C16:0)-ACP. Acyl carrier protein (ACP) is a small protein that carries the growing fatty acid chain during synthesis. Palmitoyl-ACP is elongated by a specific enzyme to form stearoyl (C18:0)-ACP, which is subsequently desaturated by a soluble chloroplast enzyme to yield oleoyl (C18:1)-ACP (Figure 2.2). Oleoyl-ACP is converted to free oleic acid (C18:1) and free ACP by a specific thioesterase (Harwood and Page, 1994). Oleic acid is then transported to the cytoplasm where it may undergo further modifications.

Figure 2.3 depicts the most common modifications of free oleic acid in rapeseed. The elongation of oleic acid by fatty acid elongase enzymes occurs in the cytoplasm and involves the addition of C_2 units. Eicosenoic acid (C20:1) and erucic acid (C22:1) are the resultant long chain fatty acids. The generation of polyunsaturated fatty acids occurs in parallel pathways in the chloroplast and cytoplasm. In developing seeds, the cytoplasmic pathway predominates (Browse and Somerville, 1991; Yadav et al. 1993). Oleic acid is first desaturated by ω-6 (Δ12) desaturase enzymes to yield linoleic acid (C18:2). The loci coding for the ω-6 enzymes are *fad6* and *fad2* in the chloroplastic and cytoplasmic pathways respectively. Next, the desaturation of linoleic acid by ω-3 (Δ15) enzymes yields linolenic acid (C18:3). The loci coding for the ω-3 enzymes are *fad7* in the chloroplast and *fad3* in the cytoplasm. The ω-6 and ω-3 designations refer to the positions of the double bond from the methyl end of the fatty acids. *fad7* has been cloned in *Arabidopsis thaliana* (Iba et al., 1993) and *fad3* has been cloned

in both *A. thaliana* and *B. napus* (Arondel et al., 1992; Yadav et al., 1993). There appears to be a high degree of sequence similarity between the *fad3* loci from the two related species, suggesting a common function (Yadav et al., 1993).

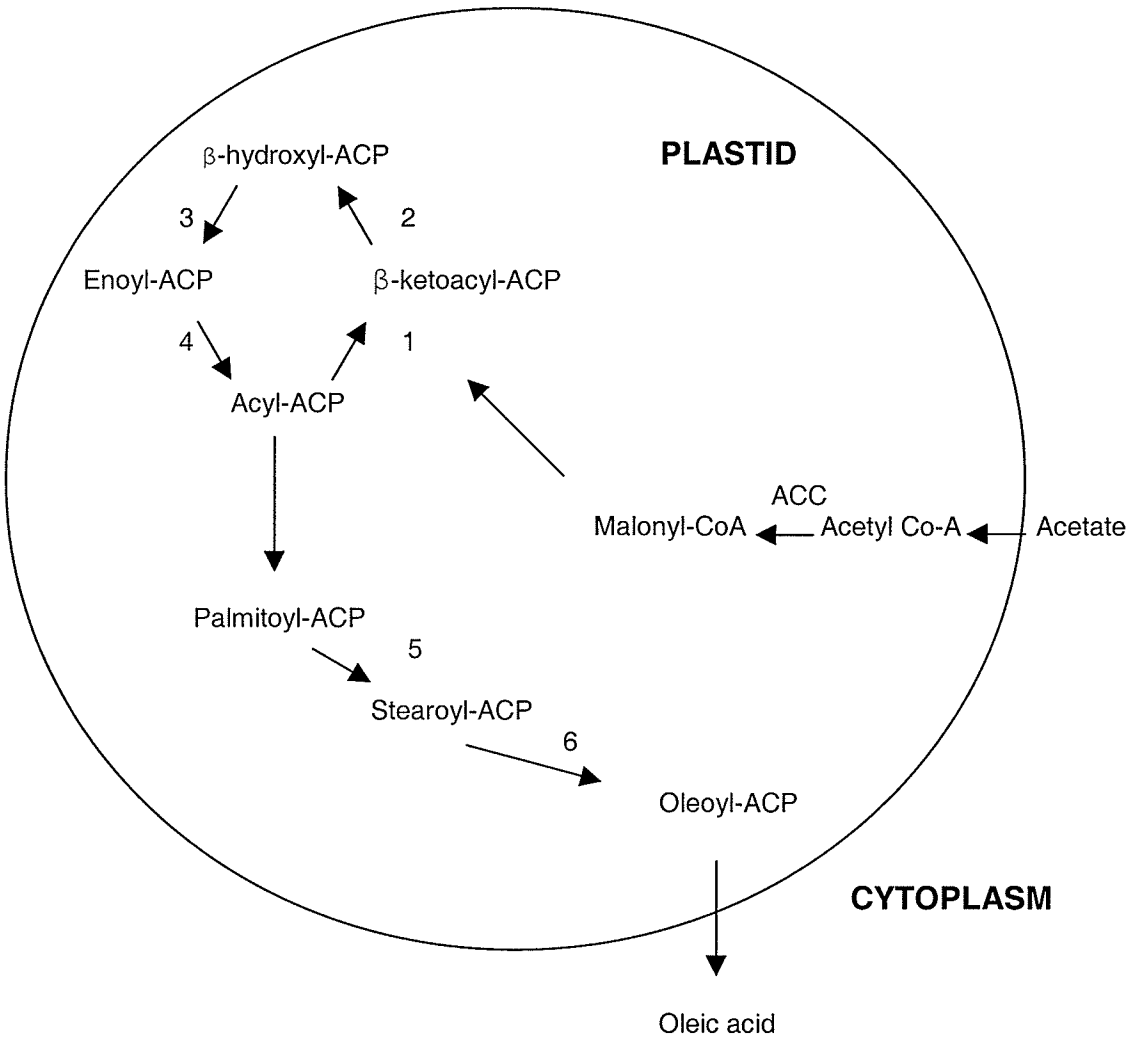

Figure 2.2. Reactions involved fatty acid synthesis (adapted from Harwood and Page, 1994). ACC = acetyl-CoA carboxylase, 1 = β-ketoacyl-ACP synthase, 2 = β-hydroxyl-ACP reductase, 3 = β-hydroxyl-ACP dehydratase, 4 = enoyl-ACP reductase, 5 = β-ketoacyl-ACP synthase II, 6 = Δ9 desaturase

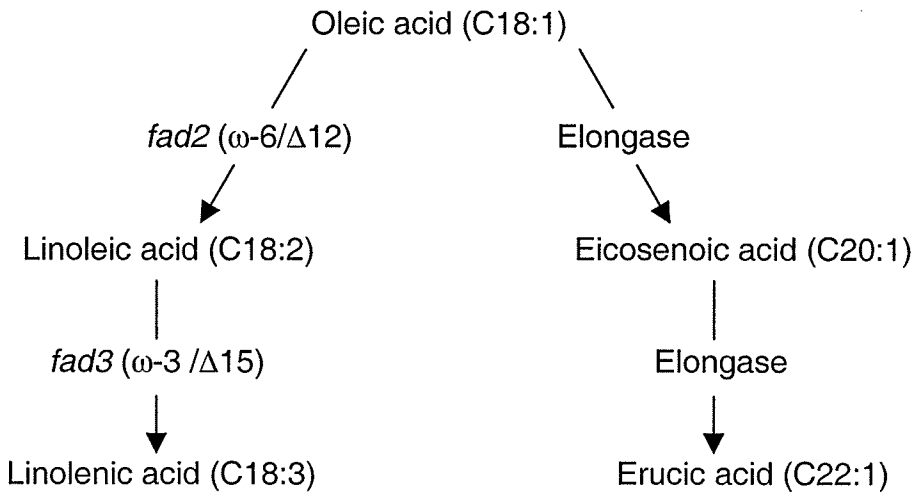

Figure 2.3. Modification reactions of long chain fatty acids in the cytoplasm (adapted from Harwood and Page, 1994).

Modified oil quality

Oil quality in rapeseed is partly determined by the fatty acid composition of the seed oil. Currently, the typical canola oil profile is as follows: 61% oleic acid, 21% linoleic acid and 11% linolenic acid (Scarth and McVetty, 1999). Although the development of near zero erucic acid rapeseed oil ('canola oil') was a key advance, many other oil modifications are also desirable. The levels and ratios of the various fatty acids influence the nutritional or industrial attributes of the oil.

Breeding for reduced levels of linolenic acid was initiated in the 1970's. The high level of linolenic acid (8-12%) found in conventional canola cultivars leads to oxidative rancidity and a distasteful flavour of the oil, and thus reduces its shelf life. Furthermore, canola oil high in linolenic acid must be hydrogenated

before use in industrial frying to reduce the level of polyunsaturated fatty acids. Hydrogenation is an expensive process and leads to the formation of *trans* fatty acid, which pose a potential health risk (Scarth et al., 1992). Low linolenic acid canola oil is more stable and more healthful. The decrease in linolenic acid is typically accompanied by an increase in linoleic acid due to the blocked desaturation pathway. Linoleic acid is considered an essential fatty acid in the human diet and has a high nutritional value (Uppstrom, 1995).

The development of canola oil with increased levels of oleic acid (C18:1) has also been a breeding objective. Oil with high levels of oleic acid can be achieved by blocking the desaturation of oleic acid to linoleic acid. Higher oleic acid in the oil, in conjunction with lower linoleic and linolenic acids, increases the oxidative stability of the oil without extensive hydrogenation (McDonald and Fitzpatrick, 1998). Mid oleic acid oil (67-75%) is marketed for food applications, while high oleic acid oil (>75%) is targeted for industrial frying end or is blended with other oils (Scarth and McVetty, 1999).

Biotechnology has produced oils high in saturated fatty acids. Oils high in stearic (C18:0) acid (40-70%) or lauric (C12:0) acid (>50%) can be blended with conventional canola oil for margarine production, used for the manufacture of cocoa butter substitutes or used as replacements for tropical oils like coconut and palm kernel oil (Murphy and Mithen, 1995).

The low saturate level of canola oil is also an important component due to the health risks associated with the consumption of high levels of saturated fat. Canola oil is currently the vegetable oil with the lowest level of saturated fatty acids (7%) (Canola Council of Canada, 2002). However, soybean germplasm with reduced levels of saturates has been developed, so a further reduction of the saturated fat level in canola oil is a breeding objective. A long term breeding goal is to reduce the levels of palmitic and stearic acid in canola oil to below 4% (Scarth and McVetty, 1999).

Rapeseed with increased levels of erucic acid (C22:1) has many industrial applications. The oil derived from HEAR (high erucic acid rapeseed) can be used in the development of plastics, nylons and clean burning fuels (Scarth et al., 1992). Conventional breeding methods produced erucic acid levels of 45-55% in the seed oil. The current objective of producing erucic acid levels over 66% in rapeseed oil is being approached through genetic engineering (Luhs et al., 1999). Oil containing fatty acids such as myristic acid (C14:0) and ricinoleic (C18:1-OH) acid have also been developed through genetic engineering and have potential industrial uses (Green et al., 1999; Rudloff et al., 1999).

As canola and rapeseed production increases nationally and globally, breeding programs will continue to improve and modify oil quality. Competition in the global vegetable oil market generates more incentive to increase the uses and market demand for rapeseed and canola oils.

Development and performance of low linolenic acid canola

Brassica germplasm originally contained very little variation in linolenic acid content. Therefore, researchers at the Institute for Plant Breeding (University of Göttingen) conducted mutation experiments to reduce levels of linolenic acid by blocking the desaturation pathway between linoleic acid and linolenic acid (Robbelen and Nitsch, 1975). Seeds of Oro, a Canadian spring *B. napus* rapeseed cultivar, were treated with the chemical mutagen ethyl methanesulphonate (EMS) and mutants with seed oil containing altered linoleic: linolenic acid ratios were selected. One of the Oro mutants, M11, was the source of the low linolenic trait in the University of Manitoba breeding program for the first low linolenic acid canola cultivar, Stellar, with a linolenic acid content of 3%, released in 1987 (Scarth et al., 1988).

The agronomic performance of the first low linolenic acid canola cultivars suggested that the source of the low linolenic acid trait might limit productivity (Scarth et al., 1992). Stellar yielded 20% lower than the check cultivar Westar, and while its meal protein content was higher, its seed oil content was 1.1% lower than Westar (Scarth, 1988). The subsequently released low linolenic acid cultivars Apollo (Scarth et al., 1995) and Allons (Scarth et al., 1997) showed improved seed yield and seed oil content. However, there remained a significant yield depression in comparison with the conventional canola check cultivars. Rucker and Robbelen (1996) studied the field performance of winter *B. napus*

and found that linolenic acid content was not associated with seed yield or oil content. Similarly, Rajcan et al. (1997) did not identify a significant association between linolenic acid content and seed yield in field trials evaluating the performance of a doubled haploid population of *B. napus* segregating for linolenic acid content. This study did, however, find higher oil content and protein content in low linolenic lines (<3.5%) compared with intermediate and high linolenic acid lines.

When compared to Westar in variety trials, Stellar and Apollo were found to mature three to four and two days later, respectively (Scarth 1988). Similarly, Rajcan et al. (1997) found that low linolenic acid lines (<3.5% linolenic acid) flowered on average two days later and matured on average three days later than intermediate or high linolenic acid lines. This study identified significant correlations between linolenic acid content and days to flowering and maturity.

Inheritance of linolenic acid content

Kondra and Thomas (1975) were the first researchers to investigate the inheritance of linolenic acid and suggested that up to four genes were involved in its control. This study was limited by the low variation in linolenic acid content among the genotypes tested, with a range from 11.2% to 15.2%. The development of low linolenic acid mutants (Robbelen and Nitsch, 1975) provided a wider range of linolenic acid genotypes for inheritance studies.

Nitsch (1975) observed a continuous variation in linolenic acid in F2 derived F3 lines, and proposed that the trait was polygenic. Jonsson and Persson (1983) used breeding lines of *B. napus* with linolenic acid levels ranging from 3% to 10% and suggested that the trait was controlled by one or two additive genes. Brunklaus-Jung and Robbelen (1987) observed segregation ratios in the progeny of crosses between low linolenic acid mutants and a high linolenic acid line and concluded that the low linolenic acid trait was controlled by monogenic recessive inheritance. Later, Chen and Beversdorf (1990) found that segregation of linolenic acid content in microspore-derived doubled haploid populations of spring *B. napus* fit a three-gene additive model.

The advent of molecular techniques has provided further knowledge of linolenic acid gene action. Molecular marker studies indicate that there are likely two major loci controlling the trait and they encode two different forms of *fad3*, the gene responsible for the desaturation of linoleic acid to linolenic acid in the cytoplasm (Jourdren et al., 1996b; Barett et al., 1999; Somers and Rakow, PTC/CA00/01140). Molecular studies are discussed in section 2.3.

In addition to the nuclear genes, several studies have supported the influence of cytoplasmic and maternal effects on the expression of linolenic acid content. An early study by Thomas and Kondra (1973), prior to the development of mutant low linolenic acid alleles, suggested that both embryo and maternal factors were involved. Bartkowiak-Broda and Krzymanski (1983) found that

embryo genotype was not involved, and that linolenic acid content was totally dependent on the maternal genotype. Pleines and Friedt (1989) found that the trait was mainly under the control of nuclear genes of the embryo, but that there was also some interaction between maternal genotype and nuclear genes. The degree of this interaction appeared to be highly influenced by temperature, with greater maternal effects observed in warm environments. This study did not identify any cytoplasmic control. Diepenbrock and Wilson (1987), however, found linolenic acid was determined by nuclear and cytoplasmic gene interaction. These results agreed with the findings of Rajcan (1996) who found differences due to cytoplasmic and nuclear gene interaction in doubled haploid populations of *B. napus*.

Environmental effects have also limited the understanding of the inheritance of linolenic acid content. In controlled environment studies, Rakow and McGregor (1973) and Pleines and Friedt (1988) found that levels of linolenic acid varied with growing conditions. Brunklaus-Jung and Robbelen (1987) and Rajcan et al. (1997) obtained similar results in field studies. Kondra and Thomas (1975) estimated heritability of linolenic acid to be from 26% to 59%. Pleines and Friedt (1988) and Rajcan et al (1997), however, obtained higher estimates of 90% and 82%, respectively.

2.3 Molecular Marker Technologies

Polygenic inheritance, as well as maternal, cytoplasmic and environmental effects make the low linolenic acid trait a good candidate for molecular analysis and for the application of marker assisted selection (MAS) to improve breeding efficiency.

Types of molecular markers

Molecular markers can be defined as heritable entities associated with economically important traits (Staub et al., 1996) and hold great potential for enhancing genetic studies and plant breeding programs. Until the advent of molecular marker technology, studies in plant breeding and model genetics relied on a phenotypic assay of genotype (Tingey and del Tufo, 1993). Genotypic analysis by molecular markers has been very informative and has allowed many advances of knowledge. Applications of markers include the identification of plant cultivars, determination of parentage, tagging of agronomically important genes, identification of chromosome-specific or genome-specific markers, the development of genetic maps and use in marker assisted selection (MAS) (Rajcan, 1996). There exist a variety of molecular marker technologies and this discussion will include a description of several types.

Restriction fragment length polymorphism (RFLP)

Restriction Fragment Length Polymorphism (RFLP) was the first type of DNA marker to be developed (Botstein et al., 1980). RFLP detects variation between genotypes using restriction enzymes that generate different sizes of DNA fragments. These variable sized fragments are separated by electrophoresis and transferred to a nitrocellulose filter. A labelled probe is then used to visualise the polymorphism. RFLP probes tend to be small (500-3000 bp) cloned DNA segments (Staub et al., 1996). RFLP analysis is very informative, as the markers are typically codominantly inherited. However, the process is very labour intensive and time consuming. Another drawback is that RFLP cannot detect polymorphisms in highly repetitive sequences (Williams et al., 1990).

Polymerase chain reaction (PCR) based markers

The development of Polymerase Chain Reaction (PCR) based markers provides an alternative to RFLP. PCR is based on the amplification of specific DNA segments located between sequences complementary to DNA synthesis primers (Mullis et al., 1986). PCR involves denaturing the DNA at a high temperature, annealing oligonucleotide primers to the template DNA, extending the primers with *Thermus aquaticus* (Taq) DNA polymerase, a thermostable DNA polymerase, in the presence of dNTPs and exponentially amplifying the DNA fragments for 25 to 45 cycles (Saiki et al., 1988, Rajcan, 1996). The technology

is simple, rapid and has become highly automated. PCR has given rise to many advances in molecular marker development.

Random amplified polymorphic DNA (RAPD) is a widely used PCR based marker technique that involves the amplification of random DNA sequences from genomic DNA using short (10 bp) primers of arbitrary nucleotide sequence (Williams et al., 1990). RAPD polymorphisms or markers, result from either base changes at the primer annealing site or insertion / deletion events in the amplified regions (Williams et al., 1993; Paran and Michelmore, 1993). RAPD markers have the advantages of PCR based technology, namely that the technique is simple, automated and relatively inexpensive and needs only small amounts of DNA. In addition, RAPD markers do not require any prior knowledge of sequence information and may be detected in regions not accessible to RFLP analysis such as highly repetitive sequences (Williams et al., 1990). However, RAPD markers are less informative due to their dominant inheritance and are more problematic in terms of repeatability. RAPD markers generally anneal to many regions of the genome and amplify several loci. Due to the short length of RAPD primers, they may not bind with complete specificity to template DNA. Other factors such as the DNA extraction procedure, template DNA concentration, Taq polymerase concentration and $MgCl_2$ concentration all have been found to contribute to low repeatability of RAPD markers (Jones et al., 1997, Mailer et al., 1994).

In order to overcome some of the difficulties associated with RAPD markers, sequence characterised amplified region (SCAR) markers were developed. A SCAR marker is a DNA fragment at a genetically defined locus that is identified by PCR amplification using a pair of specific oligonucleotide primers (Paran and Michelmore, 1993). SCAR markers are derived by cloning and sequencing the two ends of the amplified RAPD marker product. Based on this sequence information, oligonucleotide primers (22 to 24 nucleotides) can be designed to amplify a single locus. It is also possible to design SCAR markers from RFLP probes. However, since differences in RFLP are often outside the regions hybridised with the probe, these polymorphisms would not be detected with SCAR primers designed from the ends of the probes (Paran and Michelmore, 1993).

The advantage of SCAR markers over RAPD markers is that only one locus is detected under stringent annealing temperatures. Furthermore, the longer primers allow a more robust and reproducible assay than the short RAPD primers. The identification of a single locus may allow the use of a SCAR in different populations. In some cases, however, a SCAR primer may not detect the original polymorphism and may amplify the same sized fragment in both parents (Paran and Michelmore, 1993). When this occurs, allele specific amplicons (ASA) may be developed by sequencing the alternate alleles and designing allele-specific primers based on sequence divergence. ASA markers have the advantages of involving a simple PCR based assay and being much

more stringent and reproducible than RAPD markers. Furthermore, an ASA can be designed based on a gene sequence to specifically amplify the allele controlling the trait if a candidate gene for a particular trait is known (Somers et al., 1999).

Microsatellites or simple sequence repeats (SSR) are tandem repetitive DNA sequences with a repeat length of a few base pairs that are randomly dispersed throughout genomes (Maroof et al., 1994). SSR polymorphisms are due to differences in the number of repetitive di-, tri- or tetranucleotide units present (Morgante and Olivieri, 1993). The length variation of SSR markers can be detected by PCR amplification using primers based on the conserved sequences flanking the SSR region. SSR markers have the advantages of being co-dominant, widely and evenly distributed in higher plant genomes and showing high degrees of polymorphism (Powell et al., 1996). However, much labour is required to determine sequence information defining SSR loci prior to their exploitation as markers (Powell et al., 1996).

The amplified fragment length polymorphism (AFLP) technique is based on the detection of genomic restriction fragments by PCR amplification (Vos et al., 1995). The first step in the process involves restricting the DNA with two restriction enzymes and ligating double stranded oligonucleotide adapters to the ends of these DNA fragments to generate template DNA for amplification. Primers are designed based on the sequence of the adaptors and the adjacent

restriction site, and arbitrary nucleotides are added to the 3' ends of the primers. Selective amplification is achieved as primers only bind to restriction fragments in which the primer extensions match the nucleotides flanking the restriction sites. Gel analysis of the amplified products is used to detect polymorphisms. The number of selective nucleotides and the number of amplification cycles can be altered to produce different AFLP patterns. AFLP has the reliability of the RFLP technique in addition to the automation and relative simplicity of PCR. Since this marker type assays a large proportion of the genome, it is very useful in the construction of high-density genetic maps of genomes or genome segments (Vos et al., 1995; van Eck et al., 1995).

One of the most recent advances in molecular marker technology involves the exploitation of single nucleotide polymorphisms (SNP). SNPs are the simplest type of genetic polymorphism, occur frequently throughout genomes and result from single base mutations that typically substitute one base for another. SNP discovery relies on aligning highly homologous expressed sequence tags (EST) from various genotypes / sources and identifying SNP sites between ESTs. PCR primers are designed based on the nucleotide difference and therefore allow specific amplification. SNP marker development depends on the availability of EST sequence databases and one drawback to this type of marker system is the high cost of developing and sequencing ESTs. ESTs correspond to the coding region of an expressed gene and therefore, SNP markers derived from ESTs have the obvious advantage that they always

correspond to an expressed gene. By identifying the gene itself, rather than a locus linked to the gene, SNP markers provide a high degree of accuracy.

These are just a few examples of molecular markers that have been developed. The wide variety of marker technologies enables researchers to select a marker type best suited to particular studies. One important application is the use of marker assisted selection in plant breeding.

Marker assisted selection

In traditional plant breeding, selections are based on phenotypic assays of genotype. Factors such as the environment, polygenic and quantitative inheritance, and partial or complete dominance often complicate the interpretation of the genotype from the phenotypic expression (Tingey and del Tufo, 1993). Molecular marker technologies are direct genotypic assays which may help to overcome some of these difficulties, as selections are based on genotype identified by the marker. The use of molecular markers as selection tools in a breeding program is called marker-assisted selection (MAS).

Useful molecular markers are usually closely linked to the loci of interest, rather than located directly at the loci. Therefore, the value of molecular markers for MAS depends primarily on the degree of linkage between marker alleles and the loci controlling economically important traits (qualitative or quantitative) (Staub et al., 1996, Dudley, 1993). Staub et al. (1996) suggest that a linkage

distance of 10cM or less is probably sufficient for MAS of a qualitative trait. A tightly linked marker(s) co-segregates with the desired trait and thus, subsequently provides a high degree of accuracy in the identification of phenotype for selection (Mohan et al., 1997).

Most characters of economic importance in crops are quantitative traits, influenced by numerous loci (quantitative trait loci or QTL) throughout the genome (Lande and Thompson, 1990). Since the effects of the individual genes cannot be readily identified, improvement of these traits tends to be more difficult compared to simply inherited qualitative traits (Dudley, 1993). If markers can be identified for QTL, the use of MAS could be extremely valuable for such traits.

There are practical considerations that limit the potential utility of MAS in applied breeding programs. First, a large number of molecular marker loci may be required to detect all significant linkage associations. Second, large sample population sizes are required to detect QTL for traits with low heritability. MAS requires an efficient means of screening large populations for the markers and high reproducibility between laboratories (Mohan et al., 1997). Another consideration is the cross applicability of markers. A marker developed for a gene in one population may not be useful in other populations even though the same gene may be segregating in both. Ideally the marker would be the gene itself and therefore, would be applicable in different genetic backgrounds.

The implementation of MAS depends on the accuracy of phenotypic classification, the degree of linkage between the markers and the loci of interest and the cost per unit information (Staub et al., 1996, Young, 1999). Genetic maps for a variety of crop plants, including *B. napus*, are now quite densely saturated with markers (Landry et al., 1991; Ferreira et al., 1994; Foisset et al., 1996; Uzunova and Ecke, 1999; Saal et al., 2001). In *Brassica* species, molecular markers have the potential to be used in selection programs for a variety of traits including disease resistance, seed coat colour, freezing tolerance, glucosinolate content and fatty acid composition (Teutonico et al., 1995; Uzonova et al., 1995; Somers et al., 1999; Manzanares-Daulneux et al., 2000; Somers et al., 2001).

Molecular markers associated with linolenic acid content

In recent years, several research groups have identified molecular markers associated with linolenic acid content in *Brassica napus*. Hu et al. (1995) identified a RAPD marker (K01-1100) associated with linolenic acid content in an F2 population of a cross between the rapeseed cultivar Duplo and an Oro x IXLIN derived low linolenic acid line. K01-1100 accounted for 12.8% of the phenotypic variation for linolenic acid in the population, and when converted into an RFLP probe, became co-dominant and accounted for 26.5% of the genetic variation in linolenic acid content. Tanhuanpaa et al. (1995) identified a RAPD marker (25a) in an F2 population derived from a cross between Topas (canola cultivar) and R4 (low linolenic acid mutant line from Oro). This RAPD marker

accounted for 23% of the variation observed in this population. Thormann et al. (1996) used RFLP to map two QTL associated with linolenic acid content on two separate linkage groups. An F1-derived doubled haploid population from a cross between the rapeseed cultivar Major and the low linolenic acid canola cultivar Stellar was used for the mapping. The two QTL accounted for 60% of the variation observed. The QTL that explained the majority of the variance (47%) mapped near an RFLP locus detected by the *fad3* clone. This suggested that a mutation of the *fad3* gene resulted in the low linolenic acid concentrations of Stellar.

Rajcan et al. (1999) used F1-microspore derived DH populations of a cross between Reston (rapeseed cultivar) x LL09 (low linolenic acid Stellar derived line) to identify two RAPD markers, RM350 and RM574, associated with linolenic acid content. These unlinked markers accounted for 39% of the variability of linolenic acid within the population. The marker RM350 alone accounted for 25% of the variation. Jourdren et al. (1996a) identified two linkage groups, each with 6 RAPD markers for linolenic acid content, in a Stellar x Drakkar (high linolenic acid) doubled haploid population. Each linkage group contained one major QTL (L1 and L2) which together explained 71.1% of the total variation. Jourdren et al. (1996b) also developed a specific molecular marker based on the sequence of the *fad3* gene in *B. napus*. The primer pair identified a polymorphism between the *fad3* alleles of Stellar and Drakkar, the

low- and high linolenic acid parents respectively. This *fad3* gene mapped close to the L1 locus previously identified.

Somers et al. (1998) identified 16 RAPD markers distributed over three linkage groups associated with linolenic acid content in a doubled haploid population derived from a cross between Apollo (low linolenic acid) and YN90-1016 (high linolenic acid). The linkage groups individually accounted for 32%, 14% and 5% of the phenotypic variation and QTL analysis showed that collectively, the three linkage groups explained 51% of the variation for linolenic acid. Somers et al. (1998) also mapped the *B. napus fad3* gene (Arondel et al., 1992) on the linkage group controlling 14% of the variation. Hu et al. (1999) converted a RAPD marker for linolenic acid content into a SCAR marker that explained 25% of the variation in the trait. The co-dominant SCAR marker (L1L9) amplified 899bp and 641bp fragments associated with the low and high linolenic acid alleles respectively.

Barret et al. (1999) determined that the L1 locus linked to a *fad3* gene identified by Jourdren et al. (1996b) was located on the A genome of *B. napus*. In addition, it was found that a second *fad3* gene was linked to the L2 locus (Jourdren et al., 1996a) corresponding to the C genome *of B. napus*. *B. napus* is an amphidiploid species derived from *B. rapa* (AA) and *B. oleracea* (CC). Therefore, it appears that there is a *fad3* gene from each parental genome. Sequence analysis indicated that there were single base pair mutations in each

fad3 gene and these mutations were specific to Stellar in comparison to wild type lines. Specific markers were developed identify lines having the mutations. This study suggested that *fad3*A and *fad3*C are good candidates for the loci controlling linolenic acid content.

Somers and Rakow (PTC/CA00/01140) sequenced the *fad3* (A genome) locus from the low linolenic acid *B. napus* cultivar, Apollo. Results indicated that the low linolenic acid mutation in the Apollo *fad3* allele resulted in a non-conserved amino acid substitution of Cysteine for Arginine at amino acid 275. Somers and Rakow (PTC/CA00/01140) designed primers specific to the mutant allele based on the sequence divergence between the mutant and wild type *fad3* alleles.

Clearly, the development of molecular markers has enhanced knowledge of the genetic control of linolenic acid content in canola and could play an important role in breeding for low linolenic acid cultivars.

3. FIELD EVALUATION OF A DOUBLED HAPLOID POPULATION OF *Brassica napus* SEGREGATING FOR LINOLENIC ACID CONTENT

3.1 Introduction

The quality of oil derived from rapeseed (*Brassica napus* and *Brassica rapa*) is partly determined by its fatty acid composition. Although zero erucic acid rapeseed (canola) oil is considered superior due to its low levels of saturated fatty acids, its relatively high levels of linolenic acid (8-12%) lead to oxidative rancidity and loss of flavour stability of the oil especially in applications such as frying. Reducing the level of linolenic acid in canola oil to less than 3% is an important breeding objective to improve the stability of canola oil (Scarth et al., 1992).

Brassica germplasm contained little variation in linolenic acid levels. Therefore, chemical mutagenesis was used to create *B. napus* genotypes with low levels of this fatty acid. The development of these mutants prompted genetic studies examining the inheritance of linolenic acid. The trait is controlled by two or three genes, appears to be highly influenced by the environment and may involve some cytoplasmic or maternal inheritance (Brunklaus-Jung and Robbelen, 1987; Pleines and Friedt, 1989; Chen and Beversdorf, 1990). However, relatively few studies have evaluated the performance of low linolenic acid lines in field conditions (Brunklaus-Jung and Robbelen, 1987; Rucker and Robbelen, 1996; Rajcan et al., 1997). The limited information gained from these studies suggests the need for further field evaluations. The interaction between the low linolenic acid trait and agronomic

performance is an important consideration in a breeding program targeting the development of elite low linolenic acid lines.

The purpose of this study was to characterise several agronomic and quality traits in a doubled haploid (DH) population of *B. napus* segregating for linolenic acid content and to determine the stability of the low linolenic acid trait over environments.

3.2 Materials and Methods

3.2.1 Plant Material

The plant material used in the field study was an F1 microspore derived doubled haploid (DH) population of 134 lines derived from crosses between the rapeseed cultivar Reston, having 8.5% linolenic acid in the oil, and LL09, a low linolenic acid DH canola line having 3% linolenic acid in the oil. The line LL09 was derived from a cross between the canola cultivar Topas and a sister line of the low linolenic acid cultivar Stellar. The population was developed at the University of Guelph and was kindly provided by Dr. L. Kott.

Four seeds from each DH line were seeded into flats and placed in a growth room. At the 4-5 leaf stage, seedlings were transplanted into 6-inch pots (two plants/ pot) and grown in greenhouse conditions. From approximately one day prior to flowering until the end of the flowering period, plants were covered with

polyethylene isolation bags (1mm holes) to ensure self-pollination. The bags were gently shaken every two days to facilitate pollen transfer. When fully mature, each plant was threshed by hand and seed from each DH line was pooled. One hundred DH lines produced seed amounts adequate for field trials.

3.2.2 Field Trials

One hundred DH lines were grown out at two locations in two years – Winnipeg 1998 (Wpg98), Carman 1998 (Car98), Winnipeg 1999 (Wpg99) and Carman 1999 (Car99). Field trials were set up as randomised complete block design (RCBD) consisting of two replicates/location with one 3-meter row (125 seeds/row) per replicate. A check row of the low linolenic acid canola cultivar Apollo was included every fifth row in 1998 and every sixth row in 1999. Fields were hand weeded throughout the growing season. At maturity, rows were hand cut, bundled and placed in stooks in the field until dry. Each row was then individually threshed using a stationary thresher and harvested seed was placed in a drying room for approximately two weeks.

3.2.3 Data Collection

DH lines were characterised for the following agronomic and quality traits: days to flowering, plant height, days to maturity, seed oil content, seed protein content, the sum of oil and protein content and linolenic acid content. Days to flowering were determined as the number of days after planting at which 50% of the plants in the row had at least one flower open. Plant height was measured at

physiological maturity on three to five plants in each row at and the values were averaged. Days to maturity were determined as the number of days after planting at which approximately 50% of the row had reached physiological maturity. Oil, protein and fatty acid levels were measured on open-pollinated seed samples from each row. Oil content was measured on 25g of seed using a Nuclear Magnetic Resonance Analyser (Madsen, 1976). Nitrogen content was determined on 0.5g of seed using the Dumas combustion method (Williams et al., 1998). Nitrogen values were converted into protein content (Tkachuk, 1969). Fatty acid analysis of the seed oil was performed on oil extracted from 0.25g of seed (Hougen and Bodo,1973) using gas liquid chromatography. The range of fatty acid detected was from C16 to C24 and the individual fatty acids were expressed as a percentage of the total fatty acid content.

3.2.4 Data Analysis

Data was analysed through simple RCBD analysis of variance (ANOVA), and pair-wise correlations using Agrobase (Agronomix Software Inc., 1997). Means, ranges and standard error values were calculated using a standard spreadsheet program (Microsoft Excel).

3.3 Results

3.3.1 Characterisation of Quality and Agronomic Traits

Linolenic Acid Content

There was significant variation in linolenic acid content among the 100 DH lines in all four site years – Wpg98, Wpg99, Car98 and Car99 (Appendix 6). A Bartlett's test detected heterogeneity of error variances for linolenic acid content (Table 3.1) and therefore, no ANOVAs on combined site year data were conducted. Instead, simple means for DH lines over site years were compared. The mean linolenic acid content of the population at each site year ranged from 4.9% (Car98) to 5.6% (Wpg99) (Table 3.2). The mean linolenic acid content over site years was 5.2%, and the range was 2.9% - 8.7%. The distribution of linolenic acid content averaged for each DH line over site years (Figure 3.1) shows a tri-modal shape.

There was very little variation in linolenic acid content in the Apollo check rows. The mean linolenic acid content for the Apollo check rows at each site year were 1.7% ± 0.04 (Wpg98), 1.8% ± 0.03 (Car98), 1.9% ± 0.05 (Wpg99) and 1.9% ± 0.03 (Car99) (Appendix 13).

Table 3.1. Bartlett's test for heterogeneity of error variances for linolenic acid content in a DH population of *B. napus* at Wpg98, Car98, Wpg99 and Car99.

Site Year	MS error[i] (s^2)
Wpg98	0.10
Car98	0.45
Wpg99	0.18
Car99	0.18
Chi-square (χ^2) [ii]	19.80*

*, significant at P=0.05
[i], from ANOVA (Appendix 6)
[ii], using the following formulae,

$$M = \gamma \left[a(\ln \overline{s_i^2}) - \Sigma \ln s_i^2 \right]$$

$$\overline{s_i^2} = \frac{\Sigma_i \, s_i^2}{a}$$

$$C = 1 + \frac{a + 1}{3a\gamma}$$

$$\chi^2 = \frac{M}{C}$$

γ = degrees of freedom = 3
a = locations (site years) = 4

Table 3.2. Mean and range values of seed quality traits in a DH population of *B. napus* at individual site years and combined site years.

Trait	Site Year	Mean	±	Std. Error	Min.	Max.
Linolenic acid content (%)	Wpg98	5.0	±	0.14	2.8	8.1
	Car98	4.9	±	0.13	2.6	8.6
	Wpg99	5.6	±	0.16	3.0	9.7
	Car99	5.3	±	0.15	2.6	9.3
	Combined	5.2	±	0.14	2.9	8.7
Seed oil content (%)	Wpg98	45.8	±	0.18	40.5	49.4
	Car98	46.2	±	0.17	41.6	49.4
	Wpg99	47.1	±	0.15	44.0	50.2
	Car99	48.8	±	0.19	43.3	52.9
	Combined	47.0	±	0.15	43.3	50.1
Protein (whole seed) content (%)	Wpg98	29.0	±	0.01	26.5	32.4
	Car98	29.0	±	0.13	25.6	32.7
	Wpg99	29.7	±	0.14	26.2	33.4
	Car99	27.5	±	0.15	23.8	30.3
	Combined	28.8	±	0.09	26.1	30.7
Sum of oil & protein content (%)	Wpg98	74.7	±	0.25	53.3	77.6
	Car98	75.2	±	0.14	71.0	79.2
	Wpg99	76.8	±	0.14	72.5	79.6
	Car99	76.3	±	0.16	71.1	78.8
	Combined	75.8	±	0.12	73.0	78.2

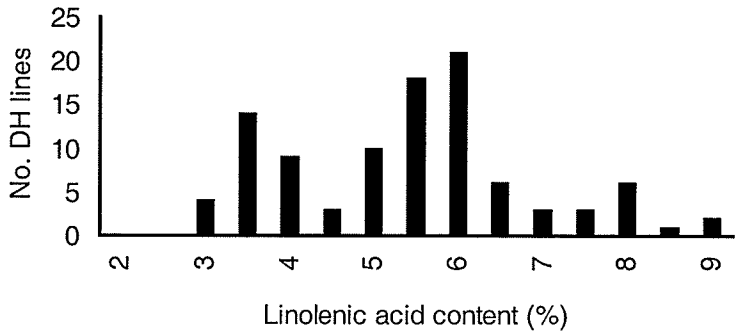

Figure 3.1. Distribution of 100 DH lines of *B. napus* segregating for linolenic acid content averaged over four site years (Wpg98, Car98, Wpg99, Car99).

Seed Quality Traits

There was significant variation for both oil content and protein content among DH lines at all site years (Appendices 7 and 8). The DH lines also showed significant variation for the sum of oil and protein content at all site years except at Wpg98 (Appendix 9). The mean values for oil content, protein content and the sum of oil and protein content over all site years were 47.0%, 28.8% and 75.8% respectively (Table 3.2).

Agronomic Traits

There was significant variation for days to flowering, plant height and maturity among the DH lines at all site years (Appendices 10-12). The mean values combined over site years for days to flowering, plant height and days to maturity were 51.7 days, 126.7 cm and 97.3 days respectively (Table 3.3).

Table 3.3. Mean and range values of agronomic traits in a DH population of *B. napus* at individual site years and combined site years.

Trait	Site Year	Mean	±	Std. Error	Min.	Max.
Days to flowering (days)	Wpg98	53.4	±	0.02	50.0	58.5
	Car98	55.4	±	0.23	50.5	60.5
	Wpg99	49.6	±	0.27	45.5	55.0
	Car99	48.3	±	0.25	44.5	54.5
	Combined	51.7	±	0.22	48.1	56.8
Plant height (cm)	Wpg98	115.8	±	1.16	77.5	147.5
	Car98	125.4	±	0.96	105.0	152.5
	Wpg99	136.4	±	1.02	112.5	160.0
	Car99	125.1	±	1.19	95.0	152.5
	Combined	126.7	±	0.84	100.0	145.6
Days to maturity (days)	Wpg98	97.9	±	0.15	94.0	100.0
	Car98	98.3	±	0.01	95.5	100.0
	Wpg99	102.4	±	0.13	98.5	105.0
	Car99	90.4	±	0.07	89.0	92.0
	Combined	97.3	±	0.09	95.0	98.9

3.3.2 Relative performance of low linolenic acid (≤3%) DH lines

The set of low linolenic acid lines consisted of seven DH lines (DH31, DH98, DH100, DH120, DH132, DH135, DH160) whose average linolenic acid content over the four site years was less than or equal to 3.0%. The set was compared to the remaining 93 DH lines. The low linolenic acid lines had, on average, slightly higher oil content (1.1%) and total oil and protein content (1.0%), and slightly lower protein content (0.1%). Days to flower and days to maturity were 0.9 days and 0.7 days later, respectively. The low linolenic acid lines were on average 4.7 cm taller than the other DH lines. Due to the lack to heterogeneity of error variances, it could not be determined if these differences between the classes were significant.

Table 3.4. Agronomic and seed quality traits of low linolenic acid (≤3.0%) DH lines relative to other DH lines (>3.0% linolenic acid) in the *B. napus* DH population.

	Trait						
	Linolenic acid content (%)	Oil content (%)	Protein content (%)	Sum of oil & protein content (%)	Days to flowering (days)	Plant height (cm)	Days to Maturity (days)
Means for low 18:3 DH lines[i]	3.0	48.0	28.7	76.7	52.5	130.0	97.9
Means for all other DH lines[ii]	5.3	46.9	28.8	75.7	51.6	125.3	97.2

[i] 7 DH lines (with average linolenic acid levels of ≤3.0%)

[ii] 93 DH lines (with average linolenic acid levels of >3.0%)

3.3.3 Correlation between agronomic/ seed quality traits and linolenic acid content

Correlations between linolenic acid content and the agronomic and seed quality traits were performed at each site year (Table 3.5). The full correlation matrices appear in Appendix 14. Correlations were not performed using combined site year data since the heterogeneity of error variances between the site years made the significance tests using combined site year data invalid (Peterson, 1994). There was a significant negative correlation between linolenic acid content and the sum of oil and protein content at Wpg99, and between protein content at Car99. There was no correlation between linolenic acid content and oil content at any site year.

There were significant negative correlations between linolenic acid content and days to flowering at all site years except Wpg99, and between linolenic acid content and days to maturity Wpg98 and Car99. There were no correlations between linolenic acid content and plant height at any site year.

3.3.4 Preliminary stability analysis of low linolenic content

The 20 DH lines with the lowest linolenic acid content were identified and comparisons were made to determine which DH lines repeatedly ranked in the lowest 5% (20/100 DH lines) of linolenic acid content. Thirteen DH lines were found to consistently rank in the lowest 5% at all site years (Table 3.6). These DH lines had mean linolenic acid contents ranging from 2.9% to 3.3%.

Table 3.5. Correlation between linolenic acid content and agronomic/ seed quality traits in 100 *B. napus* DH lines.

Parameter	Site Year	Correlation Coefficient
Days to Flowering	Wpg98	-0.22*
	Car98	-0.16*
	Wpg99	-0.14
	Car99	-0.22*
Days to Maturity	Wpg98	-0.16*
	Car98	-0.10
	Wpg99	0.01
	Car99	-0.16*
Plant Height	Wpg98	-0.01
	Car98	0.00
	Wpg99	-0.05
	Car99	-0.14
Seed Oil Content	Wpg98	-0.05
	Car98	-0.03
	Wpg99	-0.13
	Car99	0.07
Protein Content	Wpg98	-0.02
	Car98	0.01
	Wpg99	-0.12
	Car99	-0.16*
Sum of Oil & Protein Content	Wpg98	-0.03
	Car98	-0.01
	Wpg99	-0.17*
	Car99	-0.10

*, significant at P=0.05

Table 3.6. Identification of the 20 DH lines of *B. napus* with the lowest linolenic acid content at each site year.*

	Linolenic Acid Content (%)						
DH Line	Wpg98	Wpg99	Car98	Car99	Mean	±	Std. Error
3	3.2	3.0		3.2			
15				3.1			
19	3.1	3.5	3.0	3.0	3.1	±	0.12
24			3.2				
25	2.9		3.3	3.0			
27		3.4	3.4	3.3			
31	2.9	3.3	3.2	2.8	3.0	±	0.11
48		3.7					
50	2.9	3.2		2.9			
74			2.7				
80	3.2		3.3				
92	3.3	3.9					
98	2.9	3.4	2.7	2.6	2.9	±	0.17
100	3.1	3.0	3.1	3.0	3.0	±	0.03
114		3.3		3.5			
115	3.2	3.3	3.2	3.5	3.3	±	0.07
120	2.9	3.4	2.9	3.0	3.0	±	0.11
126	3.2	3.4	2.6	3.2	3.1	±	0.18
128	3.2	3.1	3.3	3.0	3.1	±	0.06
132	2.9	3.0	2.6	3.1	2.9	±	0.11
135	2.9	3.2	2.9	3.0	3.0	±	0.07
136	3.1		2.9				
149	3.2	3.3	2.9	3.1	3.1	±	0.07
153	3.3	3.5	3.3	3.1	3.3	±	0.07
155		3.4					
159	3.2			3.3			
160	2.8	3.1	2.7	2.9	2.9	±	0.09
163			3.2				

*Shading denotes DH lines that rank with the lowest linolenic acid content at all four site years

3.5 Discussion

In western Canada, days to flowering and maturity are important breeding considerations due to the short growing season, particularly in the more central and northerly canola growing regions where the number of frost free days can be less than 100 days (Canola Council of Canada, 2002). Early flowering and subsequently earlier maturity decrease the risk of seed development being prematurely halted due to frost. In this study, low linolenic acid content was associated with later flowering and later maturity. On average, low linolenic acid lines (≤3.0%) flowered and matured approximately one day later than the other lines in the population. These results are consistent with those of Rajcan et al. (1997) who evaluated the same DH population in field studies and identified significant correlations between linolenic acid content and flowering and maturity dates. The low linolenic acid lines had later flowering and maturity dates by two and three days, respectively (Rajcan et al., 1997).

The correlation between linolenic acid content and days to flowering suggests that genes controlling the two traits are linked. Ferreira et al. (1995) identified RFLP markers associated with flowering time and Thormann et al. (1996) subsequently found markers linked to linolenic acid content that mapped to the same linkage group (LG12). The linkage of quantitative trait loci (QTL) controlling these traits and the negative correlations observed in field studies suggest that breeding for low linolenic acid and early flowering simultaneously could be difficult.

Selection within a large population would be necessary to identify individuals having both of the desired traits.

Plant height is another important breeding consideration, as reduced height is associated with reduced lodging and higher yields. Shorter plants are less likely to lodge and allocate more biomass into reproductive development (Thompson and Hughes, 1986; Buzza, 1995). In this study, no correlation was found between linolenic acid content and plant height. Similar results were obtained by Rajcan et al. (1997) and Rucker and Robbelen (1996). Low linolenic acid is a neutral trait with regard to height. Selection for reduced height can be conducted in a low linolenic acid breeding program.

There was no association between seed oil content and linolenic acid content at any site year in this study. Rucker and Robbelen (1996) obtained similar results. However, Rajcan et al. (1997) found a significant, negative correlation, with low linolenic acid lines having a higher oil content. The lack of correlation in the current study is not negative from a breeding perspective, as it suggests that selecting for low linolenic acid content is neutral with regard to oil content. Protein content and the sum of oil and protein content were found to have significant negative correlations at only one site year each. While this might suggest that low linolenic acid content is associated with higher protein and a higher sum of oil and protein, conclusions could not be drawn from these results. The association with the latter parameter could be very beneficial, as the heritability of the sum of oil and protein

contents is higher than either oil or protein alone, and more advances could be made by selecting for the two traits simultaneously (Grami et al., 1977).

The second objective of this study was to determine the stability of the low linolenic acid trait over site years. Previous studies found that the environment had significant effects on the expression of linolenic acid content, both in controlled environments and in field studies (Brunklaus-Jung and Robbelen, 1987; Pleines and Freidt, 1988; Rajcan et al., 1997). The heterogeneity of error variances in this study prevented a combined analysis of variance and a determination of environmental and genetic x environmental effects (Peterson, 1994). Our preliminary stability analysis ranked the DH lines according to linolenic acid content at each site year and determined that 13 DH lines had linolenic acid levels consistently low in all environments. Although a statistical determination of stability of the low linolenic acid trait was not carried out, the stability of the 13 DH lines (with linolenic aid contents from 2.9% to 3.3%) suggested that the different environments had little effect on the expression of the trait in these lines. Further studies would be required to test this hypothesis.

The distribution of linolenic acid content in the population (Figure 3.4) showed a tri-modal distribution. The modes are not clearly defined, but the general shape would suggest that two genes are involved in the control of linolenic acid content. Two-gene inheritance has been suggested by Barret et al. (1999), whereas segregation ratios in a DH population also supported a three-gene

inheritance model (Chen and Beversdorf, 1990). There are conflicting results on the mode of inheritance but it is clear that linolenic acid content is controlled by more than one gene. Genetic inheritance is further discussed in the study described in Chapter 4 (section 4.5) with reference to the data from this study.

4. DEVELOPMENT OF AN ALLELE SPECIFIC AMPLICON ASSOCIATED WITH LINOLENIC ACID CONTENT IN *Brassica napus*

4.1 Introduction

Modification of the fatty acid profile of rapeseed (*Brassica napus and Brassica rapa*) has been a breeding objective for the past few decades. The decrease in erucic acid (C22:1) from approximately 50% to 0% (Stefansson and Downey, 1995) was one of the most important changes. Breeders have since focused on altering levels of other long chain fatty acids, including linolenic acid (C18:3). The high linolenic acid content (8-12%) found in the seed of conventional *B. napus* cultivars, leads to oxidative rancidity and a distasteful flavour of the oil. Therefore, an important breeding objective has been to reduce the levels of linolenic acid in the seed oil to less than 3% (Scarth et al., 1992).

Brassica germplasm originally contained very limited levels of linolenic acid (6-12%), so mutagenesis was used to broaden the range (2-20%) (Robbelen and Nitsch, 1975). The mutant line M11 was used to develop Stellar, the first commercial low linolenic acid canola quality *B. napus* cultivar (Scarth et al., 1988). Despite the availability of low linolenic acid genotypes, the production of low linolenic acid cultivars has been quite low, partly due to the lag in performance compared to conventional canola cultivars. Breeding for low linolenic acid content is complicated by the fact that the trait is controlled by two or three genes, and also appears to be influenced by environmental and

maternal factors (Pleines and Freidt, 1989; Chen and Beversdorf, 1990; Rajcan et al., 1996). The complex inheritance of the trait suggests that molecular markers hold potential as accurate tools for use in breeding for low linolenic acid *B. napus*.

Several research groups have identified markers associated with linolenic acid content and for the *fad3* gene, one of the genes responsible for the synthesis of linolenic acid (Hu et al., 1995; Jourdren et al., 1996b; Thormann et al., 1996; Somers et al., 1998; Hu et al., 1999; Rajcan et al., 1999; Somers and Rakow, (PTC/CA00/01140). The markers developed by Rajcan et al., (1999) were Random Amplified Polymorphic DNA (RAPD) markers. RAPD markers have been used extensively to identify markers linked to traits of interest but are not, however, locus specific. The primers are short (10 bp) and generally anneal to several regions of the genome and amplify many different loci. It is possible to convert RAPD markers into sequence characterised amplified regions (SCAR) (Paran and Michelmore, 1993) or allele specific amplicons (ASA) (Lee and Penner, 1997). These marker types specifically amplify the allele(s) of interest from a single locus and provide a more accurate identification of genotype.

The purpose of this study was to convert RM350, one of the RAPD markers for linolenic acid identified by Rajcan et al. (1999), into an ASA and to test the marker in a doubled haploid (DH) population of *B. napus* segregating for linolenic acid content.

4.2 Materials and Methods

4.2.1 Plant Material

The plant material used in this study was developed at the University of Guelph and the seed was kindly provided by Dr. L. Kott. The segregating populations arose from reciprocal crosses between a DH line of the rapeseed cultivar Reston (8.5% C18:3) and a low linolenic DH canola line LL09 (3% C18:3). Line LL09 was derived from a cross between the canola cultivar Topas and a sister line of the low linolenic acid cultivar Stellar. The F1 plants from the Reston x LL09 and LL09 x Reston crosses were used to generate DH populations by isolated microspore culture.

Two seeds from each of 134 DH lines were planted and grown out under greenhouse conditions. Polyethylene isolation bags were used to ensure self-pollination during flowering. Selfed seed harvested from these plants was used in the field study and grown to produce leaf tissue for DNA extraction.

4.2.2 Field Trials

One hundred DH lines were grown in field trials in 1998 and 1999 at Winnipeg and Carman. Each line was planted as a single 3-metre row with two replicates/location. Rows were harvested and fatty acid contents were determined on oil extracted from 0.25g of seed (Hougen and Bodo, 1973) using gas chromatography. The mean linolenic acid content for each DH line was calculated by averaging the values from the four site years (Appendix 5).

4.2.3 Sample Preparation

Seed from the greenhouse self-pollinated plants was used for the DNA analysis. Three seeds from each of the 134 DH lines were grown in a controlled environment growth room. Equal amounts of leaf tissue were sampled from each plant at the 4-5 leaf stage, placed into small paper envelopes and placed in liquid nitrogen. The envelopes were lyophilised for 48 hours, sealed in airtight bags with desiccant and stored at –20°C.

4.2.4 DNA Isolation

Approximately 20mg of dried tissue from each DH line was placed in 1.2mL wells of a 96-well microtube plate (Qiagen). Glass beads (3mm) were added to each tube and the plates were agitated by a modified paint shaker until all tissue was ground to a fine powder. DNA extraction was carried out using a Qiagen DNeasy^{TM} 96 Plant Kit according to the manufacturers instructions (Qiagen, 1999). In the final step, DNA was eluted with 150µl of the supplied elution buffer. DNA was quantified by fluorimetry using Hoecsht 33258 stain. Typical DNA yields were 15-25µg, and working dilutions of 6ng/µl were prepared and stored at –20°C. Stock DNA was stored at –80°C.

4.2.5 RAPD Amplification

PCR conditions for RM350 (Table 4.1) were optimised on a MJ Research Peltier thermal cycler (PTC 200) to produce a banding pattern that resembled the profile generated by Rajcan et al. (1999). The final 25µl reaction mixture

included: 24ng genomic DNA, 1X reaction buffer (Perkin Elmer), 1.5mM $MgCl_2$, 200μM of each dNTP, 1U Taq DNA polymerase and 20pmol of primer. All primers used in this study were synthesized by Life Technologies - Gibco BRL Custom Primers. The amplification pattern was as follows: 94°C for 2 min, 35 cycles of 94°C for 1 min, 37°C for 1 min, ramp to 72°C at 1°C/sec and 72°C for 2 min, followed by 72°C for 5 min. The amplified products were resolved in 1.6% agarose gels containing TAE (Tris/ sodim acetate/ EDTA) and ethidium bromide [2.5μl (10mg/ml)/100ml]. Gels were run in 1X TAE for 1.5 hours at 100V. A low DNA mass ladder (Gibco) or 100bp ladder (Promega) was included on each gel as a size standard. Gels were photographed on a digital gel documentation system (AlphaImager, Canberra, Inc.).

Table 4.1. Primer sequences of molecular markers amplified in a DH population of *B. napus* segregating for linolenic acid content. (F), forward primer sequence; (R), reverse primer sequence.

Primer Name	Sequence (5' to 3')	Marker Fragment Size (bp)	Marker Allele
RM350	TGA CGC GCT C	461	low C18:3
SCAR350	(F) TGA CGC GCT CCC ATA AGT TAT CTG (R)TGA CGC GCT CAT AAA CCG AGA ATC	461	n/a
ASA350	(F)CTG AAT CGG GAT CAA GGC TT (R)TGA CGC GCT CAT AAA CCG AG	315	low C18:3
fad3[i]	(F) CTA TCA ATA GTT GTT AAT CCT CCA CA (R) TTG GAC GAC CAC TTG TCA GAT T	965	low C18:3

[i] primer sequence patent pending (Somers and Rakow, PTC/CA00/01140)

Parental DNA (Reston and LL09) was not available. Therefore, DH line 126 (3.1% C18:3) and DH line 33 (10.1% C18:3) were selected based on linolenic acid levels determined at the University of Guelph to represent the low and high linolenic acid alleles. The applicability of RM350 was verified by amplification in DH126, DH33 and selected low and high linolenic acid lines of the DH population. Amplification of RM350 was carried out on all 134 DH lines.

4.2.6 Cloning and sequencing of RAPD product

The RM350 fragment amplified in DH126 was sampled from the agarose gel using the band stab PCR technique (Bjourson and Cooper, 1992) and the DNA was re-amplified using the RM350 primer. The amplified product was run on an agarose gel as above to verify that no untargeted bands were present. The re-amplified fragment was excised from the agarose gel and placed at -70°C overnight. The frozen fragment was removed from the freezer and placed between parafilm. As the fragment thawed, firm pressure was applied to it and the exuding buffer was removed with a micropipetter and placed in a clean microcentrifuge tube. DNA was extracted with CTAB/ NaCl and chloroform, and precipitated with Quik-Precip™ (Edge BioSystems). The DNA pellet was washed with ethanol, re-suspended in Tris/EDTA (TE) buffer and quantified on a 2.0% agarose gel by comparison to a DNA mass ladder.

DNA was ligated into a plasmid vector with the pGEM-T Easy Vector System (Promega) and plasmids were transformed into *Escherichia coli* (JM109

High Efficiency Competent Cells) using the heat shock transformation protocol supplied by the manufacturer (Promega, 1998). Transformed cells (100µl) were plated onto LB-Amp plates containing 40µl 5-bromo-4-chloro-3-indoyl-β-galactoside (X-Gal) (20mg/ml) and 4µl isopropyl-β-D-thiogalactoside (IPTG) (200mg/ml) and incubated overnight at 37°C. Transformed colonies were identified based on colour. Successful cloning of the insert into the plasmid interrupted the coding sequence of β-galactosidase and therefore, transformed cells produced white colonies instead of blue colonies.

A portion of each white colony was transferred to a 0.2ml microcentrifuge tube containing 10µl of sterile water. The presence of the insert was verified by colony PCR amplification with pUC M13 primers. The 25µl PCR reaction contained the following: 10µl of colony mixture, 1X reaction buffer (Gibco, BRL), 2.0mM $MgCl_2$, 200µM of each dNTP, 1.25U Taq DNA polymerase and 0.1µM of each of the M13 forward and reverse primers. The amplification pattern was as follows: 95°C for 2 min, 25 cycles of 95°C for 30s, 50°C for 45s and 72°C for 1.5 min, followed by 72°C for 7 min. Clones containing the insert were selected from the LB-Amp plates and grown at 37°C overnight in 5mL of LB-Amp broth. DNA was isolated from clones using a standard plasmid alkaline lysis miniprep procedure (Birnboim and Doly, 1979; Birnboim, 1983), digested with EcoRI, and run on an agarose gel to re-verify the presence of the insert. DNA was quantified by fluorimetry.

Six clones were sequenced by the DNA Services Laboratory (National Research Council, Plant Biotechnology Institute, Saskatoon, Canada) using dye-deoxy cycle sequencing. Clones were sequenced in both forward and reverse directions.

4.2.7 SCAR design and amplification

Two oligonucleotides were designed as sequence characterised amplified region (SCAR) primers (SCAR350F and SCAR350R) based on the sequence of RM350 (Table 4.1). Each primer contained the original ten bases of the RAPD primer and the next 14 internal bases from each end of the marker fragment (Paran and Michelmore, 1993). SCAR350 primers were tested on DH126 and DH33. The final 25µl PCR reaction mixture included: 24ng genomic DNA, 1X reaction buffer (Perkin Elmer), 1.5mM $MgCl_2$, 200µM of each dNTP, 1U Taq DNA polymerase and 10pmol of each primer. Amplification conditions were as follows: 95°C for 2 min, 35 cycles of 95°C for 1 min, 60°C for 45 sec and 72°C for 1 min, followed by 72°C for 5 minutes. The products were visualised on a 1.6% agarose gel containing TAE and ethidium bromide. The gel was run with 1X TAE for 1 hour at 100V.

4.2.8 Cloning and sequencing of SCAR fragments

The SCAR350 fragments amplified from DH126 and DH33 were excised from the gel. Extraction and cloning of the DNA was performed as described in section 4.2.6. Two clones from each of DH126 and DH33 were sequenced by the

DNA Services Laboratory (National Research Council, Plant Breeding Institute, Saskatoon, Canada).

4.2.9 Allele-Specific primer design and amplification

The sequences of the SCAR fragments were compared using LAlign software (Huang and Miller, 1991) and a site of sequence divergence between DH126 and DH33 was identified. Allele-specific primers of 20 bases were designed (ASA350F and ASA350R) (Table 4.1) with the 3' end of the forward primer located on the site of sequence divergence. ASA350 was amplified from DH126, DH33 and selected low and high linolenic acid DH lines. The 25µl PCR reaction contained the following reagents: 24ng genomic DNA, 1X reaction buffer (Perkin Elmer), 1.5mM $MgCl_2$, 200µM of each dNTP, 1U Taq DNA polymerase and 10pmol of each primer. Amplification was optimised with the following 'Touchdown' program: 95°C for 2 min, 10 cycles of 95°C for 30 sec, 65°C for 30 sec –0.7°C/cycle, 72°C for 45 seconds, followed by 20 cycles of 95°C for 30 sec, 58°C for 30 sec and 72°C for 45 sec, and finally 72°C for 5 min.

ASA350 was amplified from 134 DH lines. Segregation data was collected by scoring the marker as presence or absence of the band.

4.2.10 Amplification of the *fad3* marker

fad3 primer sequence information was kindly provided by Agriculture and Agri-Food Canada, Saskatoon Research Centre (Somers and Rakow,

PTC/CA00/01140). The *fad3* marker (Table 4.1) was amplified using the following reagents (25µl final reaction): 24ng genomic DNA, 1X reaction buffer (Perkin Elmer), 1.5mM $MgCl_2$, 200µM of each dNTP, 1U Taq DNA polymerase and 10pmol of each primer. The following "Touchdown" program was used for amplification: 95°C for 2 min, 10 cycles of 95°C for 30 sec, 65°C for 30 sec – 0.7°C/cycle, 72°C for 45 seconds, followed by 20 cycles of 95°C for 30 sec, 58°C for 30 sec and 72°C for 45 sec, and finally 72°C for 5 min. The *fad3* marker was amplified from 134 DH lines. The segregation data was scored as presence or absence of the band.

4.2.11 Data Analysis

Analyses of variance were performed using standard spreadsheet software (Microsoft Excel). Phenotypic data from 100 DH lines grown in field trails was averaged across the four site years and used for analysis.

4.3 Results

4.3.1 Doubled haploid populations and distribution of linolenic acid content

Linolenic acid content was determined for 100 DH lines – 56 lines derived from Reston x LL09 and 44 lines derived from LL09 x Reston. The mean linolenic acid levels were 5.4% and 4.9% for Reston x LL09 and LL09 x Reston respectively. When the data from the 2 populations was combined, the mean linolenic acid content was 5.2%, with the minimum being 2.9% (DH160) and the maximum being 8.7% (DH 143) (Table 4.2). Frequency distributions of the individual DH populations and the combined DH population are shown in Figures 4.1 and 4.2. The combined distribution appears to have a tri-modal shape.

Table 4.2. Mean and range values for linolenic acid content in two reciprocal *B. napus* populations segregating for linolenic acid content

		Linolenic Acid Content (%)		
Population	No. of DH Lines	Mean	Minimum	Maximum
Reston x LL09	56	5.4	3.0	8.4
LL09 x Reston	44	4.9	2.9	8.7
Combined population	100	5.2	2.9	8.7

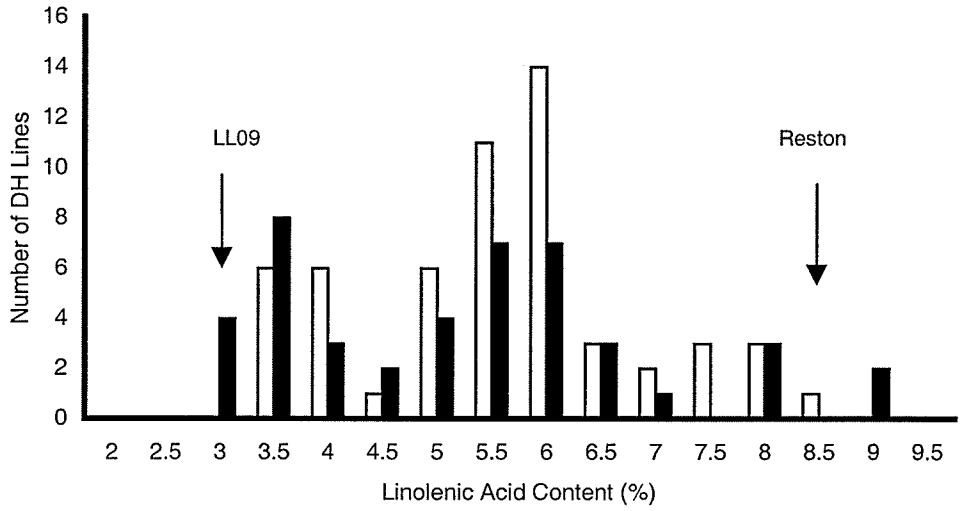

Figure 4.1. Phenotypic distribution of DH lines from two reciprocal populations of *B. napus* segregating for linolenic acid content. White bars represent the 56 lines derived from Reston x LL09. Black bars represent the 44 lines derived from LL09 x Reston.

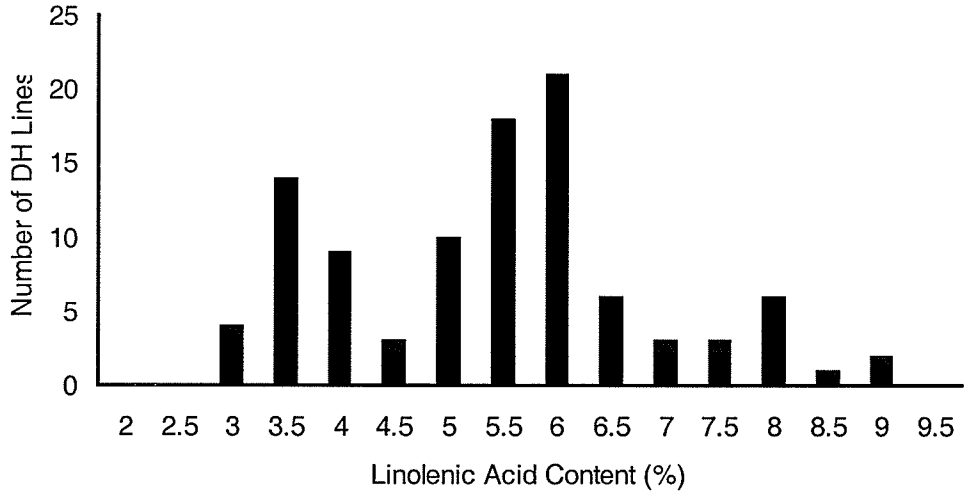

Figure 4.2. Phenotypic distribution of 100 DH lines of *B. napus* segregating for linolenic acid content. The population consists of lines derived from reciprocal crosses of Reston and LL09 (combined population).

4.3.2 Amplification and verification of RM350

RAPD marker RM350 was previously identified as a marker for linolenic acid content in the Reston x LL09 reciprocal DH populations. Rajcan et al. (1999) reported that RM350 was a fragment of approximately 470 bp and linked to the low linolenic acid allele of LL09.

The RM350 primer was tested on selected low and high linolenic acid DH lines to verify its amplification and segregation. The marker fragment was amplified in 10 out of 12 low linolenic acid lines and 1 out of 12 high linolenic acid lines (Figure 4.3).

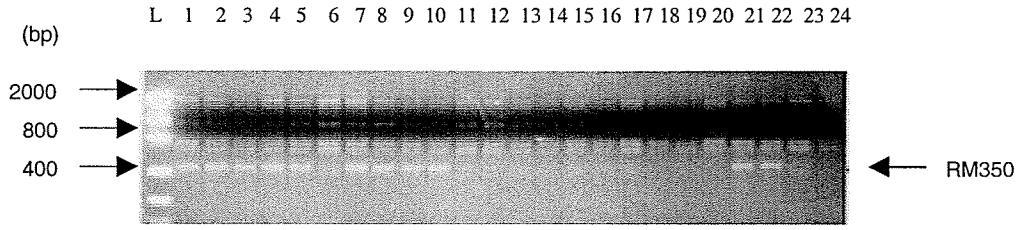

Figure 4.3. Amplification of RM350 from selected low and high linolenic acid DH lines of *B. napus.* Lane L, low DNA mass ladder; Lanes 1-11, 22, low linolenic acid DH lines; Lanes 12-21, 23, 24, high linolenic acid DH lines. Arrow at right indicates the position of RM350 at 461 bp.

It was extremely difficult to generate the desired banding pattern observed by Rajcan et al. (1999) and the RM350 marker fragment. Results were not repeatable and it was not possible to score RM350 on the 134 DH lines in the population. Therefore, there is no image of RM350 segregating in the population.

However, it was possible to excise the putative marker band from DH126 and proceed with the development of an allele specific marker. The RM350 primer was used to amplify DNA extracted from the excised band and a single fragment was generated (Figure 4.4). The RM350 fragment from DH126 was cloned and sequenced. Based on the sequence information, it was determined that the size of the RM350 fragment was 461bp (Figure 4.5).

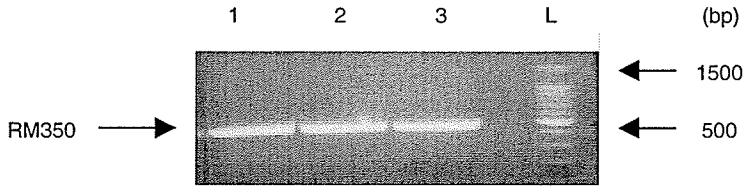

Figure 4.4. Re-amplification of RM350 using excised RM350 band (from DH126, a low linolenic acid DH line of *B. napus*) as template. Lanes 1-3, DH126; L,100bp DNA ladder. Arrow at left indicates the position of RM350 at 461 bp.

RM350

```
1    TGACGCGCTCCCATAAGTTATCTGCCATTGGAGTTAAAGCATTTATGCATCTGATCAAAC

61   CACAATTCGTATTCGATTAGAGAATTCGAAAAGCGATACCTCGACACCTTCTTTACCGGA

121  AGGTGCGGCAGAAGCAGGGGTGTAGCCGGAATCGGGATCAAGGCTCAGGTAAAGGAAGAG

181  AGCGAGGCAGAAGAATCCCAGAGCCATCAGGAAGAGGAGAGCGATGAACCAGTTCTGCTG

241  ATAAAAGGGAAATTCGGGGATCGCTTTCCTCGCCGAGGGAGTTCGTAGAGGCGAATCGGA

301  GGAAGGAGGAGGAGATGATGAGGGGGGAGGCTTCCTTCTTGAGAGAGCTTGCTGTTGCCC

361  TGACGTTGACGCCACCGTGAAGGATCAGAGTGAGATCTCGCCGGAGACAGTGGAAGGGAA
                                                RM350
421  GGTCGCTGAATCTGTTGGATTCTCGGTTTATGAGCGCGTCA
```

Figure 4.5. Sequence of the RM350 fragment amplified from DH126, a low linolenic acid DH line of *B. napus*. RM350 primer sequence indicated by shading.

4.3.3 Development and amplification of SCAR350 and ASA350

Fragments were amplified from DH126 and DH33 using SCAR350 primers (Figure 4.6). Cloning and sequencing determined that the fragments were 461 bp long. The fragments had identical sequences with the exception of one nucleotide located 166 bases from the 5' ends of the sequences. The base at position 166 was thymine (T) in DH126 and cytosine (C) in DH33 (Figure 4.7).

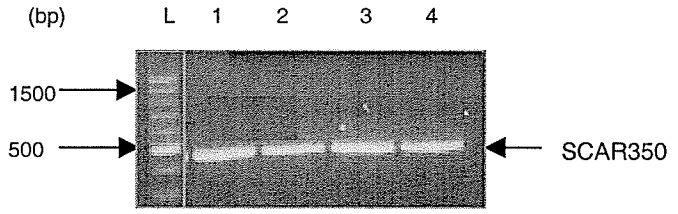

Figure 4.6. Amplification of SCAR350 from DH126 (low linolenic acid) and DH33 (high linolenic acid). L, 100 bp DNA ladder; Lanes 1-2, DH33; Lanes 3-4, DH126. Arrow at right indicates the position of SCAR350 at 461 bp.

ASA350 primers were designed based on the sequence divergence (Figure 4.7). ASA350 was amplified from DH126, DH33 and selected low and high linolenic acid lines from the DH population. The marker was present in 12 out of 12 low linolenic acid lines and in 1 out of 12 high linolenic acid lines (Figure 4.8). This segregation pattern did not exactly match that of RM350 in the selected 24 DH lines.

```
          RM350          SCAR350 (F)
          TGACGCGCTCCCATAAGTTATCTGTCATTGGAATTAAAGCATTTAAGCATCTGATCAAAC
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
1         TGACGCGCTCCCATAAGTTATCTGTCATTGGAATTAAAGCATTTAAGCATCTGATCAAAC

61        CACAATTCGTATTCGATTAGAGAATTCGAAAATCAATACCTCGACACCTTCTTTACCGGA
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
61        CACAATTCGTATTCGATTAGAGAATTCGAAAATCAATACCTCGACACCTTCTTTACCGGA

121       AGGTGCGGCGGAAGCGGAGGTGTAGCCTGAATCGGGATCAAGGCTCAGGTAGAGGAAGAG
          |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
121       AGGTGCGGCGGAAGCGGAGGTGTAGCCTGAATCGGGATCAAGGCTTAGGTAGAGGAAGAG
                                      ASA350 (F)
181       AGCGAGGCAGAAGAATCCCAGAGCCATCAGGAAGAGGAGAGCGATGAACCAGTTCTGCTG
          |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
181       AGCGAGGCAGAAGAATCCCAGAGCCATCAGGAAGAGGAGAGCGATGAACCAGTTCTGCTG

241       ATAAAAGGGAAATTCGGGGATCGGTTTCCTCGCCGAGGGAGTTCGTAGAGGCGAATCAGA
          |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
241       ATAAAAGGGAAATTCGGGGATCGGTTTCCTCGCCGAGGGAGTTCGTAGAGGCGAATCAGA

301       GGAAGGAGGAGGAGATGATGAGGGGGGAGGCTTCCTTCTTGAGAGAGCTTGTTGTTGCCC
          |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
301       GGAAGGAGGAGGAGATGATGAGGGGGGAGGCTTCCTTCTTGAGAGAGCTTGTTGTTGCCC

361       TGACGTTGACGCCACCGTGAAGGATCAGAGTGAGATCTCGCCGGAGACAGTGGAAGGGAA
          |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
361       TGACGTTGACGCCACCGTGAAGGATCAGAGTGAGATCTCGCCGGAGACAGTGGAAGGGAA
                                  SCAR350 (R)      RM350
421       GGTCGCTGAATCTGTTGGATTCTCGGTTTATGAGCGCGTCA
          ||||||||||||||||||||||||||||||||||||||||
421       GGTCGCTGAATCTGTTGGATTCTCGGTTTATGAGCGCGTCA
                                  ASA350 (R)
```

Figure 4.7. Alignment of SCAR350 fragment sequences amplified from low (DH126, lower sequence) and high (DH 33, upper sequence) linolenic acid DH lines of *B. napus*. Sequences of RM350, SCAR350 and ASA350 primers are indicated by shading.

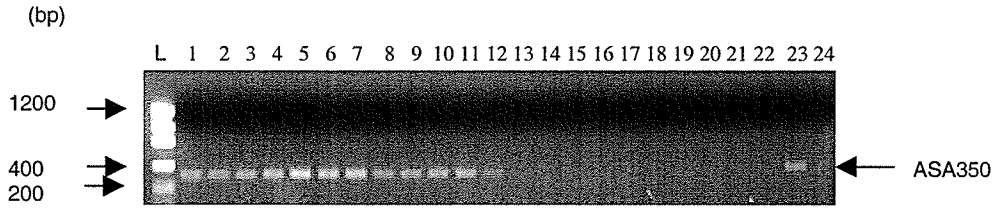

Figure 4.8. Amplification of ASA350 from selected low and high linolenic DH lines of *B. napus*. L, low DNA mass ladder; Lanes 1-12, low linolenic acid DH lines; Lanes 13-24, high linolneic acid DH lines. Arrow at right indicates the position of ASA350 at 315 bp.

ASA350 was amplified from the 134 lines in the DH population (Figure 4.9). The marker was amplified in 89 lines, and not amplified in 45 lines. Chi-square analysis showed that ASA350 did not fit the expected 1:1 segregation ratio for a single marker in the combined DH population. Segregation within the reciprocal populations did not fit the expected 1:1 ratio either (Table 4.3).

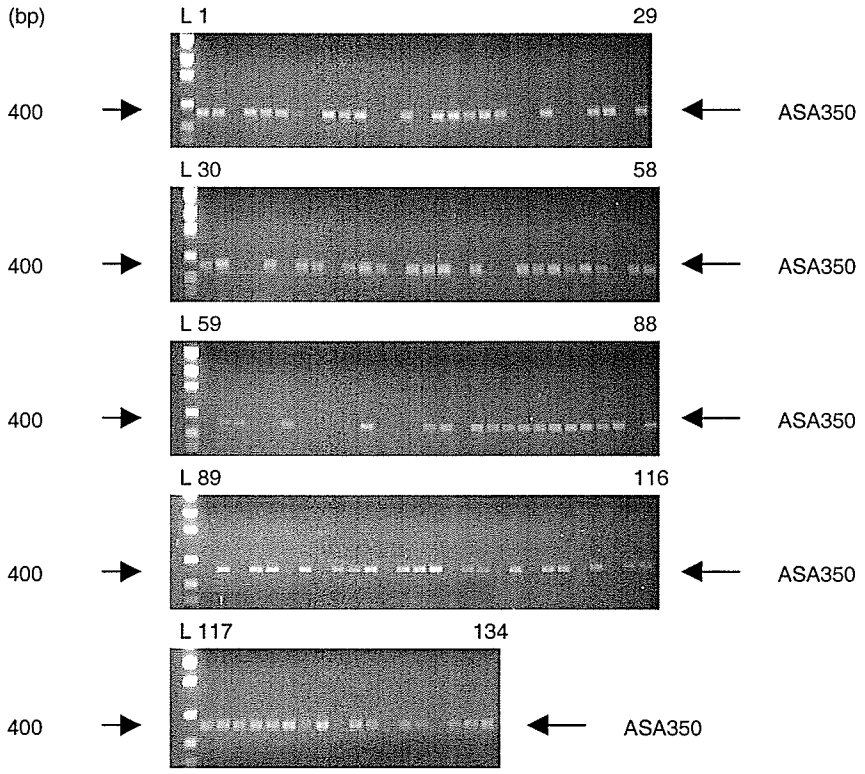

Figure 4.9. Amplification of ASA350 from 134 DH lines of *B. napus.* L, low DNA mass ladder; Lanes 1-64, 68, 69, 130-134, DH lines derived from Reston x LL09; lanes 65-67, 70-129, DH lines derived from LL09 x Reston. Arrow at right indicates the position of ASA350 at 315 bp.

Table 4.3. Chi-square tests for 1:1 segregation of markers in DH populations of *B. napus*. ("+", number of DH lines in which the marker was present; "-", number of DH lines in which the marker was absent)

Marker	Population	Observed No. (+/-)	Expected No. (+/-)	χ^2
ASA350	Combined	89/45	67/67	14.40
	Reston x LL09	47/24	35.5/35.5	7.50
	LL09 x Reston	42/21	31.5/31.5	7.00
fad3	Combined	81/53	67/67	6.75
	Reston x LL09	42/29	35.5/35.5	2.30*
	LL09 x Reston	40/23	31.5/31.5	4.59

df=1
*, significant at P=0.05

4.3.4 Amplification of the *fad3* marker

A gene specific marker, *fad3*, previously developed at the Saskatoon Research Centre, Agriculture and Agri-Food Canada (Somers and Rakow, PTC/CA00/01140) was amplified from the DH population.

The f*ad3* primers produced a marker band of 965 bp, which segregated in the selected lines. *fad3* was amplified from the combined DH population (134 DH lines) and the marker fragment was present in 81 lines and absent in 53 lines (Figure 4.10). Chi-square analysis indicated that *fad3* did not fit the expected 1:1 segregation ratio in the combined population. In the Reston x LL09 population, the marker fit the 1:1 segregation ratio, while in the LL09 x Reston population it did not (Table 4.3).

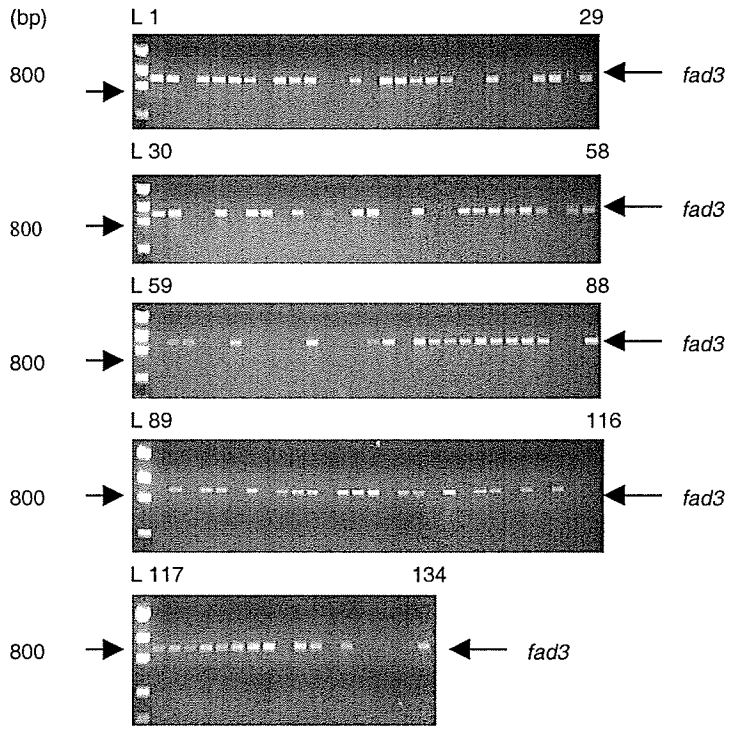

Figure 4.10. Amplification of *fad3* from 134 DH lines of *B. napus.* L, low DNA mass ladder; Lanes 1-64, 68, 69, 130-134, DH lines derived from Reston x LL09; lanes 65-67, 70-129, DH lines derived from LL09 x Reston. Arrow at right indicates the position of *fad3* at 965 bp.

4.3.5 Analysis of variance and linkage of markers associated with linolenic acid content

Regression analysis was used to describe the amount of variation in linolenic acid content explained by each marker. ASA350 and *fad3* were found to account for 28% (P=0.0001) and 41% (P=0.0001) of the variation respectively (Table 4.4). Full ANOVA results are shown in Appendix 16 & 17. Lines in which ASA350 was amplified had a mean linolenic acid content of 4.6% and lines in which *fad3* was amplified had a mean linolenic acid content of 4.4%. The mean linolenic acid content of the lines in which the marker was not amplified was 6.2% for ASA350 and 6.3% for *fad3*.

Linkage analysis showed seven recombinants out of 134 lines. This recombination frequency places ASA350 5.2cM from the *fad3* marker (Appendix 15).

Table 4.4. Analyses of variance for ASA350 and *fad3* markers in DH populations of *B. napus.*

Primer	Population	r^2	P	Means	
				Low C18:3	High C18:3
ASA350	Combined	0.27	0.0001	4.6	6.2
	Reston x LL09	0.28	0.0001	4.9	6.3
	LL09 x Reston	0.29	0.0002	4.3	6.1
Fad3	Combined	0.41	0.0001	4.4	6.3
	Reston x LL09	0.36	0.0001	4.8	6.3
	LL09 x Reston	0.50	0.0001	4.0	6.3

4.3.6 Relationship between marker determined genotypes and phenotypes of DH lines

Figures 4.11 and 4.12 show the relationship between the distribution of lines according to linolenic acid content and genotype. There appear to be three genotypic modes corresponding to the three phenotypic modes. Both ASA350 and *fad3* were amplified in all lines having <4.5% linolenic acid and were amplified in some lines having 4.6-6.5% linolenic acid. In lines having >6.5% linolenic acid, *fad3* was not amplified and ASA350 was amplified in only 3 lines.

Two-gene inheritance in a doubled haploid population is characterised by a tri-modal distribution of phenotypes. Chi-square analysis was performed to test for a 1:2:1 segregation ratio of linolenic acid classes (Table 4.5). The result (χ^2=5.5, P>0.05) was significant and supported two gene inheritance of linolenic acid.

Table 4.5. Chi-square test for 1:2:1 phenotypic distribution of 100 DH lines of *B. napus* segregating for linolenic acid content.

Phenotypic class	Observed No.	Expected No.	$\chi^{2\,(i)}$
Low C18:3 (<4.5%)	30	25	1
Intermediate C18:3 (4.6%-6.5%)	55	50	0.5
High C18:3 (>6.5%)	15	25	4
Total	100		5.5*

[i], df= 2
*, significant at P=0.05

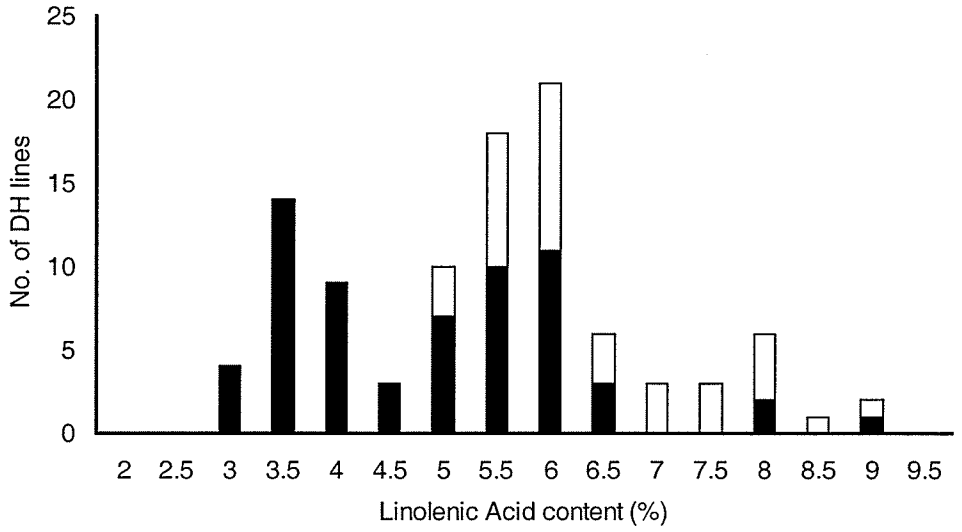

Figure 4.11. Phenotypic and genotypic (ASA350) distribution of 100 DH lines of *B. napus.* Total bar height represents the phenotypic distribution of DH lines. Black bars represent the distribution of DH lines with the ASA350 genotype.

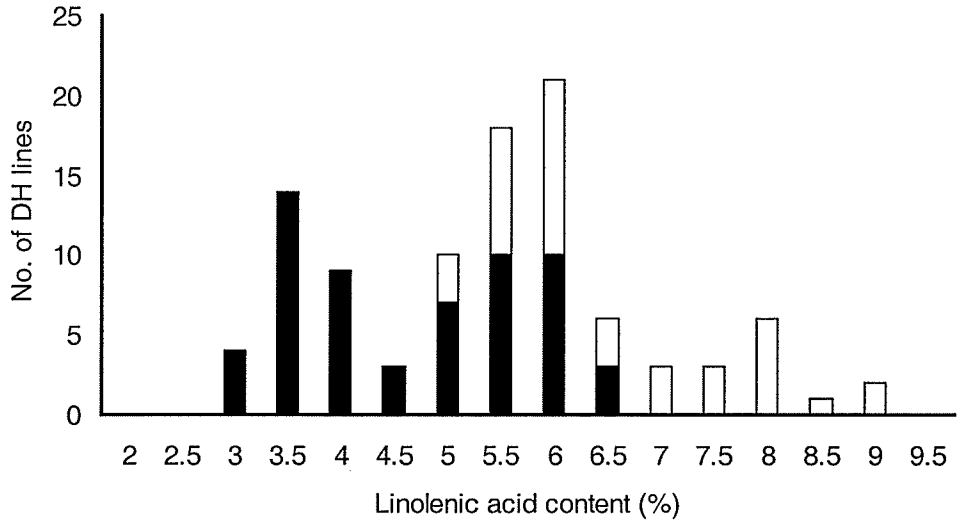

Figure 4.12. Phenotypic and genotypic (*fad3*) distribution of 100 DH lines of *B. napus*. Total bar height represents the phenotypic distribution of DH lines. Black bars represent the distribution of DH lines with the *fad3* genotype.

4.5 Discussion

The purpose of this study was to develop an ASA by sequencing a RAPD marker fragment and designing specific primers for its amplification. The RAPD marker RM350 was developed by Rajcan et al. (1999) and accounted for 25% of the variation in linolenic acid content observed in a DH population of *B. napus*. In the current study, RM350 did not produce the banding pattern observed by Rajcan et al. (1999) although the same DH population was tested under the same PCR conditions. The problem of RAPD marker reproducibility is widely recognised. Jones et al. (1997) tested RAPD markers in various laboratories and found large variations in band profiles even with adherence to stringent protocols.

Despite the difficulties with RM350 amplification, the putative linolenic acid marker band was sequenced and SCAR primers were designed by extending the original 10-bp RAPD primer sequence. However, these SCAR markers did not show the polymorphism between the low and high linolenic acid lines that was observed with RM350. Loss of polymorphism when primers are extended has been observed in other studies (Paran and Michelmore, 1993; Deng et al. 1997). RAPD polymorphisms can result from either structural rearrangements or mismatches of one or a few nucleotides in the primer binding sites. It has been suggested that most RAPD polymorphisms are due to the latter. Therefore, when longer primers, such as SCAR350F and SCAR350R, are used, the mismatch is not detected and bands are amplified from both parents.

Sequencing of the SCAR350 fragments identified a single nucleotide difference between the high and low linolenic acid lines and an ASA was designed based on the sequence divergence. In this study, the 3' terminal nucleotide of ASA350F matched the low linolenic acid allele. ASA markers designed on the basis of single nucleotide differences have been reported in other studies (Penner et al., 1995; Barret et al., 1999).

RM350 was successfully converted into an ASA (ASA350) which was found to account for 27% of the variation in linolenic acid content in the DH population. This was similar to the 25% variation found by Rajcan et al. (1999) for the original RAPD. ASA350 was superior to RM350 in that it only amplified one allele and was more robust, repeatable and facilitated the determination of individual genotypes within the population. In order to test the cross applicability of ASA350, it would be necessary to test the primers on crosses derived from parents other than Reston and LL09.

fad3 is a gene that codes for the delta–15 fatty acid desaturase enzyme that converts linoleic acid (C18:2) to linolenic acid (C18:3). It appears that mutations of the *fad3* gene cause differences in linolenic acid content. There are likely two *fad3* genes controlling linolenic acid synthesis in the cytoplasm of *B. napus* - one in the A genome and one in the C genome (Jourdren et al., 1996b;

Barret et al., 1999). *B. napus* is an amphidiploid species derived from *B. rapa* (AA) and *B. oleracea* (CC).

Somers and Rakow (PTC/CA00/01140) sequenced the *fad3* (A genome) locus from the low linolenic acid *B. napus* cultivar, Apollo. Results indicated that the low linolenic acid mutation in the Apollo *fad3* allele resulted in a non-conserved amino acid substitution of Cysteine for Arginine at amino acid 275. Somers and Rakow (PTC/CA00/01140) designed primers specific to the mutant allele based on the sequence divergence between the mutant and wild type *fad3* alleles. The *fad3* marker was amplified from the DH population in this study and accounted for 41% of the variation in linolenic acid content. This is the highest association from a single marker that has been reported for low linolenic acid content. Linkage analysis determined that the distance between ASA350 and *fad3* was 5.2 cM. Since the *fad3* marker tagged the gene of interest, it can be assumed that ASA350 is located 5.2cM from the fad3 gene of the A genome of *B. napus*.

The distributions of both ASA350 and *fad3* were skewed toward the low linolenic acid parent. A number of molecular mapping studies involving *B. napus* have reported this occurrence, with up to 35% of segregating markers demonstrating a non-Mendelian segregation (Ferreira et al., 1994; Foisset et al., 1996; Cloutier et al., 1997). Distorted segregation ratios may be due to differential viability or selection of certain genotypes at various stages of DH

development (Ferreira, et al., 1994; Foisset et al., 1996). Studies have also shown that loci with skewed segregation often cluster together on the same linkage group (Ferreira et al., 1994; Foisset et al, 1996). The results of the current study agree with this, as ASA350 and *fad3* are linked and both show skewed segregation.

Rajcan et al. (1999) did not find distorted segregation ratios and reported that RM350 fit the expected 1:1 ratio in the DH population. Unfortunately, it was not possible to score RM350 across the population in this study, and therefore, there are no results to compare to those of Rajcan et al. (1999). ASA350 was derived from RM350 so the segregation patterns should have been the same. The difference between the results could be due to inaccurate scoring of RM350 across the population in the study by Rajcan et al. (1999) due to the apparent amplification and reproducibility problems of the original RAPD marker.

Ideally, a molecular marker could be used in making selections for a particular phenotype or trait of interest. Figures 4.11 and 4.12 suggest that both ASA350 and *fad3* could be used in making selections for low linolenic acid content in the present DH population. ASA350 was amplified in all low linolenic acid ($\leq$3%) lines and in only three high linolenic acid (>6.5%) lines. The *fad3* marker was not amplified in any lines with linolenic acid levels greater than 6.5%. The greater accuracy of *fad3* is not surprising, as the marker was designed to amplify the mutant allele. However, from a selection standpoint ASA350 would

still be useful. ASA350 incorrectly tagged three high linolenic acid lines but it amplified all low linolenic acid lines, and therefore, would not have eliminated any low linolenic acid lines if it was used in marker assisted selection (MAS).

The mode of inheritance of linolenic acid content could also be inferred from the results of this study. The trimodal phenotypic distribution of the DH population (Figure 4.1) suggested that two genes were involved. These genes could be designated A and B. The first mode could have been composed of individuals with both alleles conditioning low linolenic acid (ab). Individual lines in the second mode could have possessed just one low linolenic acid allele (Ab or aB) and individual lines in the third mode could have neither low linolenic acid allele (AB). When the genotype results were overlaid on the phenotypic distributions (Figures 4.11 and 4.12), the three genotypic classes became clear, particularly for the distribution of the *fad3* marker. In the low linolenic acid class (>4.5%), all DH lines had the *fad3* marker, and thus the low linolenic acid allele. In the intermediate class (4.6-6.5%), only some individuals had the marker and in the high linolenic acid class (>6.5%), no individuals had the marker. The results of a Chi-square test indicated that the segregation fit a 1:2:1 phenotypic ratio for two-gene inheritance. This supported the initial two-gene inheritance hypothesis. These results differed from those of Chen and Beversdorf (1990) who identified four phenotypic groups for linolenic acid in a segregating DH population of *B. napus*, and proposed a three-gene inheritance model. Jourdren et al. (1996b) and Barret et al. (1999) conducted molecular studies and proposed that two

genes, *fad3*A and *fad3*C are involved in the synthesis of linolenic acid content in *B. napus*.

5. GENERAL DISCUSSION

The first objective of this research project was to characterise several agronomic and quality traits in a DH population of *B. napus* segregating for linolenic acid content. The first study (Chapter 3) concluded that low linolenic acid content was correlated with later flowering and maturity. The results supported previous agronomic and molecular studies that identified linkages between loci controlling time to flowering and linolenic acid content (Rajcan et al., 1999; Ferreira et al., 1995; Thormann et al., 1996). Developing *B. napus* cultivars with early flowering and maturity is particularly important in western Canada as the canola growing region has a relatively short frost-free period. Due to this apparent linkage, breeding for a combination of early flowering and maturity and low linolenic acid levels will require large population numbers in order to find individuals with both desired traits. Breeding for low linolenic acid levels would not have negative effects on plant height or oil and protein content as there were no consistent correlations with linolenic acid content. This study examined several important quality and agronomic traits but did not include seed yield. The association between yield and linolenic acid content would be an important extension of this project in order to fully characterise the effect of the low linolenic acid trait on field performance.

Several studies of linolenic acid content in *B. napus* have reported that the expression of the trait is highly influenced by the environment (Brunklaus-Jung

and Robbelen, 1987; Rajcan et al. 1999). Determining the genotype x environment interaction was not possible in this study, as site year data could not be combined due to heterogeneity of error variances. It was possible to do a simple stability analysis which found that 13 DH lines consistently had among the lowest linolenic acid contents at all site years, indicating that the trait was quite stable across environments. More detailed stability studies should be carried out to confirm this observation.

The second objective of this research project was to develop a molecular marker associated with linolenic acid content (Chapter 4). RM350, a RAPD marker associated with linolenic acid content, was successfully converted into an allele specific amplicon (ASA), ASA350, which was found to account for 27% of the variation in linolenic acid content. It was also determined that ASA350 tagged a locus that was located approximately 5.2cM from the *fad3* locus of the A genome (*fad3*A). Two reports have identified *fad3*A as one of the genes controlling linolenic acid content (Barret et al., 1999; Somers and Rakow, PTC/CA00/01140). The linkage between ASA350 and the *fad3* locus would make ASA350 a useful marker in making selections of low linolenic acid lines. The cross applicability of ASA350 was not examined and further studies would be needed to determine if the marker could be used in crosses derived from parents other than Reston and LL09.

An examination of the mode of inheritance of linolenic acid content *in B. napus* was not an objective of this research project. However, the phenotypic data generated in the field study (Chapter 3) and the results of the marker study (Chapter 4) can be combined to make some inferences about the mode of inheritance. The distribution of linolenic acid content in the DH population appeared to be tri-modal. When the genotype distribution was overlaid, the three modes became clearly defined and there appeared to be three phenotypic classes – low (>4.5%), intermediate (4.6-6.5%) and high (>6.5%) linolenic acid. A Chi-square test supported a 1:2:1 segregation ratio that suggests two gene inheritance in a DH population. These results agreed with those of Barret et al. (1999) who proposed that two fad3 genes, one in each genome of *B. napus*, control linolenic acid content.

In summary, knowledge of associations between linolenic acid and agronomic and quality traits will benefit the breeding programs for low linolenic acid content. The ASA marker developed in this study could be used to make selections for low linolenic acid content in *B. napus*. Furthermore, the results of this thesis will contribute towards our understanding of the mode of inheritance of linolenic acid.

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7. APPENDIX

Appendix 1. Seed quality and agronomic field results of 100 DH lines of *B. napus* grown at Wpg98.

Appendix 2. Seed quality and agronomic field results of 100 DH lines of *B. napus* grown at Car98.

Appendix 3. Seed quality and agronomic field results of 100 DH lines of *B. napus* grown at Wpg99.

Appendix 4. Seed quality and agronomic field results of 100 DH lines of *B. napus* grown at Car99.

Appendix 5. Seed quality and agronomic results of 100 DH lines of *B. napus* averaged over 4 site years (Wpg98, Car98, Wpg99, Car99).

Appendix 6. Analyses of variance for linolenic acid content on 100 DH lines of *B. napus.*

Appendix 7. Analyses of variance for seed oil content on 100 DH lines of *B. napus.*

Appendix 8. Analyses of variance for seed protein content on 100 DH lines of *B. napus.*

Appendix 9. Analyses of variance for sum of oil and protein content on 100 DH lines of *B. napus.*

Appendix 10. Analyses of variance for days to flowering on 100 DH lines of *B. napus.*

Appendix 11. Analyses of variance for plant height on 100 DH lines of *B. napus.*

Appendix 12. Analyses of variance for days to maturity on 100 DH lines of *B. napus.*

Appendix 13. Linolenic acid content values for Apollo check rows at each site year.

Appendix 14. Correlation matrices for seed quality and agronomic traits in a DH population of *B. napus.*

Appendix 15. Scores for ASA350 and *fad3* markers across 134 DH lines in a DH population of *B. napus.*

Appendix 16. Analyses of variance for ASA350 in a DH population of *B. napus* segregating for linolenic acid content.

Appendix 17. Analyses of variance for *fad3* in a DH population of *B. napus* segregating for linolenic acid content.

Appendix 1. Seed quality and agronomic field results of 100 DH lines of *B. napus* grown at Wpg98. Traits are seed oil content (OIL), seed protein content (PRO), linolenic acid content (C18:3), days to flowering (DTF), plant height (HT), days to maturity (DTM).

DH line	Replicate	OIL(%)	PRO (%)	C18:3 (%)	DTF (d)	HT (cm)	DTM (d)
3	1	46.6	29.3	3.3	50.0	110.0	95.0
3	2	47.8	28.2	3.1	52.0	120.0	97.0
7	1	47.7	30.0	5.4	52.0	115.0	98.0
7	2	46.9	30.2	5.5	52.0	120.0	99.0
10	1	44.8	28.9	5.5	52.0	125.0	95.0
10	2	42.7	31.4	4.8	52.0	130.0	100.0
11	1	44.8	29.4	5.1	55.0	110.0	99.0
11	2	44.0	31.3	4.7	55.0	110.0	99.0
12	1	45.9	29.3	5.9	52.0	115.0	97.0
12	2	46.2	30.6	5.8	53.0	125.0	99.0
13	1	47.1	28.6	5.3	56.0	115.0	99.0
13	2	46.7	30.4	5.8	56.0	135.0	100.0
14	1	41.0	30.8	5.5	53.0	100.0	99.0
14	2	40.0	34.0	5.1	55.0	120.0	99.0
15	1	44.5	31.3	3.5	55.0	140.0	100.0
15	2	46.0	28.5	3.7	57.0	155.0	99.0
16	1	46.6	27.0	4.1	52.0	70.0	95.0
16	2	47.9	28.4	4.9	55.0	110.0	97.0
17	1	46.0	27.5	6.4	54.0	125.0	99.0
17	2	46.8	28.2	6.9	55.0	135.0	99.0
18	1	44.2	29.6	5.0	51.0	110.0	99.0
18	2	47.0	28.3	5.0	52.0	100.0	94.0
19	1	42.9	29.8	2.9	55.0	120.0	99.0
19	2	43.6	30.3	3.2	55.0	110.0	100.0
20	1	44.5	29.1	5.0	50.0	115.0	97.0
20	2	44.4	28.9	5.6	53.0	100.0	99.0
21	1	40.7	31.1	7.3	55.0	110.0	99.0
21	2	43.9	29.7	6.7	55.0	125.0	99.0
24	1	44.4	28.3	4.0	52.0	105.0	99.0
24	2	40.4	31.8	3.9	54.0	100.0	99.0
25	1	43.2	28.6	2.8	55.0	115.0	97.0
25	2	42.7	29.1	2.9	59.0	90.0	98.0
27	1	44.0	30.1	3.7	52.0	120.0	99.0
27	2	45.1	31.5	3.7	55.0	105.0	99.0
28	1	49.2	28.5	4.5	52.0	120.0	98.0
28	2	48.4	28.9	4.5	52.0	130.0	99.0
31	1	49.1	26.1	3.1	55.0	105.0	99.0
31	2	47.2	29.8	2.7	55.0	115.0	99.0
33	1	45.3	29.5	5.4	52.0	75.0	99.0
33	2	44.6	31.3	7.1	53.0	80.0	99.0
35	1	44.1	28.1	5.5	55.0	125.0	97.0
35	2	43.4	30.4	5.8	55.0	125.0	99.0
36	1	44.1	30.4	6.0	52.0	110.0	97.0
36	2	46.6	29.1	5.8	55.0	125.0	94.0
37	1	45.0	27.8	7.4	52.0	95.0	94.0
37	2	44.5	30.4	7.2	53.0	120.0	94.0
38	1	45.4	29.1	5.4	55.0	130.0	97.0
38	2	45.7	31.0	5.3	56.0	125.0	99.0
40	1	46.2	29.3	5.8	54.0	130.0	99.0
40	2	46.0	30.4	5.7	55.0	110.0	99.0
41	1	45.5	29.5	5.6	50.0	110.0	97.0
41	2	46.1	30.9	5.1	50.0	110.0	97.0
42	1	46.3	28.2	5.2	50.0	120.0	94.0
42	2	46.2	30.0	4.7	50.0	120.0	97.0

Appendix 1 continued

DH line	Replicate	OIL(%)	PRO (%)	C18:3 (%)	DTF (d)	HT (cm)	DTM (d)
44	1	46.1	30.3	5.1	52.0	100.0	99.0
44	2	45.1	28.8	7.2	53.0	100.0	99.0
45	1	44.9	29.8	5.4	54.0	135.0	99.0
45	2	46.9	28.4	5.3	55.0	100.0	99.0
46	1	42.2	31.4	4.0	58.0	105.0	99.0
46	2	43.9	29.3	4.2	59.0	95.0	99.0
47	1	46.4	27.5	5.1	50.0	110.0	94.0
47	2	46.2	28.6	5.0	50.0	115.0	94.0
48	1	47.0	28.5	3.6	56.0	115.0	99.0
48	2	47.6	27.4	3.5	58.0	105.0	100.0
49	1	47.4	29.4	7.5	52.0	125.0	99.0
49	2	47.4	28.0	6.9	55.0	135.0	99.0
50	1	47.4	29.1	2.8	51.0	80.0	99.0
50	2	47.6	28.3	3.0	52.0	95.0	99.0
52	1	47.3	28.5	4.6	51.0	125.0	97.0
52	2	46.6	29.8	4.0	52.0	120.0	97.0
53	1	41.3	31.5	5.5	50.0	115.0	97.0
53	2	40.6	31.1	5.1	50.0	125.0	97.0
54	1	46.3	25.3	5.4	51.0	110.0	95.0
54	2	45.4	27.6	4.9	52.0	120.0	97.0
56	1	44.9	26.8	7.7	55.0	120.0	97.0
56	2	43.0	30.3	7.8	55.0	130.0	99.0
57	1	48.0	28.9	5.7	55.0	140.0	99.0
57	2	47.0	31.3	5.9	56.0	135.0	99.0
59	1	46.6	25.6	5.6	50.0	110.0	95.0
59	2	45.5	28.4	5.7	51.0	115.0	94.0
61	1	49.0	28.5	7.6	54.0	115.0	99.0
61	2	48.6	28.4	7.4	55.0	110.0	95.0
62	1	47.6	27.8	5.7	50.0	120.0	94.0
62	2	47.5	26.8	5.8	50.0	120.0	95.0
64	1	47.5	27.9	5.3	52.0	125.0	97.0
64	2	47.9	26.7	5.3	53.0	130.0	99.0
65	1	45.2	29.2	5.3	50.0	105.0	95.0
65	2	46.2	30.2	5.5	52.0	115.0	94.0
66	1	42.4	29.9	3.5	58.0	120.0	99.0
66	2	43.2	30.1	3.8	58.0	105.0	99.0
67	1	45.4	27.5	3.6	55.0	125.0	99.0
67	2	44.5	28.8	3.6	55.0	150.0	99.0
68	1	46.6	29.8	5.3	55.0	140.0	97.0
68	2	45.3	29.4	5.4	55.0	105.0	99.0
69	1	44.4	29.6	5.2	52.0	105.0	94.0
69	2	45.6	29.2	5.4	54.0	90.0	97.0
71	1	46.5	29.4	5.6	55.0	125.0	99.0
71	2	47.5	30.5	4.9	56.0	115.0	101.0
72	1	50.1	25.1	7.6	52.0	100.0	99.0
72	2	47.4	28.2	7.6	52.0	120.0	99.0
74	1	41.8	30.2	4.6	52.0	110.0	98.0
74	2	41.4	30.8	3.9	55.0	95.0	99.0
76	1	46.7	28.2	4.2	52.0	120.0	99.0
76	2	46.3	29.3	4.2	52.0	135.0	99.0
78	1	44.0	29.6	4.7	50.0	130.0	99.0
78	2	45.9	29.1	4.1	52.0	110.0	95.0
79	1	45.3	31.0	6.5	50.0	105.0	97.0
79	2	46.9	27.9	7.1	51.0	120.0	99.0
80	1	47.5	28.0	3.1	57.0	125.0	99.0
80	2	46.7	30.4	3.2	58.0	125.0	101.0
82	1	48.7	27.1	5.4	51.0	115.0	99.0

Appendix 1 continued

DH line	Replicate	OIL(%)	PRO (%)	C18:3 (%)	DTF (d)	HT (cm)	DTM (d)
82	2	46.8	29.1	4.9	52.0	105.0	97.0
83	1	42.6	29.6	8.2	50.0	115.0	95.0
83	2	42.6	30.9	8.0	51.0	110.0	94.0
84	1	44.3	30.2	6.6	52.0	115.0	99.0
84	2	44.5	30.2	6.8	55.0	125.0	99.0
89	1	46.6	29.6	4.6	50.0	110.0	95.0
89	2	43.7	28.2	4.9	52.0	110.0	99.0
91	1	45.2	28.3	5.6	51.0	115.0	94.0
91	2	43.3	30.9	5.3	53.0	115.0	94.0
92	1	46.9	26.5	3.5	55.0	110.0	99.0
92	2	45.8	29.6	3.1	55.0	110.0	99.0
94	1	48.3	26.3	5.4	50.0	120.0	95.0
94	2	46.9	28.6	5.4	52.0	115.0	99.0
96	1	49.6	25.9	4.8	50.0	110.0	99.0
96	2	49.1	27.5	4.9	50.0	115.0	99.0
98	1	44.1	30.6	2.7	52.0	130.0	99.0
98	2	45.0	30.6	3.0	53.0	110.0	99.0
100	1	48.8	26.7	3.0	50.0	110.0	99.0
100	2	50.0	28.0	3.1	52.0	110.0	97.0
104	1	46.1	29.0	5.5	51.0	120.0	94.0
104	2	44.6	29.4	5.4	52.0	115.0	98.0
107	1	47.4	28.6	7.1	50.0	110.0	97.0
107	2	47.9	28.9	8.0	52.0	110.0	99.0
110	1	43.5	30.8	5.2	51.0	115.0	97.0
110	2	43.6	30.1	5.4	52.0	105.0	98.0
113	1	46.9	29.6	6.1	50.0	120.0	94.0
113	2	47.5	27.5	6.8	50.0	120.0	95.0
114	1	47.6	28.3	4.9	55.0	105.0	95.0
114	2	47.0	27.1	4.9	55.0	115.0	99.0
115	1	49.2	26.1	3.1	55.0	140.0	99.0
115	2	46.7	28.5	3.2	55.0	140.0	99.0
116	1	46.7	30.5	8.2	54.0	125.0	99.0
116	2	46.3	29.3	7.5	55.0	130.0	100.0
120	1	48.0	26.4	2.8	58.0	110.0	99.0
120	2	48.9	27.7	3.0	58.0	100.0	99.0
122	1	47.2	27.7	7.0	50.0	130.0	97.0
122	2	46.7	29.5	6.1	54.0	130.0	99.0
123	1	45.5	27.7	5.0	54.0	125.0	99.0
123	2	45.4	27.7	5.2	55.0	125.0	99.0
124	1	46.3	28.2	5.5	50.0	115.0	97.0
124	2	46.8	29.4	5.2	52.0	125.0	99.0
126	1	45.7	29.0	3.1	58.0	135.0	99.0
126	2	47.2	29.6	3.3	59.0	115.0	97.0
127	1	47.7	27.1	5.7	54.0	100.0	99.0
127	2	46.0	28.9	6.0	55.0	105.0	99.0
128	1	46.4	27.7	3.0	54.0	130.0	97.0
128	2	46.5	29.5	3.3	55.0	145.0	99.0
129	1	47.6	27.8	4.2	52.0	100.0	97.0
129	2	48.2	26.8	4.9	52.0	95.0	99.0
131	1	46.3	29.5	5.0	54.0	125.0	99.0
131	2	46.1	30.6	4.9	54.0	125.0	97.0
132	1	47.4	28.8	2.8	53.0	135.0	97.0
132	2	47.9	29.3	2.9	55.0	120.0	99.0
133	1	47.8	26.1	4.9	52.0	95.0	99.0
133	2	43.8	31.0	4.6	52.0	115.0	99.0
135	1	47.2	28.5	2.8	54.0	130.0	99.0
135	2	46.4	29.3	2.9	55.0	130.0	100.0

Appendix 1 continued

DH line	Replicate	OIL(%)	PRO (%)	C18:3 (%)	DTF (d)	HT (cm)	DTM (d)
136	1	43.8	30.4	3.1	50.0	95.0	97.0
136	2	42.9	31.3	3.1	50.0	95.0	99.0
138	1	45.7	29.9	6.7	52.0	95.0	98.0
138	2	46.4	29.3	6.5	53.0	100.0	99.0
141	1	45.3	28.9	4.4	53.0	125.0	97.0
141	2	44.9	30.7	4.3	55.0	130.0	99.0
143	1	44.2	28.4	8.2	55.0	110.0	100.0
143	2	43.3	28.9	7.9	56.0	110.0	100.0
147	1	45.4	29.3	8.2	57.0	115.0	99.0
147	2	45.6	30.7	7.5	57.0	135.0	99.0
149	1	46.8	27.2	3.2	54.8	117.7	98.7
149	2	46.6	28.3	3.1	55.0	120.0	99.0
151	1	48.4	27.7	4.4	54.0	105.0	99.0
151	2	46.4	30.6	4.9	55.0	120.0	99.0
152	1	47.0	27.8	5.8	56.0	130.0	99.0
152	2	47.9	28.1	6.0	56.0	125.0	100.0
153	1	46.1	29.8	3.5	51.0	125.0	97.0
153	2	45.9	27.5	3.0	52.0	95.0	94.0
154	1	45.3	28.8	6.2	52.0	100.0	95.0
154	2	45.2	29.1	6.0	55.0	100.0	97.0
155	1	46.5	29.5	3.2	55.0	110.0	99.0
155	2	45.2	30.3	3.7	55.0	120.0	99.0
157	1	47.8	27.2	5.4	52.0	120.0	98.0
157	2	47.6	28.3	5.3	55.0	125.0	99.0
159	1	43.3	29.1	3.1	55.0	110.0	99.0
159	2	43.6	29.4	3.3	55.0	110.0	99.0
160	1	47.6	30.9	2.8	58.0	140.0	99.0
160	2	47.2	27.7	2.8	58.0	120.0	100.0
161	1	45.6	27.0	5.8	52.0	115.0	95.0
161	2	44.8	29.2	5.6	52.0	100.0	97.0
163	1	45.5	29.2	3.3	55.0	120.0	97.0
163	2	44.8	30.2	3.6	55.0	125.0	99.0

Appendix 2. Seed quality and agronomic field results of 100 DH lines of *B. napus* grown at Car98. Traits are seed oil content (OIL), seed protein content (PRO), linolenic acid content (C18:3), days to flowering (DTF), plant height (HT), days to maturity (DTM).

DH line	Replicate	OIL(%)	PRO (%)	C18:3 (%)	DTF (d)	HT (cm)	DTM (d)
3	1	48.5	29.3	3.2	53.0	130.0	99.0
3	2	47.0	28.7	6.2	57.0	140.0	99.0
7	1	48.3	29.1	5.6	56.0	125.0	99.0
7	2	49.0	27.9	5.7	56.0	130.0	100.0
10	1	47.2	28.2	6.6	54.0	125.0	98.0
10	2	41.9	34.2	5.5	53.0	115.0	100.0
11	1	47.1	29.0	4.5	57.0	135.0	97.0
11	2	45.0	30.3	5.4	58.0	120.0	100.0
12	1	47.9	24.2	1.6	50.0	110.0	97.0
12	2	47.3	28.5	5.7	55.0	130.0	98.0
13	1	50.0	26.9	5.3	59.0	150.0	96.0
13	2	45.4	30.7	5.5	59.0	155.0	99.0
14	1	43.2	28.2	4.5	57.0	100.0	99.0
14	2	40.0	32.9	5.8	58.0	120.0	99.0
15	1	45.2	30.5	3.0	57.0	130.0	99.0
15	2	42.2	30.4	6.2	58.0	130.0	99.0
16	1	47.6	27.4	5.1	54.0	110.0	98.0
16	2	48.0	26.9	5.5	54.0	110.0	98.0
17	1	47.3	26.4	6.6	57.0	140.0	99.0
17	2	45.5	28.9	6.1	57.0	150.0	100.0
18	1	44.3	30.8	5.3	54.0	130.0	97.0
18	2	44.2	29.8	5.5	55.0	130.0	98.0
19	1	45.1	28.7	2.8	57.0	130.0	99.0
19	2	43.3	32.3	3.2	59.0	125.0	99.0
20	1	45.4	30.0	5.3	52.0	120.0	96.0
20	2	46.3	30.3	5.4	54.0	120.0	99.0
21	1	46.5	31.9	3.0	58.0	135.0	98.0
21	2	41.7	29.7	5.5	57.0	115.0	99.0
24	1	41.8	31.9	3.2	56.9	112.3	98.8
24	2	41.8	30.9	3.1	57.0	115.0	99.0
25	1	42.1	29.0	3.2	56.0	120.0	98.0
25	2	41.9	29.0	3.3	57.0	110.0	98.0
27	1	48.0	28.9	3.4	57.0	125.0	98.0
27	2	47.1	28.3	3.3	57.0	145.0	99.0
28	1	47.8	29.1	4.5	53.0	125.0	98.0
28	2	47.7	29.7	4.6	56.0	130.0	99.0
31	1	46.7	29.9	3.1	55.0	115.0	99.0
31	2	46.1	30.5	3.2	57.0	130.0	99.0
33	1	45.4	31.6	6.5	53.0	115.0	97.0
33	2	46.7	28.2	6.3	53.0	115.0	98.0
35	1	44.3	27.1	5.9	57.0	130.0	98.0
35	2	43.3	29.8	6.1	58.0	135.0	98.0
36	1	46.3	28.9	5.5	56.0	125.0	99.0
36	2	46.0	29.6	5.7	56.0	120.0	99.0
37	1	44.1	29.3	6.3	56.0	115.0	96.0
37	2	43.8	28.6	6.8	56.0	125.0	97.0
38	1	46.8	27.9	5.2	59.0	140.0	98.0
38	2	45.3	30.2	4.8	57.0	135.0	99.0
40	1	47.5	32.3	6.0	55.9	124.3	96.5
40	2	47.5	31.3	5.9	56.0	127.0	96.7
41	1	46.1	28.5	5.6	51.0	115.0	95.0
41	2	47.0	27.9	5.5	51.0	110.0	96.0
42	1	47.2	28.5	4.6	50.0	140.0	97.0
42	2	47.7	29.3	4.7	51.0	120.0	99.0

Appendix 2 continued

DH line	Replicate	OIL(%)	PRO (%)	C18:3 (%)	DTF (d)	HT (cm)	DTM (d)
44	1	46.3	28.3	6.0	57.0	120.0	99.0
44	2	45.3	29.0	5.2	58.0	100.0	99.0
45	1	47.1	28.7	5.0	55.0	130.0	98.0
45	2	45.3	30.7	5.1	57.0	150.0	100.0
46	1	42.5	32.5	3.4	59.0	110.0	98.0
46	2	43.0	26.6	3.7	59.0	100.0	98.0
47	1	47.9	25.8	6.3	51.0	120.0	96.0
47	2	45.4	28.8	4.5	54.0	145.0	98.0
48	1	47.7	31.2	3.4	59.0	110.0	99.0
48	2	47.6	27.7	3.5	59.0	130.0	100.0
49	1	45.8	29.7	6.5	55.0	120.0	98.0
49	2	46.4	30.4	7.4	56.0	130.0	99.0
50	1	46.8	29.7	5.7	56.0	135.0	98.0
50	2	46.3	32.2	2.7	51.0	90.0	99.0
52	1	46.5	29.6	4.7	54.0	130.0	97.0
52	2	47.5	28.5	4.7	54.0	125.0	98.0
53	1	43.0	29.7	5.5	50.0	110.0	98.0
53	2	42.2	31.0	5.4	51.0	125.0	100.0
54	1	45.7	26.8	5.8	51.0	140.0	96.0
54	2	47.6	24.4	5.5	53.0	125.0	98.0
56	1	46.7	26.8	8.0	57.0	115.0	95.0
56	2	44.6	28.6	7.9	56.0	145.0	99.0
57	1	48.2	28.8	5.9	57.0	120.0	99.0
57	2	47.9	29.0	5.7	57.0	150.0	99.0
59	1	44.5	28.8	5.8	52.0	130.0	98.0
59	2	42.7	28.4	5.5	51.0	130.0	99.0
61	1	47.8	28.9	7.3	57.0	120.0	98.0
61	2	47.1	30.1	7.4	57.0	135.0	100.0
62	1	47.7	29.8	5.8	51.0	110.0	96.0
62	2	48.1	25.7	5.6	50.0	120.0	97.0
64	1	47.4	28.0	5.2	56.0	130.0	98.0
64	2	47.9	28.4	5.4	56.0	135.0	99.0
65	1	48.4	26.4	5.3	52.0	105.0	95.0
65	2	47.8	25.9	5.5	53.0	135.0	97.0
66	1	45.4	28.4	3.6	59.0	130.0	99.0
66	2	43.0	29.7	3.2	59.0	130.0	99.0
67	1	44.9	28.8	3.6	57.0	140.0	99.0
67	2	44.1	31.2	3.5	58.0	150.0	100.0
68	1	45.2	30.3	4.7	57.0	140.0	98.0
68	2	45.3	30.6	5.6	59.0	140.0	100.0
69	1	43.6	29.9	5.3	54.0	105.0	98.0
69	2	44.3	30.3	4.8	57.0	110.0	99.0
71	1	47.0	30.5	4.9	57.0	115.0	99.0
71	2	45.4	29.8	4.7	58.0	110.0	100.0
72	1	48.8	29.3	7.0	55.0	115.0	98.0
72	2	47.8	27.9	7.2	56.0	140.0	99.0
74	1	41.3	33.3	3.5	55.0	105.0	96.0
74	2	45.8	26.3	1.9	56.0	130.0	99.0
76	1	48.7	27.0	4.7	54.0	110.0	97.0
76	2	48.7	26.0	4.6	54.1	112.7	97.2
78	1	46.7	26.5	4.4	53.0	125.0	96.0
78	2	45.5	28.5	4.7	54.0	140.0	98.0
79	1	45.4	30.5	7.1	53.0	130.0	99.0
79	2	43.9	30.9	6.5	54.0	120.0	99.0
80	1	46.9	28.5	3.2	58.0	140.0	99.0
80	2	45.9	29.8	3.4	58.0	135.0	99.0
82	1	47.8	29.1	4.7	56.0	130.0	99.0

Appendix 2 continued

DH line	Replicate	OIL(%)	PRO (%)	C18:3 (%)	DTF (d)	HT (cm)	DTM (d)
82	2	48.5	27.4	4.9	55.0	130.0	100.0
83	1	44.4	28.7	8.3	52.0	115.0	96.0
83	2	47.6	28.0	5.9	54.0	130.0	98.0
84	1	44.4	30.1	5.9	57.0	115.0	99.0
84	2	44.2	30.7	6.1	57.0	125.0	100.0
89	1	49.8	26.0	5.2	52.0	110.0	95.0
89	2	47.9	28.1	5.3	53.0	120.0	98.0
91	1	43.5	28.7	5.7	56.0	120.0	97.0
91	2	43.8	30.9	5.8	54.0	130.0	98.0
92	1	47.5	28.3	3.4	56.0	115.0	98.0
92	2	46.9	27.5	3.4	57.0	125.0	100.0
94	1	48.4	26.5	5.1	53.0	120.0	98.0
94	2	47.8	27.2	4.7	53.0	135.0	99.0
96	1	48.5	28.2	4.6	51.0	135.0	100.0
96	2	48.7	27.4	4.8	51.0	130.0	100.0
98	1	43.5	30.0	2.7	56.0	125.0	98.0
98	2	43.3	31.1	2.7	57.0	140.0	99.0
100	1	48.8	28.6	3.2	51.0	105.0	99.0
100	2	48.3	28.4	3.0	51.0	140.0	99.0
104	1	46.9	25.7	5.1	55.0	130.0	98.0
104	2	47.0	28.3	5.4	55.0	120.0	99.0
107	1	48.0	29.2	7.2	54.0	120.0	99.0
107	2	47.8	29.5	7.4	54.0	120.0	99.0
110	1	45.2	30.2	4.8	51.0	120.0	98.0
110	2	45.2	29.0	5.0	51.0	130.0	98.0
113	1	46.1	29.9	6.7	53.0	125.0	97.0
113	2	46.4	29.3	6.4	53.0	140.0	97.0
114	1	46.5	28.2	5.0	57.0	120.0	99.0
114	2	45.7	28.1	5.0	57.0	130.0	99.0
115	1	48.9	26.8	3.5	57.0	140.0	98.0
115	2	48.6	25.6	2.9	58.0	150.0	98.0
116	1	47.6	28.8	6.9	57.0	130.0	99.0
116	2	46.9	30.3	7.2	57.0	130.0	100.0
120	1	47.8	26.1	2.8	59.0	130.0	99.0
120	2	47.6	28.1	3.0	60.0	130.0	100.0
122	1	47.5	28.5	2.8	53.0	140.0	98.0
122	2	47.3	28.7	5.9	54.0	130.0	98.0
123	1	43.9	30.3	5.1	57.0	115.0	98.0
123	2	47.0	29.6	5.2	57.0	125.0	99.0
124	1	47.8	27.8	5.3	54.0	135.0	97.0
124	2	44.7	27.2	6.1	54.0	120.0	99.0
126	1	48.8	27.6	2.6	59.0	140.0	99.0
126	2	45.9	28.3	2.5	59.0	145.0	100.0
127	1	48.1	27.5	4.7	52.0	125.0	97.0
127	2	47.5	27.8	5.9	54.0	100.0	99.0
128	1	48.1	27.3	3.7	57.0	140.0	97.0
128	2	47.3	27.9	2.9	57.0	140.0	98.0
129	1	48.9	26.2	4.7	52.0	125.0	97.0
129	2	49.8	28.2	4.5	54.0	120.0	97.0
131	1	47.5	30.8	5.1	57.0	120.0	98.0
131	2	47.5	28.6	5.2	56.0	120.0	99.0
132	1	48.4	29.0	2.7	56.0	120.0	99.0
132	2	46.3	29.3	2.5	55.0	110.0	100.0
133	1	45.3	30.1	4.9	56.0	125.0	97.0
133	2	45.2	28.9	4.9	56.0	130.0	97.0
135	1	46.8	28.7	3.2	52.0	125.0	99.0
135	2	46.1	28.5	2.6	57.0	120.0	99.0

Appendix 2 continued

DH line	Replicate	OIL(%)	PRO (%)	C18:3 (%)	DTF (d)	HT (cm)	DTM (d)
136	1	45.5	29.9	2.8	52.0	115.0	97.0
136	2	45.3	29.6	3.0	53.0	115.0	98.0
138	1	45.2	30.1	6.7	57.0	120.0	99.0
138	2	45.1	30.0	6.5	57.0	135.0	100.0
141	1	45.4	28.7	5.3	51.0	115.0	96.0
141	2	45.2	28.4	4.7	56.0	150.0	98.0
143	1	45.7	28.6	8.3	57.0	120.0	99.0
143	2	44.5	27.1	8.8	57.0	120.0	100.0
147	1	44.5	30.4	6.5	56.0	120.0	99.0
147	2	44.2	31.1	7.0	59.0	140.0	99.0
149	1	47.6	27.9	2.8	57.0	140.0	99.0
149	2	46.4	29.8	3.0	58.0	125.0	99.0
151	1	47.2	28.7	4.2	57.0	140.0	97.0
151	2	46.1	30.7	4.5	57.0	130.0	100.0
152	1	47.6	29.3	4.8	54.0	110.0	99.0
152	2	45.7	31.4	6.5	57.0	130.0	100.0
153	1	46.9	28.4	3.7	53.0	105.0	96.0
153	2	46.0	27.1	2.9	53.0	105.0	96.0
154	1	45.5	30.4	6.2	55.0	130.0	97.0
154	2	45.4	29.3	5.8	56.0	140.0	97.0
155	1	47.3	29.8	4.1	57.0	105.0	97.0
155	2	45.3	29.7	3.4	57.0	110.0	100.0
157	1	49.1	26.2	5.0	56.0	125.0	98.0
157	2	47.6	30.0	5.1	55.0	115.0	99.0
159	1	43.9	37.6	3.5	56.0	120.0	97.0
159	2	43.5	27.8	3.2	57.0	120.0	99.0
160	1	49.2	27.3	2.6	60.0	140.0	99.0
160	2	46.4	29.6	2.7	61.0	135.0	100.0
161	1	46.1	28.6	5.8	53.0	115.0	97.0
161	2	45.4	28.3	6.3	54.0	130.0	98.0
163	1	46.0	28.2	3.4	57.0	125.0	98.0
163	2	45.2	30.2	3.0	58.0	130.0	99.0

Appendix 3. Seed quality and agronomic field results of 100 DH lines of *B. napus* grown at Wpg99. Traits are seed oil content (OIL), seed protein content (PRO), linolenic acid content (C18:3), days to flowering (DTF), plant height (HT), days to maturity (DTM).

DH line	Replicate	OIL(%)	PRO (%)	C18:3 (%)	DTF (d)	HT (cm)	DTM (d)
3	1	49.3	29.3	3.1	46.0	140.0	103.0
3	2	47.5	29.7	2.8	47.0	125.0	103.0
7	1	50.0	29.1	6.0	46.0	130.0	101.0
7	2	48.8	31.1	6.3	46.0	135.0	103.0
10	1	47.4	29.5	5.7	46.0	125.0	101.0
10	2	48.6	28.3	5.7	47.0	135.0	103.0
11	1	49.3	29.2	5.8	53.0	130.0	101.0
11	2	48.8	28.6	6.0	55.0	160.0	105.0
12	1	48.6	29.4	6.3	46.0	130.0	101.0
12	2	47.2	26.9	6.0	46.0	125.0	102.0
13	1	46.8	32.1	6.2	55.0	160.0	103.0
13	2	48.5	30.4	6.2	54.0	160.0	105.0
14	1	49.9	29.1	6.4	47.0	130.0	102.0
14	2	47.5	30.5	6.2	47.0	140.0	103.0
15	1	44.4	34.2	3.6	55.0	155.0	103.0
15	2	45.5	32.6	4.3	55.0	155.0	103.0
16	1	48.0	28.8	5.3	49.0	115.0	102.0
16	2	49.0	29.4	4.6	50.0	130.0	103.0
17	1	47.2	29.1	8.2	50.0	140.0	104.0
17	2	48.6	26.8	8.6	51.0	145.0	105.0
18	1	46.4	29.4	5.7	48.0	120.0	100.0
18	2	45.0	32.5	5.7	49.0	130.0	101.0
19	1	49.4	27.6	3.7	52.0	110.0	103.0
19	2	45.7	31.9	3.3	53.0	140.0	103.0
20	1	47.0	27.3	6.8	47.0	135.0	102.0
20	2	46.5	28.8	6.5	47.0	140.0	102.0
21	1	45.8	30.7	6.6	53.0	145.0	102.0
21	2	42.4	31.9	5.9	51.0	130.0	104.0
24	1	46.5	31.1	4.9	49.0	155.0	103.0
24	2	44.3	30.1	4.1	50.0	105.0	105.0
25	1	47.4	29.0	3.9	49.0	135.0	99.0
25	2	45.5	30.9	4.0	52.0	150.0	102.0
27	1	46.7	28.7	3.4	53.0	150.0	101.0
27	2	47.1	29.4	3.3	51.0	145.0	103.0
28	1	49.9	29.0	5.4	47.0	135.0	101.0
28	2	49.6	28.5	5.1	49.0	130.0	102.0
31	1	48.3	29.7	3.2	50.0	145.0	102.0
31	2	47.4	31.0	3.3	51.0	150.0	103.0
33	1	47.0	28.2	7.2	47.0	100.0	100.0
33	2	49.0	27.6	7.5	48.0	125.0	102.0
35	1	46.0	28.0	6.8	52.0	140.0	103.0
35	2	44.5	30.0	6.4	54.0	160.0	103.0
36	1	45.4	31.5	7.3	50.0	145.0	103.0
36	2	43.0	33.5	6.4	49.0	125.0	104.0
37	1	43.6	32.4	8.8	50.0	130.0	102.0
37	2	45.0	31.9	6.7	50.0	130.0	103.0
38	1	48.7	29.4	5.8	50.0	135.0	102.0
38	2	46.8	30.5	5.8	51.0	140.0	103.0
40	1	47.2	30.6	6.4	50.0	130.0	100.0
40	2	46.0	31.8	6.1	55.0	130.0	103.0
41	1	48.5	28.3	5.6	46.0	140.0	97.0
41	2	48.0	31.4	6.2	46.0	120.0	100.0
42	1	47.8	27.2	5.2	47.0	135.0	101.0
42	2	46.5	31.5	5.7	47.0	125.0	102.0

Appendix 3 continued

DH line	Replicate	OIL(%)	PRO (%)	C18:3 (%)	DTF (d)	HT (cm)	DTM (d)
44	1	48.0	27.9	6.1	50.0	130.0	103.0
44	2	48.1	29.9	5.9	50.0	140.0	105.0
45	1	46.5	31.7	6.1	48.0	145.0	97.0
45	2	48.7	29.0	5.8	49.0	125.0	103.0
46	1	46.3	29.1	5.2	55.0	150.0	103.0
46	2	46.1	30.4	4.8	55.0	145.0	105.0
47	1	46.8	28.1	5.6	46.0	130.0	101.0
47	2	47.8	28.5	5.2	47.0	125.0	101.0
48	1	46.4	30.7	3.4	51.0	135.0	103.0
48	2	47.2	31.3	4.0	54.0	150.0	105.0
49	1	48.0	30.5	8.4	51.0	130.0	103.0
49	2	48.0	30.6	8.3	51.0	150.0	103.0
50	1	47.4	29.2	3.1	46.0	130.0	102.0
50	2	47.5	32.2	3.2	46.0	130.0	103.0
52	1	48.1	30.4	5.4	46.0	130.0	101.0
52	2	49.3	29.3	5.1	47.0	115.0	102.0
53	1	44.5	27.8	6.5	46.0	130.0	100.0
53	2	44.7	27.9	6.4	46.0	125.0	101.0
54	1	49.3	25.7	6.3	47.0	135.0	100.0
54	2	46.2	28.9	6.6	47.0	140.0	101.0
56	1	47.6	27.7	9.3	50.0	150.0	103.0
56	2	45.6	29.6	9.3	51.0	140.0	103.0
57	1	46.8	32.4	6.3	53.0	140.0	103.0
57	2	47.1	32.8	6.0	51.0	150.0	104.0
59	1	46.0	30.1	6.7	47.0	115.0	101.0
59	2	47.3	26.4	6.6	47.0	140.0	102.0
61	1	49.9	26.3	7.9	53.0	140.0	103.0
61	2	50.0	27.8	8.1	52.0	140.0	104.0
62	1	49.0	27.6	5.9	47.0	120.0	101.0
62	2	47.0	29.0	5.9	47.0	120.0	102.0
64	1	49.3	28.1	5.3	49.0	145.0	102.0
64	2	47.4	28.9	5.6	49.0	125.0	102.0
65	1	48.4	28.0	4.8	46.0	130.0	99.0
65	2	47.3	30.2	5.3	49.0	130.0	103.0
66	1	43.7	31.2	3.7	54.0	120.0	103.0
66	2	44.2	31.8	4.5	53.0	140.0	105.0
67	1	44.1	32.9	4.4	51.0	160.0	99.0
67	2	44.5	32.4	4.2	51.0	120.0	103.0
68	1	46.5	31.3	5.6	53.0	140.0	103.0
68	2	45.7	32.1	5.7	53.0	145.0	103.0
69	1	46.6	30.5	6.3	50.0	125.0	102.0
69	2	46.2	30.7	6.3	50.0	110.0	103.0
71	1	45.6	32.7	5.4	54.0	155.0	104.0
71	2	48.0	30.8	5.8	54.0	165.0	105.0
72	1	47.2	30.6	8.7	49.0	140.0	105.0
72	2	47.0	29.8	8.4	50.0	125.0	105.0
74	1	44.8	30.0	4.1	45.0	130.0	100.0
74	2	43.3	31.3	4.0	46.0	140.0	101.0
76	1	50.0	26.4	4.7	47.0	135.0	100.0
76	2	49.4	26.0	4.8	47.0	165.0	103.0
78	1	46.5	30.8	5.4	47.0	150.0	97.0
78	2	45.4	32.1	5.5	48.0	130.0	103.0
79	1	47.2	29.6	6.8	47.0	130.0	103.0
79	2	47.2	29.0	7.5	48.0	145.0	103.0
80	1	46.0	32.8	7.7	47.0	155.0	103.0
80	2	47.4	29.4	6.9	49.0	150.0	103.0
82	1	48.7	29.6	5.3	46.0	120.0	103.0

Appendix 3 continued

DH line	Replicate	OIL(%)	PRO (%)	C18:3 (%)	DTF (d)	HT (cm)	DTM (d)
82	2	47.5	30.4	5.6	46.0	120.0	103.0
83	1	46.4	27.5	9.8	46.0	130.0	101.0
83	2	43.5	31.1	9.5	46.0	140.0	102.0
84	1	46.0	30.7	6.8	47.0	105.0	102.0
84	2	49.2	27.7	7.2	50.0	135.0	102.0
89	1	45.5	33.3	5.5	46.0	125.0	103.0
89	2	47.8	29.4	5.4	47.0	120.0	103.0
91	1	47.0	26.5	6.3	49.0	130.0	100.0
91	2	44.7	31.7	6.2	49.0	130.0	101.0
92	1	46.5	28.9	3.9	49.0	130.0	99.0
92	2	46.9	28.6	3.8	49.0	130.0	101.0
94	1	49.0	27.7	5.7	47.0	125.0	102.0
94	2	47.5	30.4	5.5	49.0	130.0	103.0
96	1	49.2	28.4	4.9	46.0	140.0	99.0
96	2	50.5	28.3	5.3	47.0	120.0	103.0
98	1	45.2	31.4	3.2	48.0	130.0	103.0
98	2	46.2	30.8	3.5	49.0	150.0	103.0
100	1	49.8	28.8	3.0	45.0	140.0	102.0
100	2	50.5	27.1	3.0	47.0	130.0	102.0
104	1	45.3	30.4	5.8	47.0	135.0	103.0
104	2	46.0	28.3	5.6	49.0	125.0	103.0
107	1	49.8	29.9	7.7	47.0	140.0	103.0
107	2	49.2	29.9	7.8	49.0	135.0	104.0
110	1	46.0	31.6	5.8	47.0	125.0	100.0
110	2	46.0	30.1	5.9	46.0	130.0	103.0
113	1	46.4	31.0	7.5	47.0	140.0	97.0
113	2	48.1	29.7	7.3	47.0	125.0	102.0
114	1	48.0	29.9	3.4	51.0	140.0	101.0
114	2	47.0	30.6	3.1	52.0	140.0	103.0
115	1	48.7	29.0	3.4	54.0	140.0	104.0
115	2	47.4	29.6	3.2	52.0	150.0	105.0
116	1	48.7	29.4	8.5	53.0	150.0	103.0
116	2	48.3	29.3	8.0	54.0	135.0	103.0
120	1	48.0	26.8	3.3	53.0	165.0	103.0
120	2	48.0	28.2	3.4	55.0	145.0	103.0
122	1	49.0	29.0	5.3	50.0	140.0	103.0
122	2	47.0	28.6	5.8	50.0	140.0	103.0
123	1	45.0	30.0	6.7	49.0	140.0	103.0
123	2	44.4	30.8	6.7	50.0	145.0	103.0
124	1	48.0	29.1	5.7	47.0	120.0	100.0
124	2	47.6	30.4	6.3	45.0	135.0	103.0
126	1	46.5	31.2	3.3	50.0	150.0	105.0
126	2	47.0	31.3	3.4	56.0	160.0	105.0
127	1	46.1	29.8	6.8	50.0	100.0	103.0
127	2	46.6	28.7	6.8	50.0	130.0	103.0
128	1	47.0	31.8	3.0	54.0	150.0	104.0
128	2	47.0	30.1	3.2	53.0	165.0	105.0
129	1	49.0	27.6	4.8	48.0	130.0	100.0
129	2	48.2	29.7	5.0	47.0	140.0	103.0
131	1	47.4	26.0	2.2	48.0	135.0	102.0
131	2	49.2	28.9	5.8	49.0	140.0	102.0
132	1	49.2	30.3	2.9	50.0	130.0	103.0
132	2	47.7	29.0	3.1	50.0	150.0	103.0
133	1	43.2	32.4	8.3	50.0	120.0	103.0
133	2	45.0	32.4	5.9	51.0	155.0	103.0
135	1	47.3	30.8	3.1	50.0	120.0	102.0
135	2	48.4	29.3	3.2	51.0	165.0	104.0

Appendix 3 continued

DH line	Replicate	OIL(%)	PRO (%)	C18:3 (%)	DTF (d)	HT (cm)	DTM (d)
136	1	45.7	29.5	5.9	47.0	150.0	98.0
136	2	45.0	32.3	6.2	47.0	125.0	100.0
138	1	47.5	31.4	5.9	49.0	130.0	101.0
138	2	47.7	29.2	5.5	49.0	130.0	102.0
141	1	46.0	31.2	5.4	48.0	145.0	104.0
141	2	44.4	32.0	5.5	50.0	150.0	105.0
143	1	44.4	27.9	9.2	51.0	160.0	100.0
143	2	44.1	29.0	9.5	50.0	145.0	105.0
147	1	47.1	28.6	8.2	52.0	145.0	104.0
147	2	45.8	30.4	8.6	56.0	155.0	105.0
149	1	49.3	28.6	3.4	50.0	140.0	103.0
149	2	48.2	25.7	3.1	54.0	150.0	103.0
151	1	49.9	28.6	4.8	51.0	145.0	102.0
151	2	48.3	29.3	5.2	51.0	140.0	102.0
152	1	47.7	27.7	6.5	50.0	160.0	100.0
152	2	48.7	28.0	5.9	49.0	150.0	104.0
153	1	46.2	27.8	3.4	47.0	115.0	97.0
153	2	47.0	31.0	3.5	50.0	115.0	103.0
154	1	46.8	29.2	6.5	49.0	120.0	100.0
154	2	47.2	30.0	6.5	48.0	125.0	103.0
155	1	46.8	29.0	2.9	50.0	130.0	103.0
155	2	47.0	31.8	3.9	49.0	135.0	104.0
157	1	48.2	28.7	5.6	52.0	145.0	103.0
157	2	47.5	29.0	5.6	55.0	135.0	104.0
159	1	47.2	28.6	3.7	54.0	135.0	102.0
159	2	45.8	30.1	4.5	54.0	145.0	103.0
160	1	49.2	29.0	3.1	53.0	130.0	102.0
160	2	49.8	28.6	3.1	51.0	145.0	103.0
161	1	46.0	29.5	6.7	47.0	135.0	102.0
161	2	46.1	28.5	6.6	48.0	135.0	102.0
163	1	45.5	31.9	4.0	54.0	130.0	103.0
163	2	46.0	26.6	3.8	55.0	150.0	103.0

Appendix 4. Seed quality and agronomic field results of 100 DH lines of *B. napus* grown at Car99. Traits are seed oil content (OIL), seed protein content (PRO), linolenic acid content (C18:3), days to flowering (DTF), plant height (HT), days to maturity (DTM).

DH line	Replicate	OIL(%)	PRO (%)	C18:3 (%)	DTF (d)	HT (cm)	DTM (d)
3	1	48.3	29.3	3.2	46.0	110.0	91.0
3	2	48.7	29.2	3.1	47.0	110.0	91.0
7	1	49.7	29.3	5.9	46.0	120.0	90.0
7	2	49.2	29.2	5.9	46.0	130.0	91.0
10	1	51.4	24.6	6.1	44.0	105.0	89.0
10	2	50.1	28.7	5.8	45.0	120.0	89.0
11	1	49.6	27.2	5.4	49.0	150.0	90.0
11	2	49.7	27.4	5.4	54.0	110.0	92.0
12	1	48.9	27.2	5.9	45.0	125.0	89.0
12	2	49.3	27.2	5.9	46.0	110.0	90.0
13	1	48.7	30.0	6.4	51.0	130.0	90.0
13	2	50.8	27.2	5.7	54.0	160.0	91.0
14	1	52.7	25.3	5.8	45.0	130.0	90.0
14	2	53.0	24.2	5.8	46.0	115.0	90.0
15	1	50.4	27.1	3.0	53.0	130.0	90.0
15	2	48.3	29.4	3.1	53.0	130.0	90.0
16	1	50.6	24.4	4.7	47.0	95.0	89.0
16	2	46.2	29.8	5.3	47.0	95.0	92.0
17	1	49.7	25.9	7.1	52.0	120.0	89.0
17	2	48.2	28.2	7.5	53.0	140.0	91.0
18	1	48.0	29.0	5.5	46.0	110.0	90.0
18	2	50.0	25.6	5.6	47.0	120.0	89.0
19	1	48.1	30.4	3.1	52.0	125.0	92.0
19	2	48.4	28.0	2.9	53.0	140.0	91.0
20	1	49.6	25.4	6.3	46.0	110.0	90.0
20	2	46.8	29.0	6.0	46.0	120.0	90.0
21	1	43.5	30.3	6.0	51.0	130.0	91.0
21	2	44.4	30.2	6.0	51.0	135.0	91.0
24	1	47.0	28.6	4.7	47.0	140.0	91.0
24	2	49.6	25.5	3.9	48.0	125.0	90.0
25	1	49.8	26.6	3.3	47.0	120.0	90.0
25	2	43.4	30.2	2.7	51.0	120.0	90.0
27	1	51.0	25.2	3.2	47.0	120.0	91.0
27	2	50.1	26.4	3.3	47.0	140.0	92.0
28	1	47.4	28.5	5.6	47.0	125.0	90.0
28	2	49.2	28.2	5.7	47.0	140.0	90.0
31	1	51.5	26.3	2.7	49.0	150.0	90.0
31	2	47.8	29.7	2.9	50.0	130.0	91.0
33	1	47.4	30.0	6.7	47.0	95.0	89.0
33	2	49.5	26.3	8.2	47.0	95.0	90.0
35	1	48.2	24.6	6.0	49.0	145.0	90.0
35	2	46.8	27.6	6.1	49.0	130.0	92.0
36	1	48.7	27.0	6.4	46.0	130.0	90.0
36	2	44.9	21.6	5.8	47.0	105.0	92.0
37	1	46.0	28.9	7.3	48.0	100.0	90.0
37	2	45.4	29.3	7.7	48.0	130.0	90.0
38	1	46.3	29.2	5.4	49.0	120.0	90.0
38	2	48.3	27.2	5.6	52.0	140.0	91.0
40	1	50.2	28.4	5.7	48.0	145.0	91.0
40	2	50.8	27.8	6.2	49.0	140.0	92.0
41	1	50.2	27.1	5.9	45.0	110.0	89.0
41	2	47.5	28.8	5.4	47.0	95.0	89.0
42	1	48.2	30.2	5.1	46.0	100.0	89.0
42	2	48.9	28.3	5.1	46.0	140.0	90.0

Appendix 4 continued

DH line	Replicate	OIL(%)	PRO (%)	C18:3 (%)	DTF (d)	HT (cm)	DTM (d)
44	1	50.3	24.8	6.1	47.0	105.0	90.0
44	2	49.8	25.2	6.1	48.0	110.0	91.0
45	1	49.5	27.4	5.5	48.0	140.0	90.0
45	2	52.6	24.7	5.7	50.0	135.0	90.0
46	1	45.1	30.2	5.1	53.0	120.0	92.0
46	2	44.3	29.4	3.6	56.0	125.0	91.0
47	1	49.9	25.4	5.4	45.0	120.0	89.0
47	2	47.8	26.1	5.4	45.0	130.0	89.0
48	1	45.8	29.4	3.6	51.0	135.0	92.0
48	2	51.6	24.5	3.7	53.0	135.0	91.0
49	1	51.5	26.6	8.1	48.0	135.0	90.0
49	2	52.4	25.9	8.2	49.0	120.0	91.0
50	1	51.5	27.0	3.0	46.0	125.0	90.0
50	2	46.0	29.7	2.8	46.0	115.0	90.0
52	1	50.7	28.0	5.3	46.0	100.0	90.0
52	2	51.0	26.4	5.0	47.0	120.0	91.0
53	1	45.9	28.7	6.4	46.0	120.0	88.0
53	2	46.0	28.6	6.2	46.0	100.0	90.0
54	1	49.6	25.3	5.8	46.0	130.0	89.0
54	2	49.0	24.4	5.8	46.0	130.0	90.0
56	1	45.0	30.1	8.7	49.0	130.0	90.0
56	2	47.0	27.8	8.8	49.0	145.0	90.0
57	1	49.3	29.0	5.9	49.0	120.0	92.0
57	2	52.6	25.1	5.7	50.0	120.0	90.0
59	1	48.4	26.1	6.4	45.0	110.0	90.0
59	2	47.0	28.0	6.2	46.0	120.0	90.0
61	1	52.2	26.1	7.5	51.0	120.0	90.0
61	2	52.8	23.6	7.7	53.0	115.0	90.0
62	1	51.3	23.3	5.9	45.0	125.0	90.0
62	2	49.9	24.2	5.8	46.0	120.0	89.0
64	1	50.6	24.7	5.2	47.0	145.0	90.0
64	2	49.6	26.0	4.7	53.0	90.0	91.0
65	1	50.7	26.0	5.5	45.0	110.0	90.0
65	2	47.5	28.8	4.9	47.0	140.0	90.0
66	1	46.6	28.2	3.5	54.0	100.0	91.0
66	2	44.2	31.3	4.4	54.0	110.0	92.0
67	1	46.2	29.1	3.9	49.0	140.0	90.0
67	2	46.5	28.0	3.7	49.0	145.0	91.0
68	1	48.2	27.1	5.3	50.0	130.0	89.0
68	2	45.1	32.2	4.8	54.0	140.0	92.0
69	1	49.0	28.6	5.8	47.0	90.0	90.0
69	2	47.7	29.9	5.7	49.0	115.0	90.0
71	1	51.6	25.3	5.1	52.0	130.0	92.0
71	2	47.8	29.8	4.9	53.0	160.0	91.0
72	1	50.0	28.4	8.0	46.0	120.0	91.0
72	2	51.9	26.3	7.9	48.0	110.0	90.0
74	1	44.1	30.1	4.1	44.0	125.0	90.0
74	2	44.0	29.8	3.8	45.0	130.0	90.0
76	1	45.4	28.9	7.6	45.0	130.0	91.0
76	2	50.1	26.0	4.7	47.0	120.0	90.0
78	1	51.9	25.3	5.4	47.0	130.0	89.0
78	2	49.1	28.4	5.6	47.0	125.0	90.0
79	1	49.5	28.7	8.0	46.0	110.0	91.0
79	2	51.5	25.4	7.4	47.0	110.0	89.0
80	1	50.1	27.1	5.9	48.0	140.0	91.0
80	2	49.9	28.7	6.5	48.0	130.0	91.0
82	1	49.2	29.0	5.5	45.0	120.0	91.0

Appendix 4 continued

DH line	Replicate	OIL(%)	PRO (%)	C18:3 (%)	DTF (d)	HT (cm)	DTM (d)
82	2	52.4	25.7	5.5	47.0	130.0	91.0
83	1	46.2	26.5	9.0	46.0	115.0	90.0
83	2	45.5	27.3	9.6	46.0	135.0	90.0
84	1	47.1	29.5	6.6	47.0	140.0	92.0
84	2	47.9	29.0	6.3	48.0	135.0	91.0
89	1	50.1	27.5	5.1	46.0	130.0	89.0
89	2	45.7	32.1	4.8	47.0	125.0	89.0
91	1	48.6	24.8	6.1	47.0	120.0	88.0
91	2	48.6	27.0	5.7	48.0	80.0	91.0
92	1	48.5	26.9	3.9	47.0	120.0	91.0
92	2	48.2	26.8	3.4	48.0	140.0	91.0
94	1	50.6	24.8	5.2	46.0	105.0	90.0
94	2	51.8	24.7	5.4	47.0	120.0	90.0
96	1	52.1	25.8	5.3	46.0	130.0	90.0
96	2	53.0	23.0	5.5	47.0	120.0	91.0
98	1	48.0	28.9	2.6	48.0	140.0	90.0
98	2	47.7	28.9	2.6	48.0	110.0	91.0
100	1	48.8	29.4	3.0	45.0	125.0	91.0
100	2	49.5	28.7	2.9	46.0	120.0	90.0
104	1	46.0	30.3	5.5	45.0	130.0	91.0
104	2	49.0	27.9	5.3	46.0	110.0	91.0
107	1	51.0	28.2	7.5	46.0	115.0	90.0
107	2	52.3	26.0	7.6	47.0	125.0	90.0
110	1	44.0	31.2	5.3	46.0	120.0	91.0
110	2	49.0	26.7	5.9	47.0	110.0	90.0
113	1	51.5	26.1	6.9	46.0	125.0	90.0
113	2	47.0	30.0	6.6	47.0	100.0	91.0
114	1	49.8	29.0	3.5	48.0	120.0	90.0
114	2	51.3	25.8	3.5	48.0	140.0	90.0
115	1	48.0	28.1	3.1	51.0	155.0	92.0
115	2	50.3	27.3	3.8	53.0	145.0	91.0
116	1	52.5	24.4	8.0	49.0	130.0	91.0
116	2	52.4	24.6	7.8	51.0	140.0	91.0
120	1	49.1	27.1	2.8	52.0	115.0	91.0
120	2	49.5	25.7	3.2	52.0	145.0	91.0
122	1	47.4	28.3	5.9	47.0	165.0	91.0
122	2	49.0	27.6	5.9	48.0	140.0	90.0
123	1	47.9	26.7	5.7	47.0	140.0	91.0
123	2	50.8	23.0	5.8	48.0	120.0	90.0
124	1	51.5	24.5	5.9	45.0	110.0	90.0
124	2	49.6	27.8	5.5	46.0	110.0	90.0
126	1	48.0	29.1	3.2	51.0	150.0	92.0
126	2	48.7	29.1	3.1	54.0	110.0	92.0
127	1	46.8	30.5	6.0	47.0	120.0	91.0
127	2	49.0	28.5	3.4	54.0	140.0	92.0
128	1	48.0	28.6	2.9	49.0	170.0	91.0
128	2	45.2	30.7	3.1	56.0	110.0	90.0
129	1	51.7	25.7	4.7	46.0	125.0	89.0
129	2	51.5	25.3	4.8	46.0	140.0	90.0
131	1	52.5	24.2	5.6	47.0	125.0	90.0
131	2	50.1	27.4	5.7	47.0	115.0	90.0
132	1	51.2	25.6	3.0	48.0	120.0	90.0
132	2	48.5	30.0	3.2	48.0	115.0	91.0
133	1	47.5	29.7	5.4	48.0	125.0	90.0
133	2	48.2	26.7	5.4	49.0	130.0	90.0
135	1	50.7	27.5	3.0	48.0	140.0	91.0
135	2	50.0	28.6	2.9	48.0	140.0	92.0

Appendix 4 continued

DH line	Replicate	OIL(%)	PRO (%)	C18:3 (%)	DTF (d)	HT (cm)	DTM (d)
136	1	49.4	26.6	3.2	47.0	110.0	90.0
136	2	47.4	28.3	5.4	49.0	110.0	92.0
138	1	50.4	27.0	5.4	46.0	135.0	90.0
138	2	47.8	31.6	5.2	46.0	120.0	91.0
141	1	49.2	26.6	5.3	47.0	125.0	90.0
141	2	48.1	27.1	5.1	48.0	130.0	90.0
143	1	47.2	26.5	8.5	49.0	120.0	91.0
143	2	48.0	26.2	9.2	49.0	130.0	92.0
147	1	49.0	27.0	7.7	53.0	160.0	92.0
147	2	50.0	26.4	7.5	54.0	130.0	91.0
149	1	48.8	26.7	3.0	48.0	140.0	91.0
149	2	47.9	27.1	3.1	53.0	120.0	91.0
151	1	52.2	25.0	5.1	49.0	130.0	90.0
151	2	47.7	29.3	4.6	53.0	120.0	92.0
152	1	40.8	26.8	5.4	48.0	140.0	91.0
152	2	45.8	30.4	5.9	52.0	130.0	91.0
153	1	47.4	28.0	3.2	47.0	110.0	89.0
153	2	46.3	27.9	3.0	47.0	105.0	91.0
154	1	47.1	29.3	6.0	47.0	140.0	90.0
154	2	47.8	29.5	6.1	47.0	110.0	91.0
155	1	49.5	27.3	3.6	46.0	125.0	91.0
155	2	48.5	28.9	4.1	46.0	130.0	91.0
157	1	51.2	26.4	5.3	48.0	120.0	90.0
157	2	50.6	26.8	5.6	48.0	130.0	91.0
159	1	48.5	26.8	3.5	52.0	120.0	90.0
159	2	45.3	29.0	3.1	53.0	150.0	91.0
160	1	49.8	27.8	2.8	51.0	145.0	91.0
160	2	53.1	24.7	3.0	51.0	160.0	92.0
161	1	45.2	27.5	6.1	46.0	135.0	90.0
161	2	48.1	25.5	5.6	47.0	120.0	90.0
163	1	49.2	25.2	3.5	48.0	135.0	90.0
163	2	49.6	25.5	3.7	51.0	130.0	89.0

Appendix 5. Seed quality and agronomic field results of 100 DH lines of *B. napus* averaged over 4 site years (Wpg98, Car98, Wpg99, Car99). Traits are seed oil content (OIL), seed protein content (PRO), sum of oil and protein contents (SUM), linolenic acid content (C18:3), days to flowering (DTF), plant height (HT), days to maturity (DTM).

DH line	OIL(%)	PRO (%)	SUM (%)	C18:3 (%)	DTF (d)	HT (cm)	DTM (d)
3	48.0	29.1	77.1	3.5	49.8	123.1	97.3
7	48.7	29.5	78.2	5.8	50.0	125.6	97.6
10	46.8	29.2	76.0	5.7	49.1	122.5	96.9
11	47.3	29.1	76.3	5.3	54.5	128.1	97.9
12	47.7	27.9	75.6	5.4	49.1	121.3	96.6
13	48.0	29.5	77.5	5.8	55.5	145.6	97.9
14	45.9	29.4	75.3	5.6	51.0	119.4	97.6
15	45.8	30.5	76.3	3.8	55.4	140.6	97.9
16	48.0	27.8	75.8	4.9	51.0	104.4	96.8
17	47.4	27.6	75.0	7.2	53.6	136.9	98.3
18	46.1	29.4	75.5	5.4	50.3	118.8	96.0
19	45.8	29.9	75.7	3.1	54.5	125.0	98.3
20	46.3	28.6	74.9	5.9	49.4	120.0	96.9
21	43.6	30.7	74.3	5.9	53.9	128.1	97.9
24	44.5	29.8	74.3	4.0	51.7	119.7	98.1
25	44.5	29.1	73.6	3.3	53.3	120.0	96.5
27	47.4	28.6	76.0	3.4	52.4	131.3	97.8
28	48.7	28.8	77.5	5.0	50.4	129.4	97.1
31	48.0	29.1	77.1	3.0	52.8	130.0	97.8
33	46.9	29.1	76.0	6.9	50.0	100.0	96.8
35	45.1	28.2	73.3	6.1	53.6	136.3	97.5
36	45.6	29.0	74.6	6.1	51.4	123.1	97.3
37	44.7	29.8	74.5	7.3	51.6	118.1	95.8
38	46.7	29.3	76.0	5.4	53.6	133.1	97.4
40	47.7	30.2	77.8	6.0	52.9	129.5	97.2
41	47.4	29.1	76.4	5.6	48.3	113.8	95.0
42	47.4	29.2	76.5	5.0	48.4	125.0	96.1
44	47.4	28.0	75.4	6.0	51.9	113.1	98.1
45	47.7	28.8	76.5	5.5	52.0	132.5	97.0
46	44.2	29.9	74.0	4.3	56.8	118.8	98.1
47	47.3	27.4	74.7	5.3	48.5	124.4	95.3
48	47.6	28.8	76.5	3.6	55.1	126.9	98.6
49	48.4	28.9	77.3	7.7	52.1	130.6	97.8
50	47.6	29.7	77.2	3.3	49.3	112.5	97.5
52	48.4	28.8	77.2	4.9	49.6	120.6	96.6
53	43.5	29.5	73.1	5.9	48.1	118.8	96.4
54	47.4	26.1	73.4	5.8	49.1	128.8	95.8
56	45.6	28.5	74.0	8.4	52.8	134.4	97.0
57	48.4	29.7	78.0	5.9	53.5	134.4	98.1
59	46.0	27.7	73.7	6.1	48.6	121.3	96.1
61	49.7	27.5	77.1	7.6	54.0	124.4	97.4
62	48.5	26.8	75.3	5.8	48.3	119.4	95.5
64	48.5	27.3	75.8	5.3	51.9	128.1	97.3
65	47.7	28.1	75.8	5.3	49.3	121.3	95.4
66	44.1	30.1	74.2	3.8	56.1	119.4	98.4
67	45.0	29.8	74.9	3.8	53.1	141.3	97.5
68	46.0	30.4	76.3	5.3	54.5	135.0	97.6
69	45.9	29.8	75.8	5.6	51.6	106.3	96.6
71	47.4	29.9	77.3	5.2	54.9	134.4	98.9
72	48.8	28.2	77.0	7.8	51.0	121.3	98.3
74	43.3	30.2	73.5	3.7	49.8	120.6	96.6
76	48.2	27.2	75.4	4.9	49.8	128.5	97.0
78	46.9	28.8	75.7	5.0	49.8	130.0	95.9

Appendix 5 continued

DH line	OIL(%)	PRO (%)	SUM (%)	C18:3 (%)	DTF (d)	HT (cm)	DTM (d)
79	47.1	29.1	76.2	7.1	49.5	121.3	97.5
80	47.6	29.3	76.9	5.0	52.9	137.5	98.3
82	48.7	28.4	77.1	5.2	49.8	121.3	97.9
83	44.9	28.7	73.6	8.5	48.9	123.8	95.8
84	46.0	29.8	75.7	6.5	51.6	124.4	98.0
89	47.1	29.3	76.4	5.1	49.1	118.8	96.4
91	45.6	28.6	74.2	5.8	50.9	117.5	95.4
92	47.2	27.9	75.0	3.6	52.0	122.5	97.3
94	48.8	27.0	75.8	5.3	49.6	121.3	97.0
96	50.1	26.8	76.9	5.0	48.5	125.0	97.6
98	45.4	30.3	75.7	2.9	51.4	129.4	97.8
100	49.3	28.2	77.5	3.0	48.4	122.5	97.4
104	46.4	28.7	75.0	5.5	50.0	123.1	97.1
107	49.2	28.8	78.0	7.5	49.9	121.9	97.6
110	45.3	30.0	75.3	5.4	48.9	119.4	96.9
113	47.5	29.1	76.6	6.8	49.1	124.4	95.4
114	47.9	28.4	76.2	4.2	52.9	126.3	97.0
115	48.5	27.6	76.1	3.3	54.4	145.0	98.3
116	48.7	28.3	77.0	7.8	53.8	133.8	98.3
120	48.4	27.0	75.4	3.0	55.9	130.0	98.1
122	47.6	28.5	76.1	5.6	50.8	139.4	97.4
123	46.2	28.2	74.5	5.7	52.1	129.4	97.8
124	47.8	28.1	75.8	5.7	49.1	121.3	96.9
126	47.2	29.4	76.6	3.1	55.8	138.1	98.6
127	47.2	28.6	75.8	5.7	52.0	115.0	97.9
128	46.9	29.2	76.1	3.1	54.4	143.8	97.6
129	49.4	27.2	76.5	4.7	49.6	121.9	96.5
131	48.3	28.3	76.5	4.9	51.5	125.6	97.1
132	48.3	28.9	77.2	2.9	51.9	125.0	97.8
133	45.8	29.7	75.4	5.5	51.8	124.4	97.3
135	47.9	28.9	76.8	3.0	51.9	133.8	98.3
136	45.6	29.7	75.4	4.1	49.4	114.4	96.4
138	47.0	29.8	76.8	6.1	51.1	120.6	97.5
141	46.1	29.2	75.3	5.0	51.0	133.8	97.4
143	45.2	27.8	73.0	8.7	53.0	126.9	98.4
147	46.5	29.2	75.7	7.7	55.5	137.5	98.5
149	47.7	27.7	75.4	3.1	53.7	131.6	98.0
151	48.3	28.7	77.0	4.7	53.4	128.8	97.6
152	46.4	28.7	75.1	5.9	52.8	134.4	98.0
153	46.5	28.4	74.9	3.3	50.0	109.4	95.4
154	46.3	29.5	75.7	6.2	51.1	120.6	96.3
155	47.0	29.5	76.6	3.6	51.9	120.6	98.0
157	48.7	27.8	76.5	5.4	52.6	126.9	97.8
159	45.1	29.8	74.9	3.5	54.5	126.3	97.5
160	49.0	28.2	77.2	2.9	55.4	139.4	98.3
161	45.9	28.0	73.9	6.1	49.9	123.1	96.4
163	46.5	28.4	74.9	3.5	54.1	130.6	97.3

Appendix 6. Analyses of variance for linolenic acid content on 100 DH lines of *B. napus* (2 replicates/location). (Performed using Agrobase software.)

Wpg98

Source	df	SS	MS	F-value	Pr>F
Total	199	414.28			
Replicate	1	0.65	0.65	6.75	0.01
DH line	99	404.10	4.08	42.40	0.00
Residual	99	9.53	0.10		

Car98

Source	df	SS	MS	F-value	Pr>F
Total	199	405.41			
Replicate	1	1.05	1.05	2.33	0.13
DH line	99	359.78	3.63	8.07	0.00
Residual	99	44.57	0.45		

Wpg99

Source	df	SS	MS	F-value	Pr>F
Total	199	540.47			
Replicate	1	0.74	0.74	4.25	0.04
DH line	99	522.37	5.28	30.10	0.00
Residual	99	17.36	0.18		

Car99

Source	df	SS	MS	F-value	Pr>F
Total	199	488.08			
Replicate	1	0.02	0.02	0.12	0.73
DH line	99	470.37	4.75	26.60	0.00
Residual	99	17.68	0.18		

Appendix 7. Analyses of variance for seed oil content on 100 DH lines of *B. napus* (2 replicates/location). (Performed using Agrobase software.)

Wpg98

Source	df	SS	MS	F-value	Pr>F
Total	199	734.05			
Replicate	1	1.66	1.66	2.05	0.16
DH line	99	652.27	6.59	8.14	0.00
Residual	99	80.12	0.81		

Car98

Source	df	SS	MS	F-value	Pr>F
Total	199	723.15			
Replicate	1	0.03	0.03	0.02	0.88
DH line	99	598.00	6.04	4.78	0.00
Residual	99	125.12	1.26		

Wpg99

Source	df	SS	MS	F-value	Pr>F
Total	199	547.48			
Replicate	1	2.14	2.14	2.21	0.14
DH line	99	449.43	4.50	4.69	0.00
Residual	99	95.91	0.97		

Car99

Source	df	SS	MS	F-value	Pr>F
Total	199	1017.73			
Replicate	1	14.63	14.63	5.51	0.02
DH line	99	739.98	7.48	2.81	0.00
Residual	99	263.12	2.66		

Appendix 8. Analyses of variance for seed protein content on 100 DH lines of *B. napus* (2 replicates/location). (Performed using Agrobase software.)

Wpg98

Source	df	SS	MS	F-value	Pr>F
Total	199	399.54			
Replicate	1	57.67	57.67	60.74	0.00
DH line	99	247.86	2.50	2.64	0.00
Residual	99	94.00	0.95		

Car98

Source	df	SS	MS	F-value	Pr>F
Total	199	596.06			
Replicate	1	54.29	54.29	27.78	0.00
DH line	99	348.32	3.52	1.80	0.00
Residual	99	193.45	1.95		

Wpg99

Source	df	SS	MS	F-value	Pr>F
Total	199	564.44			
Replicate	1	1.11	1.11	0.59	0.44
DH line	99	378.10	3.82	2.04	0.00
Residual	99	185.23	1.87		

Car99

Source	df	SS	MS	F-value	Pr>F
Total	199	763.08			
Replicate	1	7.45	7.45	2.40	0.12
DH line	99	448.15	4.53	1.46	0.03
Residual	99	307.48	3.11		

Appendix 9. Analyses of variance for sum of oil and protein content on 100 DH lines of *B. napus* (2 replicates/location). (Performed using Agrobase software.)

Wpg98

Source	df	SS	MS	F-value	Pr>F
Total	199	2211.97			
Replicate	1	10.44	10.44	1.12	0.29
DH line	99	1277.18	12.90	1.38	0.05
Residual	99	924.35	9.34		

Car98

Source	df	SS	MS	F-value	Pr>F
Total	199	617.28			
Replicate	1	51.82	51.82	33.90	0.00
DH line	99	414.12	4.18	2.74	0.00
Residual	99	151.34	1.53		

Wpg99

Source	df	SS	MS	F-value	Pr>F
Total	199	493.09			
Replicate	1	6.37	6.37	5.29	0.02
DH line	99	367.53	3.71	3.08	0.00
Residual	99	119.18	1.20		

Car99

Source	df	SS	MS	F-value	Pr>F
Total	199	612.51			
Replicate	1	1.22	1.22	0.89	0.35
DH line	99	476.22	4.81	3.53	0.00
Residual	99	135.07	1.36		

Appendix 10. Analyses of variance for days to flowering on 100 DH lines of *B. napus* (2 replicates/location). (Performed using Agrobase software.)

Wpg98

Source	df	SS	MS	F-value	Pr>F
Total	199	1071.40			
Replicate	1	15.79	15.79	17.33	0.00
DH line	99	965.38	9.75	10.70	0.00
Residual	99	90.23	0.91		

Car98

Source	df	SS	MS	F-value	Pr>F
Total	199	1193.31			
Replicate	1	0.20	0.20	0.16	0.69
DH line	99	1071.30	10.82	8.79	0.00
Residual	99	121.82	1.23		

Wpg99

Source	df	SS	MS	F-value	Pr>F
Total	199	1526.88			
Replicate	1	1.81	1.81	1.44	0.23
DH line	99	1401.38	14.16	11.33	0.00
Residual	99	123.70	1.25		

Car99

Source	df	SS	MS	F-value	Pr>F
Total	199	1396.48			
Replicate	1	8.00	8.00	4.35	0.04
DH line	99	1206.48	12.19	6.63	0.00
Residual	99	182.00	1.84		

Appendix 11. Analyses of variance for plant height on 100 DH lines of *B. napus* (2 replicates/location). (Performed using Agrobase software.)

Wpg98

Source	df	SS	MS	F-value	Pr>F
Total	199	35140.70			
Replicate	1	214.87	214.87	2.57	0.11
DH line	99	26650.56	269.20	3.22	0.00
Residual	99	8275.28	83.59		

Car98

Source	df	SS	MS	F-value	Pr>F
Total	199	27959.02			
Replicate	1	359.39	359.39	3.73	0.06
DH line	99	18073.09	182.56	1.90	0.00
Residual	99	9526.55	96.23		

Wpg99

Source	df	SS	MS	F-value	Pr>F
Total	199	32958.00			
Replicate	1	3698.00	3698.00	41.83	0.00
DH line	99	20508.00	207.15	2.34	0.00
Residual	99	8752.00	88.40		

Car99

Source	df	SS	MS	F-value	Pr>F
Total	199	45273.88			
Replicate	1	2346.13	2346.13	15.39	0.00
DH line	99	27836.38	281.18	1.84	0.00
Residual	99	15091.38	152.44		

Appendix 12. Analyses of variance for days to maturity on 100 DH lines of *B. napus* (2 replicates/location). (Performed using Agrobase software.)

Wpg98

Source	df	SS	MS	F-value	Pr>F
Total	199	621.82			
Replicate	1	0.026	0.026	0.02	0.90
DH line	99	464.77	4.695	2.96	0.00
Residual	99	157.02	1.586		

Car98

Source	df	SS	MS	F-value	Pr>F
Total	199	281.70			
Replicate	1	1.38	1.38	1.49	0.23
DH line	99	188.64	1.91	2.06	0.00
Residual	99	91.68	0.93		

Wpg99

Source	df	SS	MS	F-value	Pr>F
Total	199	551.20			
Replicate	1	1.45	1.45	0.71	0.40
DH line	99	348.70	3.52	1.73	0.00
Residual	99	201.06	2.03		

Car99

Source	df	SS	MS	F-value	Pr>F
Total	199	153.16			
Replicate	1	0.61	0.61	1.05	0.31
DH line	99	95.66	0.97	1.68	0.01
Residual	99	56.90	0.58		

Appendix 13. Linolenic acid content values for Apollo check rows at each site year. Note: Apollo was planted every 5th row in 1998, and every 6th row in 1999. Data sorted in ascending order.

Linolenic acid content (%)			
Wpg98	Car98	Wpg99	Car99
1.4	1.4	1.5	1.5
1.4	1.4	1.5	1.6
1.4	1.4	1.6	1.6
1.4	1.5	1.6	1.6
1.5	1.5	1.6	1.6
1.5	1.5	1.6	1.6
1.5	1.5	1.6	1.7
1.5	1.5	1.7	1.7
1.5	1.5	1.7	1.7
1.5	1.5	1.7	1.7
1.5	1.5	1.7	1.7
1.5	1.5	1.7	1.7
1.5	1.6	1.8	1.7
1.5	1.6	1.8	1.8
1.5	1.6	1.8	1.8
1.5	1.6	1.8	1.8
1.5	1.6	1.8	1.8
1.6	1.6	1.8	1.8
1.6	1.6	1.8	1.8
1.6	1.6	1.8	1.8
1.6	1.6	1.9	1.8
1.6	1.7	1.9	1.8
1.6	1.7	1.9	1.9
1.6	1.7	1.9	1.9
1.6	1.7	2.0	1.9
1.7	1.7	2.0	1.9
1.7	1.7	2.0	1.9
1.7	1.7	2.0	1.9
1.7	1.7	2.0	1.9
1.7	1.7	2.0	2.0
1.7	1.7	2.0	2.0
1.7	1.7	2.0	2.0
1.7	1.7	2.0	2.0
1.7	1.8	2.1	2.0
1.7	1.8	2.1	2.0
1.7	1.8	2.2	2.1
1.7	1.8	2.2	2.2
1.7	1.8	2.3	2.2
1.7	1.8	2.8	2.3
1.7	1.8	2.9	2.4
1.8	1.8		
1.8	1.8		
1.8	1.8		
1.8	1.8		
1.8	1.8		
1.8	1.8		
1.8	1.8		
1.9	1.8		
1.9	1.8		
1.9	1.8		
1.9	1.8		
1.9	1.9		
1.9	1.9		
2.0	1.9		
2.0	1.9		
2.1	2.0		
2.1	2.0		
2.1	2.0		
2.1	2.0		
2.1	2.1		
2.2	2.2		
2.3	2.4		
3.3	3.2		

Appendix 14. Correlation matrices for seed quality and agronomic traits in a DH population of *B. napus*. Traits examined were seed oil content (OIL), seed protein content (PRO), sum of oil and protein (SUM), days to flowering (DTF), plant height (HT) and days to maturity (DTM), linolenic acid content (C18:3). (Upper number, correlation coefficient. Lower number, probablility)

Wpg98

	OIL	PRO	SUM	DTF	HT	DTM
PRO	0.12					
	0.09					
SUM	0.39	0.34				
	0.00	**0.00**				
DTF	-0.05	0.10	0.08			
	0.45	**0.15**	**0.26**			
HT	0.11	0.19	0.10	0.20		
	0.11	**0.01**	**0.18**	**0.01**		
DTM	0.00	0.19	0.09	0.51	0.16	
	0.96	**0.01**	**0.18**	**0.00**	**0.02**	
C18:3	-0.05	-0.02	-0.03	-0.22	-0.01	-0.16
	0.50	**0.77**	**0.68**	**0.00**	**0.86**	**0.02**

Car98

	OIL	PRO	SUM	DTF	HT	DTM
PRO	0.07					
	0.35					
SUM	0.56	0.86				
	0.00	**0.00**				
DTF	-0.17	0.12	0.01			
	0.01	**0.09**	**0.87**			
HT	0.14	0.00	0.07	0.29		
	0.05	**0.98**	**0.32**	**0.00**		
DTM	-0.12	0.18	0.08	0.43	0.21	
	0.10	**0.01**	**0.24**	**0.00**	**0.00**	
C18:3	-0.03	0.01	-0.01	-0.16	0.00	-0.10
	0.67	**0.91**	**0.87**	**0.03**	**0.97**	**0.14**

Wpg99

	OIL	PRO	SUM	DTF	HT	DTM
PRO	0.00					
	0.96					
SUM	0.46	0.89				
	0.00	**0.00**				
DTF	-0.13	0.12	0.06			
	0.07	**0.08**	**0.44**			
HT	-0.01	0.06	0.05	0.46		
	0.92	**0.39**	**0.46**	**0.00**		
DTM	-0.03	0.22	0.18	0.48	0.25	
	0.66	**0.00**	**0.01**	**0.00**	**0.00**	
C18:3	-0.13	-0.12	-0.17	-0.14	-0.05	0.00
	0.06	**0.09**	**0.02**	**0.06**	**0.45**	**0.98**

Appendix 14 continued

Car99

	OIL	PRO	SUM	DTF	HT	DTM	
PRO	-0.07						
	0.31						
SUM	0.55	0.79					
	0.00	**0.00**					
DTF	-0.12	0.09	0.00				
	0.09	**0.22**	**0.96**				
HT	0.01	0.03	0.03	0.25			
	0.87	**0.67**	**0.68**	**0.00**			
DTM	-0.17	0.19	0.05	0.44	0.23		
	0.02	**0.01**	**0.45**	**0.00**	**0.00**		
C18:3	0.07	-0.16	-0.10	-0.22	-0.13	-0.16	
	0.33	**0.02**	**0.18**	**0.00**	**0.06**	**0.02**	

Appendix 15. Scores for ASA350 and *fad3* markers across 134 DH lines in a DH population of *B. napus.* "+", presence of marker band; "-", absence of marker band. Shading denotes recombinant lines.

DH line	ASA350	*fad3*
3	+	+
4	+	+
7	-	-
9	+	+
10	+	+
11	+	+
12	+	+
13	-	-
14	+	+
15	+	+
16	+	+
17	-	-
18	-	-
19	+	+
20	-	-
21	+	+
24	+	+
25	+	+
26	+	+
27	+	+
28	-	-
30	-	-
31	+	+
33	-	-
34	-	-
35	+	+
36	+	+
37	-	-
38	+	+
39	+	+
40	+	+
41	-	-
42	-	-
43	+	+
44	-	-
45	+	+
46	+	+
47	-	-
48	+	+
49	+	-
50	+	+
51	-	-
52	+	+
53	+	+
54	+	-
56	-	-
57	+	+
59	-	-
60	+	-
61	-	-
62	+	+
63	-	-
64	+	+
65	+	+
66	+	+
67	+	+
68	+	+
69	-	-
70	+	+
71	+	+
72	-	-
74	+	+
76	+	+
78	-	-
79	-	-
80	+	+
81	-	-
82	-	-
83	-	-
84	-	-
85	+	-
86	+	-
87	+	+
88	+	+
89	-	-
90	-	-
91	-	-
92	+	+
93	+	+
94	-	-
95	+	+
96	+	+
97	+	+
98	+	+
100	+	+
101	+	+
103	+	+
104	+	+
105	+	+
107	+	-
108	-	-
109	+	+
110	-	-
111	+	+
113	-	-
114	+	+
115	+	+
116	-	-
117	+	+
118	-	-
120	+	+
121	+	+
122	+	+
123	-	-
124	+	+
125	+	+
126	+	+
127	-	-
128	+	+
129	+	+
131	-	-
132	+	+
133	-	-
135	+	+
136	+	+
138	-	-
139	+	+
140	-	-
141	+	+
143	+	-
147	-	-
149	+	+
150	+	+
151	+	+
152	+	+
153	+	+
154	+	+
155	+	+
156	+	+
157	-	-
159	+	+
160	+	+
161	-	-
163	+	+

Appendix 16. Analyses of variance for ASA350 in DH populations of *B. napus* segregating for linolneic acid content

Combined population (100 DH lines)

Groups	*Count*	*Sum*	*Average*	*Variance*
A (+)	64	295.59	4.618594	1.74125
B (-)	36	222.14	6.170556	1.06832

ANOVA

Source	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
b/w Groups	55.49381	1	55.49381	36.97325	2.31E-08	3.938112
w/in Groups	147.09	98	1.500918			
Total	202.5838	99				

Reston x LL09 (56 DH lines)

Groups	*Count*	*Sum*	*Average*	*Variance*
A (+)	37	181.03	4.892703	1.211615
B (-)	19	119.44	6.286316	1.094747

ANOVA

Source	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
b/w Groups	24.38101	1	24.38101	20.79123	2.98E-05	4.01954
w/in Groups	63.32357	54	1.172659			
Total	87.70458	55				

LL09 x Reston (44 DH lines)

Groups	*Count*	*Sum*	*Average*	*Variance*
A (+)	28	120.35	4.298214	2.288845
B (-)	16	96.91	6.056875	1.138636

ANOVA

Source	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
b/w Groups	31.49122	1	31.49122	16.76799	0.000188	4.07266
w/in Groups	78.87835	42	1.878056			
Total	110.3696	43				

Appendix 17. Analyses of variance for *fad3* in DH populations of *B. napus* segregating for linolneic acid content

Combined population (100 DH lines)

Groups	*Count*	*Sum*	*Average*	*Variance*
A (+)	60	265.93	4.432167	1.218102
B (-)	40	251.8	6.295	1.21621

ANOVA

Source	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
b/w Groups	83.28355	1	83.28355	68.41386	6.67E-13	3.938112
w/in Groups	119.3002	98	1.217349			
Total	202.5838	99				

Reston x LL09 (56 DH lines)

Groups	*Count*	*Sum*	*Average*	*Variance*
A (+)	34	161.82	4.759412	1.024206
B (-)	22	138.65	6.302273	1.052866

ANOVA

Source	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
b/w Groups	31.79561	1	31.79561	30.70997	9.16E-07	4.01954
w/in Groups	55.90897	54	1.035351			
Total	87.70458	55				

LL09 x Reston (44 DH lines)

Groups	*Count*	*Sum*	*Average*	*Variance*
A (+)	26	104.11	4.004231	1.186673
B (-)	18	113.15	6.286111	1.489378

ANOVA

Source	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
b/w Groups	55.38331	1	55.38331	42.30328	7.48E-08	4.07266
w/in Groups	54.98626	42	1.309197			
Total	110.3696	43				