1. Introduction

1.1. The importance of rapeseed

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

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

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

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

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

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

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

1.2. The genetic background of important traits in rapeseed

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

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

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

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

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

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

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

1.3. QTL mapping

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

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

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

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

1.4. Intervarietal substitution lines

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

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

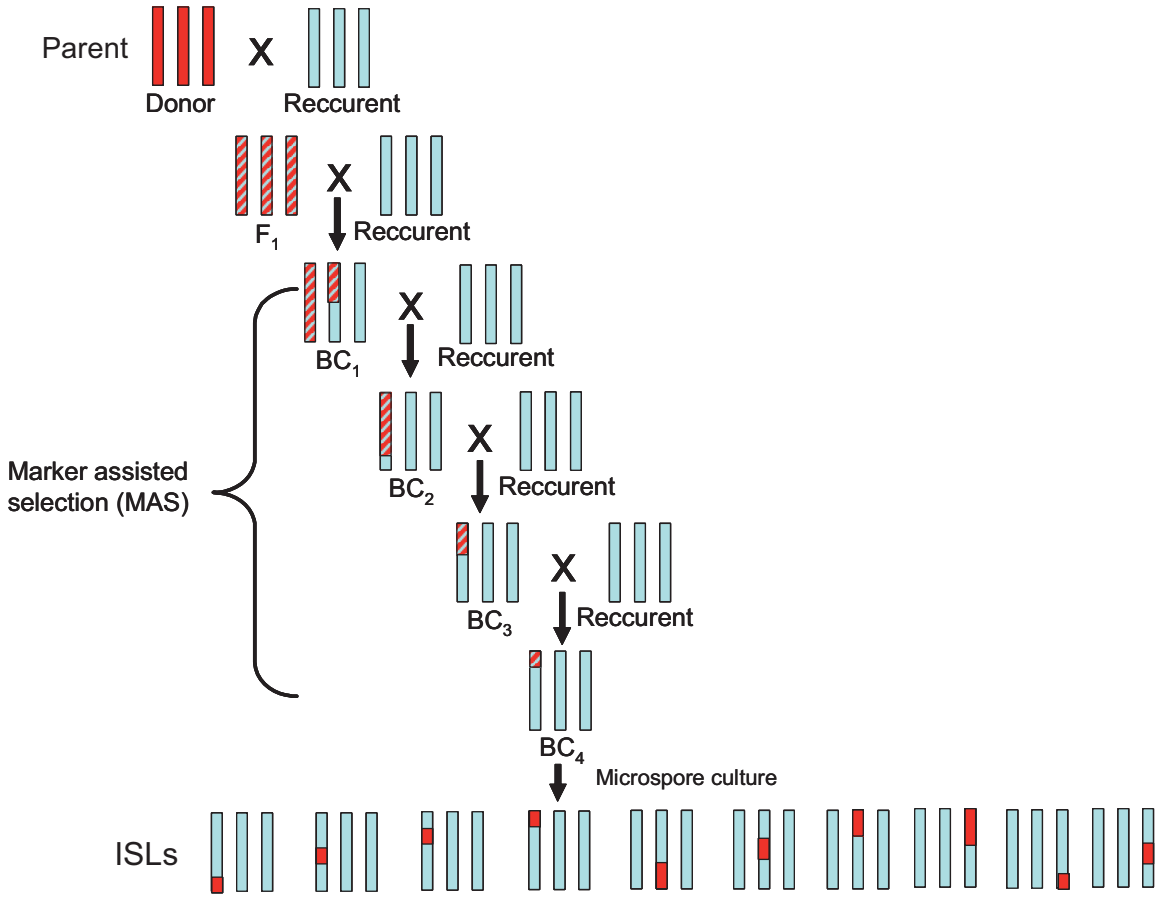

Figure 1 An illustration of the development scheme of intervarietal substitution lines (ISLs). ISLs are developed through several backcross generations. Using marker assisted selection, lines carrying a complementary set of donor segments are selected. Donor: donor parent; Reccurent: recurrent parent;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

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

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

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

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

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

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

1.5. QTL mapping using intervarietal substitution lines

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

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

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